UNIVERSIDADE FEDRAL DE MATO GROSSO DO SUL

FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE DESENVOLVIMENTO NA REGIÃO CENTRO-OESTE

HUDMAN CUNHA ORTIZ

AVALIAÇÃO DOS EFEITOS DO EXTRATO ETANÓLICO DE *Alternanthera littoralis, Salvia lachnostachys Benth* E *Serjania erecta* NO DESEMPENHO REPRODUTIVO, DESENVOLVIMENTO EMBRIOFETAL E INTEGRIDADE DO DNA EM CAMUNDONGOS *Swiss*

CAMPO GRANDE
2023

HUDMAN CUNHA ORTIZ

AVALIAÇÃO DOS EFEITOS DO EXTRATO ETANÓLICO DE *Alternanthera littoralis, Salvia lachnostachys Benth* E *Serjania erecta* NO DESEMPENHO REPRODUTIVO, DESENVOLVIMENTO EMBRIOFETAL E INTEGRIDADE DO DNA EM CAMUNDONGOS *Swiss*

Tese apresentada ao Programa de Pós-Graduação em Saúde e Desenvolvimento na Região Centro-Oeste, da Faculdade de Medicina da Universidade Federal de Mato Grosso do Sul, como parte dos requisitos para obtenção do título de Doutor em Saúde e Desenvolvimento na Região Centro-Oeste. Linha de Pesquisa: A Biodiversidade do Pantanal e Cerrado e suas relações e aplicações na saúde.

Orientador: prof. Dr. Rodrigo Juliano Oliveira

CAMPO GRANDE
2023

HUDMAN CUNHA ORTIZ

AVALIAÇÃO DOS EFEITOS DO EXTRATO ETANÓLICO DE *Alternanthera littoralis, Salvia lachnostachys Benth* E *Serjania erecta* NO DESEMPENHO REPRODUTIVO, DESENVOLVIMENTO EMBRIOFETAL E INTEGRIDADE DO DNA EM CAMUNDONGOS *Swiss*

Tese apresentada ao Programa de Pós-Graduação em Saúde e Desenvolvimento na Região Centro-Oeste, da Faculdade de Medicina da Universidade Federal de Mato Grosso do Sul, como parte dos requisitos para obtenção do título de Doutor em Saúde e Desenvolvimento na Região Centro-Oeste. Linha de Pesquisa: A Biodiversidade do Pantanal e Cerrado e suas relações e aplicações na saúde.

Orientador: prof. Dr. Rodrigo Juliano Oliveira

BANCA EXAMINADORA NOTA/Conceito

_____ _____
Rodrigo Juliano Oliveira – Faculdade de Medicina/UFMS

_____ _____
Paula Felipe Martinez – Instituto Integrado de Saúde/UFMS

_____ _____
Larissa Côrrea Hermeto – Faculdade de Veterinária/UFMS

_____ _____
Leandro Antero da Silva – Faculdade de Medicina/UEMS

_____ _____
Adélia Correia Lucio Girardi – HUMAP / EBSERH

_____ _____
Rodrigo Re Pop – HUMAP/EBSERH

AVALIAÇÃO FINAL: ( ) Aprovado ( ) Reprovado

AGRADECIMENTOS

A Deus por ter me dados forças para não desistir, perseverar e concluir mais esta etapa da minha caminhada.

Ao professor Alberto Ferreira Donatti que me ajudou, e muito, na formulação do projeto com o qual ingressei no doutorado e ao professor Albert Schiaveto de Souza por iniciar comigo esta jornada.

Ao professor Rodrigo Juliano Oliveira ter me recebido de braços abertos, pela atenção e por todo o conhecimento compartilhado.

A Silvia Cordeiro das Neves e Thais de Andrade Farias Rodrigues pela parceria ao longo de toda pesquisa.

A Giovana Martins Dantas de Oliveira, Maria Julia Costa e Silva e Thais Arruda de Santana, minhas colegas de laboratório que me auxiliaram e muito na finalização desta jornada.

A todos o meu enorme e sincero agradecimento!!

"O que mais na vida me apaixona é contribuir para uma realidade mais durável do que eu" .

M. Waltmann

RESUMO

Desde o início da história da humanidade há registro de que a medicina tradicional se utiliza de plantas medicinais para tratar doenças inclusive durante a gestação e o parto. Essas práticas são cada vez mais frequentes e podem ser incorporadas às práticas médicas modernas. *Alternanthera littoralis* é empregada na medicina popular brasileira para o tratamento de doenças inflamatórias e infecciosas. A *Salvia lachnostachys Benth* é nativa do Brasil e é utilizada de maneira popular como anti-inflamatória, anti-artrítica e anti-hiperalgésica. Já o chá das folhas de *Serjania erecta* é popularmente utilizada no Brasil contra úlcera e suas raízes contra hipertensão, além da indicação contra distúrbios estomacais. Não há na literatura registros dos efeitos do uso prolongado destas plantas sobre a gestante e o feto. Portanto o presente estudo teve como objetivo avaliar os efeitos dos Extratos Etanólicos de *Alternanthera littoralis* (EEAl), de *Salvia lachnostachys Benth* (EESl) e de *Serjania erecta* (EESe) sobre o desempenho reprodutivo, o desenvolvimento embriofetal e a integridade do DNA de camundongos fêmeas *Swiss* prenhes. No primeiro e segundo estudo, *Alternanthera littoralis* e *Salvia lachnostachys Benth*, respectivamente, as fêmeas prenhes foram distribuídas aleatoriamente em 3 grupos experimentais (n = 10): Grupo controle tratado com veículo e grupos EEAl/EESl 100 e EEAl/EESl 1000 tratados com doses de 100 e 1000 mg/kg, respectivamente. Já no terceiro estudo, com *Serjania erecta*, as fêmeas prenhes foram distribuídas aleatoriamente em 4 grupos experimentais (n = 10): Grupo controle tratado com veículo e grupos EESe 500, EESe 1000 e EESe 2000 tratados com as doses 500, 1000 e 2000 mg/kg, respectivamente. Em todos os estudos o tratamento ocorreu por gavagem durante todo o período gestacional até o 18° dia. Após foram avaliados parâmetros relacionados ao desempenho reprodutivo, desenvolvimento embriofetal e integridade do DNA. Os resultados indicaram que o EEAl não apresentou efeitos adversos já que não alterou o desempenho reprodutivo e nem mesmo o desenvolvimento embriofetal de forma significativa nos grupos tratados, além de não ser genotóxico; EESl não alterou os parâmetros do desempenho reprodutivo, e não é genotóxico. No entanto, alterou o desenvolvimento embriofetal por meio da redução do peso placentário, redução do peso fetal, aumento da frequência de fetos pequenos para a idade gestacional e aumento na frequência de malformações externas, viscerais e esqueléticas. No que se refere ao EESe, foram encontradas alterações estatisticamente significativas nos parâmetros biométricos de órgãos como coração e pulmão. Observou-se aumento no número e da taxa de reabsorções, além do aumento discreto de malformações externa, visceral e óssea, porém não apresentou genotoxicidade. Diante do exposto, considera-se que o EEAl não é maternotóxico, não altera o desempenho reprodutivo, não apresenta potencial teratogênico e nem genotoxicidade. O EESl não é maternotóxico, não altera o desempenho reprodutivo, não é genotóxico, mas altera o desenvolvimento embriofetal, e devido ao seu potencial teratogênico desaconselha-se o seu uso no período gestacional. Sobre o EESe observou-se que ele foi maternotóxico na dose de 2.000mg/Kg pois levou todos os indivíduos à morte, e em doses menores não apresentou maternotoxicidade e nem genotoxicidade. O EESe é embriofetotóxico já que aumentou o número e a taxa de reabsorção e possui baixo potencial teratogênico. Logo, contraindica-se o seu consumo no período gestacional.

Descritores: teratogênese, mutagênese, genotoxicidade, plantas medicinais.

ABSTRACT

Since the beginning of human history, there have been records that traditional medicine has used medicinal plants to treat diseases, including during pregnancy and childbirth. These practices are increasingly common and can be incorporated into modern medical practices. *Alternanthera littoralis* is used in Brazilian folk medicine for the treatment of inflammatory and infectious diseases. *Salvia lachnostachys Benth* is native to Brazil and is popularly used as anti-inflammatory, anti-arthritic and anti-hyperalgesic. *Serjania erecta* leaf tea is popularly used in Brazil against ulcers and its roots against hypertension, in addition to being used against stomach disorders. There are no records in the literature of the effects of prolonged use of these plants on the pregnant woman and the fetus. Therefore, the present study aimed to evaluate the effects of Ethanol Extracts of *Alternanthera littoralis* (EEAl), *Salvia lachnostachys Benth* (EESl) and *Serjania erecta* (EESe) on reproductive performance, embryofetal development and DNA integrity of female mice swiss pregnant. In the first and second studies, *Alternanthera littoralis* and *Salvia lachnostachys Benth*, respectively, pregnant females were randomly assigned to 3 experimental groups (n = 10): Control group treated with vehicle and groups EEAl/EESl 100 and EEAl/EESl 1000 treated with doses of 100 and 1000 mg/kg, respectively. In the third study, with Serjania erecta, pregnant females were randomly distributed into 4 experimental groups (n = 10): Control group treated with vehicle and groups EESe 500, EESe 1000 and EESe 2000 treated with doses 500, 1000 and 2000 mg /kg, respectively. In all studies, treatment occurred by gavage throughout the gestational period until the 18th day. Afterwards, parameters related to reproductive performance, embryofetal development and DNA integrity were evaluated. The results indicated that EEAl did not present adverse effects since it did not change the reproductive performance or even the embryofetal development in a significant way in the treated groups, in addition to not being genotoxic; EESl did not alter reproductive performance parameters and is not genotoxic. However, it altered embryofetal development by reducing placental weight, reducing fetal weight, increasing the frequency of small-for-gestational-age fetuses, and increasing the frequency of external, visceral, and skeletal malformations. With regard to the EESe, statistically significant changes were found in the biometric parameters of organs such as the heart and lungs. An increase in the number and rate of resorptions was observed, in addition to a slight increase in external, visceral and bone malformations, but it did not show genotoxicity. Given the above, it is considered that EEAl is not maternal toxic, does not alter reproductive performance, does not have teratogenic potential or genotoxicity. EESl is not maternal toxic, does not alter reproductive performance, is not genotoxic, but alters embryo-fetal development, and due to its teratogenic potential, its use in the gestational period is not recommended. Regarding EESe, it was observed that it was maternal toxic at a dose of 2,000mg/Kg, as it led all individuals to death, and at lower doses, it did not show maternal toxicity or genotoxicity. EESe is embryofetotoxic as it increased the number and rate of reabsorption and has low teratogenic potential. Therefore, its consumption during pregnancy is contraindicated.

Key words: teratogenesis, mutagenesis, genotoxicity, medicinal plants.

SUMÁRIO

LISTA DE FIGURAS

1. **INTRODUÇÃO**

Plantas medicinais são utilizadas para fins preventivos e terapêuticos desde tempos imemoriais e esse conhecimento tradicional é utilizado pela indústria farmacêutica, pois há grande potencial para a descoberta de novos medicamentos e até aperfeiçoamento de fórmulas já existentes (ARAÚJO *et al*., 2016).

O uso de plantas medicinais cresceu em todo o mundo nas últimas duas décadas e a Organização Mundial de Saúde (OMS) estima que 65% - 80% da população mundial principalmente dos países em desenvolvimento, depende de plantas medicinais para fins de saúde. Logo, elas são vistas como uma forma essencial, e muitas vezes a única, para tratar doenças na ausência da medicina convencional (medicamentos alopáticos) (BRUNO *et al*., 2018; AHMED, *et al*., 2021; BALARASTAGHI *et al.*, 2021).

Devido ao fácil acesso às plantas medicinais, à sua disponibilidade e à crença geral de que tratamentos naturais não causam efeitos adversos, acredita-se que o uso plantas é seguro e não causam riscos à saúde. Essa falsa ideia de segurança demonstra ainda mais a necessidade de pesquisas neste campo (HYACIENTH *et al.*, 2020).

Em contrapartida, tais plantas podem associar-se a efeitos nocivos como resultado de toxicidade direta, interações erva-droga, constituintes tóxicos, contaminação ou adulteração com metais tóxicos ou mesmo medicamentos convencionais não divulgados (por exemplo, esteroides, anti-inflamatórios não esteroides) (KAM; BARNETT; DOUGLAS, 2019). Além disso, muitas espécies são consumidas e vendidas livremente em mercados e feiras com pouca ou nenhuma comprovação de suas atividades farmacológicas e sem controle de qualidade e sanitário e, em geral, esses produtos comercializados livremente também não possuem teste de segurança (USTULIN, *et al.,* 2009).

A literatura apresenta estudos clínicos e pré-clínicos que identificaram efeitos teratogênicos e/ou abortivos de algumas plantas que são comumente usadas ou de seus derivados. Esse fato serve de alerta contra a suposição de que as plantas e seus derivados não são inofensivos apenas porque são naturais. Logo, há necessidade de ampliar a base de pesquisa sobre as plantas medicinais e de medicamentos usados durante a gestação para gerar dados científicos de efeitos terapêuticos e deletérios (BERNSTEIN *et al.*, 2020).

O Brasil, um país composto por diversos biomas, possui grande biodiversidade e, portanto, pode ser uma importante fonte de novos produtos terapêuticos. Ao que se sabe, apenas uma minoria das plantas que compõem a biodiversidade brasileira foi cientificamente estudada quanto a sua qualidade, segurança e eficácia, sobretudo no que diz respeito à gestação

(MARTINS *et al.,* 2015). Dentre as plantas da biodiversidade brasileira destaca-se a *Alternanthera littoralis,* a *Salvia lachnostachys benth* e a *Serjania erecta* que são nossos objetos de estudo.

Antes dos ensaios clínicos em seres humanos, o primeiro passo no desenvolvimento das diretrizes sobre a avaliação da qualidade, segurança e eficácia do medicamento, é testar em animais. É bem conhecido que a maioria dos estudos de teratogenicidade, geralmente, usa animais para identificar a potencial toxicidade ou interferência de produtos químicos ou drogas no desenvolvimento embrionário. Assim, pode-se supor o que aconteceria em seres humanos visto que consequências teratogênicas identificadas em animais também são encontradas em seres humanos (LI *et al.,* 2019).

Na literatura não foram encontrados dados sobre os efeitos dos extratos etanólicos da *Alternanthera littoralis* (EEAl), da *Salvia lachnostachys benth* (EESl) ou da *Serjania erecta* (EESe) no período gestacional. Também não foram encontrados estudos que garantam a segurança do desenvolvimento embriofetal e isso é imprescindível para a saúde da gestante e deu seu feto. Desta forma, o presente estudo teve como objetivo avaliar os efeitos do EEAl, EESl e do EESe sobre desempenho reprodutivo, desenvolvimento embriofetal e integridade do DNA de camundongos fêmeas prenhes.

2. REVISÃO DE LITERATURA

2.1 Plantas Medicinais

Todas as culturas e civilizações antigas desenvolveram e promoveram seus próprios sistemas terapêuticos, fazendo uso de recursos biológicos disponíveis localmente com base em observações empíricas e suas inferências. Plantas medicinais ainda são utilizadas mundialmente para o tratamento de mal-estar e doenças, e este conhecimento representou no passado a única opção terapêutica para muitas comunidades e ainda hoje é a única alternativa para comunidades mais isoladas (TRIBESS *et al*., 2015; SPONCHIADO *et al*., 2016).

O termo planta medicinal refere-se a uma variedade de plantas que possuem propriedades terapêuticas. A Organização Mundial da Saúde (OMS) define planta medicinal como materiais vegetais naturais que são utilizados na ausência de processamento industrial para o tratamento de doenças em escala local ou regional. Reconhece os benefícios das plantas medicinais e as define como "a melhor e maior fonte de medicamentos para a humanidade" (JAMSHIDI-KIA; LORIGOOINI; AMINI-KHOEI, 2018; NETO *et al.*, 2020).

Considera-se que são uma rica fonte de compostos ativos, que têm efeitos fisiológicos nos organismos vivos e são a base para o desenvolvimento e síntese de medicamentos. Diferentes partes da planta podem ser utilizadas para este fim, tais como sementes, raiz, folha, fruto, casca, flores ou até mesmo a planta inteira (JAMSHIDI-KIA; LORIGOOINI; AMINI-KHOEI, 2018; ROCHA *et al*., 2019).

O uso de plantas medicinais aumentou substancialmente, seja como agentes empregados na medicina tradicional e/ou como fonte de matéria-prima para a produção de suplementos alimentares, tanto na cultura ocidental quanto na asiática (SPONCHIADO *et al*., 2016). A OMS afirma que 80% da população dos países em desenvolvimento, incluindo o Brasil, utiliza práticas tradicionais em seus cuidados básicos de saúde e 85% utilizam plantas medicinais ou preparações delas. Assim, é intenção da OMS incentivar o uso da fitoterapia na atenção básica (ARAÚJO *et al*., 2016).

No Brasil, 66% da população não tem acesso a medicamentos comerciais e o uso popular de medicamentos à base de plantas, muitas vezes, se deve à precariedade da assistência médica e farmacêutica e ao alto custo do tratamento com medicamentos convencionais (MAZZARI; PRIETO, 2014).

A presença de diferentes biomas brasileiros, tais como Florestas e Savanas Tropicais Pluviais (1 - Bioma Floresta Amazônica e suas subdivisões; 2 - Bioma Savana Amazônica ou

Campinarana; 3- Bioma Floresta Atlântica e suas subdivisões), Florestas e Savanas Tropicais Estacionais (1 - Bioma Floresta Tropical e suas subdivisões - Ciliar, Galeria e Mata Atlântica; 2 - Bioma Savana Tropical Estacional - Cerrado; 3 - Bioma Savana Tropical Estacional Semiárida - Caatinga do Nordeste) e Florestas Quente-Temperadas Úmidas (Bioma Floresta Quente-Temperada Úmida - Mata Atlântica - e suas subdivisões), além de sistemas complexos como o Pantanal e os Campos Sulinos (COUTINHO, 2016), caracterizam o Brasil como o país com uma das maiores biodiversidades do planeta com números superiores a 55.000 espécies descritas, correspondendo a 22% do total de espécies no mundo (BOLSON *et al*., 2015). Essa rica biodiversidade é acompanhada por uma longa aceitação das plantas medicinais e conhecimentos tradicionais passados de forma verbal de geração em geração (CARVALHO *et al*., 2014; CARVALHO *et al*., 2019).

De todo o potencial fitoterápico brasileiro, apenas 8% das 55.000 espécies de plantas catalogadas já foram estudadas para identificação de compostos bioativos e apenas 1.100 dessas espécies foram avaliadas por suas propriedades medicinais (NETO *et al*., 2020).

A população brasileira tem uma longa tradição no uso de plantas medicinais para o tratamento de diversas doenças agudas e crônicas e o conhecimento tradicional relacionado às plantas medicinais é a base da medicina popular no Brasil que é derivada de uma mistura de culturas indígenas brasileiras, influências europeias e africanas do período de colonização (BOLSON *et al*., 2015; DUTRA *et al*., 2016).

Algumas plantas brasileiras foram incluídas, décadas atrás, em diferentes Farmacopeias por possuírem substâncias utilizadas na prática médica em todo o mundo. Exemplos são *Carapichea ipecacuanha* (Brot.) *L. Andersson* (ipecac) conhecida popularmente como Raiz preta ou Ipeca de mato grosso, fonte do alcaloide emético e amebicida emetina e *Pilocarpus microphyllus Stapf ex Wardlew*, conhecida popularmente como Jaborandi, fonte do antiglaucomatoso pilocarpina (PALHARES *et al*., 2021).

Pelo fato de as plantas medicinais serem amplamente utilizadas pela população mundial e serem fonte em potencial de fármacos terapêuticos, avaliações de segurança são cruciais para validar o seu uso contínuo e de fitoterápicos. Dentre as avaliações, estudar o potencial 13enotóxicos de plantas que são consumidas como medicamento é essencial para garantir que sejam realmente seguras para uso (SPONCHIADO *et al*., 2016).

2.2 *Alternanthera littoralis*

A família Aramanthaceae compreende aproximadamente 65 gêneros e 1.000 espécies de plantas herbáceas anuais e perenes, arbustos e algumas árvores que ocorrem em regiões tropicais, subtropicais e temperadas (SOUZA *et al*., 2007). Essa família é utilizada na nutrição e na medicina popular (SALVADOR *et al*., 2004ª; SOUZA *et al*., 2007).

De acordo com Singla *et al.* (2022), o gênero *Alternanthera* compreende 139 espécies, incluindo 14 espécies usadas tradicionalmente para o tratamento de várias doenças, tais como a hipertensão arterial sistêmica, dor, inflamação, diabetes, câncer, infecções microbianas e transtornos mentais. Segundo Aquino *et al*. (2015), esse gênero possui apenas 80 espécies das quais 30 são encontradas no Brasil e estão localizadas nas praias arenosas da costa leste (Figura 1).

Figura 1: Mapa de distribuição da *Alternanthera littoralis* no Brasil; Fonte: Sistema de informação sobre Biodiversidade Brasileira; Disponível em: https://ala-bie.sibbr.gov.br/ala-bie/species/283602.

Alternanthera littoralis P. Beauv. (Figura 2), conhecida popularmente como Perequito-da-praia, possui sinônimos botânicos (heterotípicos) e, portanto, pode ser encontrada na literatura como *Alternanthera littoralis var. marítima* (*Mart.*) *Pedersen*, *Alternanthera marítima Mart.* ou *Telanthera marítima (Mart.) Moq.* (SENNA, 2020). Apesar dos sinônimos, a classificação *Alternanthera littoralis* P. Beauv é a mais atual e, portanto, essa será utilizada no presente estudo.

Figura 2: *Alternanthera littoralis;* disponível em: https://tropical.theferns.info/viewtropical.php?id=Alternanthera%20littoralis

A. littoralis é empregada na medicina popular brasileira para o tratamento de doenças inflamatórias e infecciosas (SOUZA *et al.*, 2007), e é fonte de metabólitos secundários como vitexina (A14), 2″-O-α-Lrhamnopiranosil-vitexina (A18) e 2″ -O- β-D - glucopiranosil-vitexina (A19), que podem ser encontrados na fração enriquecida de flavonas glicosiladas de extratos etanólicos dessa planta (SALVADOR; DIAS, 2004; KASSUYA *et al*., 2021). Tais frações ou compostos podem ser responsáveis pelos resultados obtidos nos estudos descritos a seguir.

No de estudo Salvador *et al.* (2004b) demonstraram que a *Alternanthera littoralis* e seus constituintes isolados (esteroides, saponinas e flavonoides) apresentam atividade antimicrobiana *in vitro*. Assim, eles têm potencial para serem usados para fins médicos ou como aditivos naturais antimicrobianos nas indústrias cosmética e alimentícia ou possivelmente como alternativas seguras aos antimicrobianos sintéticos.

Salvador *et al.* (2004a) complementaram o primeiro estudo e avaliaram a atividade antibacteriana e antifúngica dos extratos brutos de *Alternanthera littoralis* (Amaranthaceae), de duas coletas distintas e obtidos por cultura de células, buscando-se averiguar a manutenção da atividade antimicrobiana dos extratos obtidos da planta *in vivo* e *in vitro*. Observaram que os extratos *in natura* inibiram o desenvolvimento de diferentes microrganismos (bactérias, leveduras e dermatófitos) e os extratos obtidos da cultura de calos desenvolvidos em duas condições de cultivo diferentes, também se mantiveram bioativos, e sugerem novos estudos para entender seu mecanismo de ação e avaliar sua toxicidade, visando uma possível aplicação farmacêutica.

Já Souza *et al*. (2007) demonstraram em seu trabalho que *A. littoralis* pode ser considerada uma fonte natural promissora de compostos antioxidantes para usos terapêuticos ou preventivos contra doenças crônicas dependentes de ROS.

Foi observado por Gasparetto *et al.* (2010) que a fotoativação dos extratos hexânico e etanólico das partes aéreas de *A. littoralis* por irradiação produz efeito antimicrobiano contra cepas de *C. dubliniensis* e que seus extratos são promissores como fotossensibilizadores naturais em terapia fotodinâmica.

Aquino *et al*. (2015) concluiram em sua pesquisa que o extrato etanólico das folhas de *A. littoralis* tem efeitos anti-inflamatórios e anti-hiperalgésicos no edema, pleurisia e hiperalgesia induzidos por carragenina (Cg) em camundongos. A 2″-O-α-L-ramnopiranosilvitexina, um flavonoide encontrado na planta que parece ser responsável pelos efeitos do seu extrato. Quando testado em doses baseadas no rendimento do extrato, tanto por via oral quanto local, foi capaz de reduzir o edema, a migração de leucócitos, o extravasamento de proteínas e a hiperalgesia induzida pela Cg.

Apesar dos estudos acima citados, não foram encontrados na literatura ensaios que indiquem se esta planta medicinal pode ser utilizada de maneira segura durante a gestação.

2.3 *Salvia lachnostachys Benth*

A palavra Salvia significa saúde em Latim e, no século X, médicos pertencentes à comunidade árabe acreditavam que a sálvia ofereceria uma solução definitiva para a morte que ocorria em todo o mundo (KANDMIR *et al*., 2022). A Lamiaceae é uma das maiores famílias do reino vegetal e inclui mais de 240 gêneros que são aromáticos. Algumas espécies de *Salvia L*. têm propriedades farmacológicas significativas e são utilizadas na medicina tradicional desde os tempos antigos. Elas são consumidas como chá e utilizadas nas indústrias de cosméticos, aromatizantes e perfumaria (EMRE; KURSAT, 2022).

A espécie *Salvia lachnostachys Benth* (Figura 3) é uma erva perene do Brasil. Ocorre de forma endêmica apenas nas regiões Sul e Sudeste do país (Figura 4) (OLIVEIRA *et al*., 2022). É considerada uma espécie ornamental (LEAL; BIONDI, 2006), portanto, com possibilidades de distribuição para outras regiões do país. Esta planta é conhecida como melissa e é utilizada para o tratamento de espasmos, gripes e insônia na medicina popular (NEGRELLE; FORNAZZARI, 2007).

Figura 3 - *Salvia lachnostachys Benth*. Dispopnivel em: https://docplayer.com.br/161697231-Universidade-federal-do-parana-marianna-erbano.html

Figura 4 – Mapa da distribuição da *Salvia lachnostachys Benth* no Brasil; Fonte: Sistema de informação sobre Biodiversidade Brasileira; Disponível em: https://ala-bie.sibbr.gov.br/ala-bie/species/334317

O óleo essencial das flores e folhas de *S. lachnostachys* possui compostos alifáticos saturados e uma fração sesquiterpênica (KASSUYA *et al*., 2009). O perfil cromatográfico do seu extrato etanólico também foi obtido por cromatografia líquida de alta frequência e os seguintes compostos foram identificados: ácido rosmarínico, demetilfruticulina A, ácidos oleanólico e ursólico, fruticulina A e fruticulina B (OLIVEIRA *et al*., 2016; CORSO *et al*., 2019; OLIVEIRA *et al*., 2021,).

O extrato etanólico de *S. lachnostachys* já foi descrito com atividades anti-inflamatória, citotóxica, antiartrítica, analgésica, antidepressiva e antinociceptiva e acredita-se que a Fructiculina A seja um dos compostos que auxiliam nessas propriedades, sendo citada como

anti-inflamatório, anti-hiperalgésico, antidepressivo, antinociceptivo e como antineoplásica em modelo pré-clínico *in vitro* e *in vivo* (PICCINELLI *et al*., 2104; OLIVEIRA *et al*., 2016; SANTOS *et al*., 2017; RADAI *et al*., 2018; CORSO *et al*., 2020). Além disso, outros componentes como dimetilfruticulina A, fruticulina B e demetilfruticulina B apresentaram atividade antioxidante (OLIVEIRA *et al*., 2021), e o extrato de *S. lachnostachys* foi caracterizado na literatura como quimiopreventivo e antitumoral em modelos de Ehrlich (CORSO *et al*., 2019).

Em relação à toxicidade Corso *et al.* (2019) realizaram a Avaliação de Toxicidade Aguda do extrato etanólico de *S. lachnostachys* em camundongos *Swiss*, de acordo com a Organização para Cooperação e Desenvolvimento Econômico (OECD 423, 2002) e apontam que a LD50 via oral, com a utilização deste extrato, é superior a 2.000 mg/kg para esta espécie. Não foram observados sinais de toxicidade durante os 14 dias de tratamento em camundongos com dose única de 2.000 mg/kg deste extrato.

Para considerar a possibilidade de usar o extrato etanólico de *S. lachnostachys* para o tratamento de artrite reumatoide ou inflamação crônica, Radai *et al.* (2018) testaram a genotoxicidade 72 h após a administração do extrato e evidenciaram que as diferentes doses de do extrato utilizadas em seu estudo não causaram danos ao DNA.

Considerando o exposto, infere-se que o extrato de *S. lachnostachys* pode ser usado para o tratamento primário de processos inflamatórios, dor e depressão com segurança. No entanto, como mulheres grávidas são afetadas por todas essas condições há a necessidade de expandir os ensaios toxicológicos para avaliação do período gestacional. Soma-se a isso o fato de que anti-inflamatórios e analgésicos são amplamente utilizados durante a gravidez, como apontam os estudos de Fontoura *et al*. (2014) e Lima *et al*. (2022).

Baseado nas propriedades terapêuticas existentes dessa planta, torna-se necessária a investigação de sua ação durante o período gestacional, já que nenhum dado a respeito foi encontrado na literatura até o momento.

2.4 *Serjania erecta*

O grupo *Paullinieae*, pertence à família Sapindaceae, apresenta distribuição neotropical e compreende aproximadamente 450 espécies distribuídas em sete gêneros: *Cardiospermum, Houssayanthus, Lophostigma, Paullinia, Serjania, Urvillea e Thinouia*. O gênero *Serjania* possui cerca de 231 espécies e o maior grupo são as *Paullinieae* (CARDOSO *et al*., 2013). No

entanto, *Serjania erecta* (Figura 5) é uma planta medicinal pertencente à família Sapindaceae e à Ordem Sapindales (GUARIM-NETO, SANTANA e SILVA, 2000).

Figura 5: *Serjania erecta*. Disponível em: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:784978-1

Ela é encontrada em áreas mais secas, ao longo de bordas de florestas e em áreas acidentadas e com ervas daninhas (CARDOSO *et al*., 2013), e está amplamente distribuída nas regiões tropicais do mundo e algumas espécies são encontradas no Brasil, principalmente nos estados de Mato Grosso, Mato Grosso do Sul, Tocantins, Goiás e Distrito Federal (Figura 6). Esta planta é popularmente conhecida como Cinco Folhas ou Cipó-cinco-folhas (GUARIM-NETO; SANTANA; SILVA, 2000).

Figura 6: Mapa da distribuição da *Serjania erecta* no Brasil; Fonte: Sistema de informação sobre Biodiversidade Brasileira; Disponível em: https://ala-bie.sibbr.gov.br/ala-bie/species/296678

De acordo com Pott e Pott (1994), as folhas de *S. erecta* tem indicação popular contra úlcera e as raízes contra hipertensão arterial sistêmica. Guarim-Neto, Santana e Silva (2000) reforçam essa mesma indicação popular e indicam o uso na forma de chá. Já Martins *et al.* (2015) descrevem o uso popular de *S. erecta* contra distúrbios estomacais.

A análise fitoquímica qualitativa do extrato hidroalcoólico obtido do caule e folhas de *S. erecta* mostrou a presença de saponinas, flavonóides, triterpenóides, esteróides, taninos e catequinas. Estudos apontam que seu extrato e/ou alguns dos seus compostos isolados tem potencial anti-inflamatório tópico (GOMIG *et al.,* 2008), tem efeito contra dores estomacais, doenças ulcerativas, hipertensão arterial sistêmica (ARRUDA *et al.,* 2009) e apresenta atividade antimicrobiana (CORDOVA *et al.,* 2010; CARDOSO *et al.,* 2013). Porém, não influencia na motilidade gástrica (POTRICH *et al*., 2014).

Broggnini *et al.* (2010) e Guimarães *et al*. (2015) demonstraram em suas pesquisas que o extrato aquoso das folhas de *S. erecta* atua no sistema nervoso central, inibindo a perda de memória e estimulando a aquisição e evocação de memória em roedores. Além disso, exerce atividade anticolinesterase *in vitro*. Essas ações, associadas à baixa toxicidade dos extratos tornam a *S. erecta* uma fonte promissora de novos compostos para o tratamento da Doença de Alzheimer.

Fernandes *et al*. (2011) observaram em seu estudo que essa planta apresenta atividade antiofídica devido à presença de compostos que são capazes de inibir as atividades tóxicas do veneno de *Bothrops jararacuçu,* pois inibem a ação de enzimas envolvidas nos distúrbios da coagulação sanguínea responsáveis por processos hemorrágicos.

Em relação à toxicidade, Arruda *et al.* (2009) realizaram uma única administração oral de extrato clorofórmico na dose de 5.000 mg/kg não produziu sinais nem sintomas de toxicidade aguda nos animais tratados. Durante os 14 dias após a administração do extrato, nenhum animal morreu e não foram observadas alterações significativas no peso diário do corpo ou dos órgãos até o final deste período. Assim, este resultado indica que o extrato não apresenta efeito toxicológico agudo quando administrado por via oral em camundongos machos e fêmeas.

Broggnini *et al.* (2010) realizaram estudos de toxicidade aguda, com extrato bruto de *Serjania erecta* conforme RE 90 da Agência Nacional de Vigilância Sanitária (ANVISA, 2004) utilizando 100 camundongos *Swiss* machos e fêmeas, com administração via oral e intraperitoneal, e seus dados de DL50 mostraram que levou à morte a 1250 mg/kg apenas por via intraperitoneal.

No entanto nenhum dado foi encontrado sobre os efeitos de seu uso prolongado durante a gestação, sobre a gestante ou sobre o feto.

2.5 Gestação e Plantas medicinais

As mulheres são reconhecidas como as principais usuárias de plantas medicinais, e esse uso generalizado se estende até a gravidez (AHMED *et al*., 2021). A gravidez é um período crítico para os cuidados médicos, durante o qual o bem-estar da mulher e do feto devem ser considerados (SPIESS *et al*., 2021).

Desde o início da história humana, está documentado que a medicina tradicional utiliza remédios à base de plantas para tratar complexidades e desafios durante a gravidez, parto e pós-parto, e incluem o uso para fertilidade feminina, amenorreia, controle de natalidade, puerpério e lactação (AHMED *et al*., 2018).

Estudos mostraram que a maioria das mulheres usa ervas durante a gravidez para aliviar náuseas e vômitos, aumentar o tônus uterino ou tratar infecções, incluindo candidíase e infecções do trato urinário (ASSOGBA *et al.,* 2021; DOSOKY; SETZER, 2021) por ficarem apreensivas quanto aos potenciais efeitos adversos dos medicamentos convencionais consumidos durante a gravidez e, por isso, consomem produtos à base de plantas porque acredita-se que sejam inofensivos. No entanto, a crença de sua eficácia e segurança na gravidez não é baseada em evidências (KAM; BARNETT; DOUGLAS, 2019).

Devido às inúmeras situações clínicas relacionadas à saúde da mulher e assistência médica precária em países em desenvolvimento, um grande repertório de plantas é utilizado como medicamentos populares o que gera riscos à saúde das usuárias, pois testes toxicológicos ainda são insuficientes para garantir sua segurança (YAZBEK *et al*., 2016).

Como exemplo podemos citar o estudo realizado por Araújo *et al.* (2016) no nordeste do Brasil, que constatou que 30,9% das gestantes utilizavam plantas medicinais, sendo o boldo a mais citada (35,4%). Entre as plantas utilizadas com alta frequência pelas gestantes *(Peumus boldus* - Boldo, *Foeniculum vulgare* - Funcho, *Cymbopogon citratus* – Capim Cidreira, *Matricaria chamomilla* – Camomila, *Baccharis trimera* – Carqueja e *Mentha piperita L*.- Menta), todas, com exceção apenas da Erva-Cidreira (*Melissa officinalis*), apresentavam possíveis efeitos tóxicos na gestação.

Como as plantas medicinais têm um papel significativo nos tratamentos associados à saúde da mulher, os dados etnofarmacológicos sobre elas podem contribuir para a redução das taxas de mortalidade, seja por meio de estudos farmacológicos para comprovar sua eficácia ou estudos de toxicidade para avaliar sua segurança antes de serem introduzidas na terapêutica (YAZBEK *et al.,* 2016; HYACIENTH *et al.,* 2020).

No caso das gestantes, a taxa de mortalidade pode se tornar ainda maior quando há o uso concomitante de medicamentos industrializados e de preparações artesanais obtidas de plantas medicinais ou drogas vegetais. Nessas situações aumenta-se a possibilita do surgimento de interações medicamentosas desconhecidas na mãe e prejuízos ao desenvolvimento fetal (CARDOSO; AMARAL, 2019).

De fato, certas plantas utilizadas no tratamento tradicional de doenças em humanos podem causar efeitos indesejáveis. Estudos indicam que algumas plantas têm potencial tóxico, teratogênico e/ou abortivo, pois alguns de seus princípios ativos são capazes de atingir o feto, principalmente no primeiro trimestre de gestação, especialmente, se forem consumidas em doses excessivas (ARAÚJO *et al*., 2016; BALARASTAGHI, *et al.*, 2021).

A falta de conhecimento sobre os efeitos embriotóxico, teratogênico e abortífero que algumas plantas medicinais podem apresentar, em associação com o seu uso indiscriminado durante a gestação, evidenciam a necessidade de estudos que investiguem quais delas oferecem risco durante este período. Além disso, este conhecimento precisa ser repassado para profissionais da área da saúde e para as próprias gestantes (GORRIL *et al.*, 2016).

2.6 Danos no DNA e malformações

A genotoxicidade constitui a área da toxicologia que estuda processos que alteram o DNA, denominado mutagênese. Os agentes genotóxicos lesam o DNA, modificam sua estrutura e/ou função e comprometem sua replicação e transmissão genética. Tais lesões podem ser reparadas fisiologicamente, porém quando persistem, ocorrem mutações que são herdadas pelas células filhas durante a replicação (EASTMOND *et al*., 2009).

As mutações no DNA acometem todos os seres vivos, por razões naturais, fatores endógenos ou exógenos ao organismo, o que resulta na variedade genética da população. Quando benéficas, as alterações no DNA podem melhorar condições de sobrevivência e reprodução em um determinado ambiente, garantindo adaptação e a evolução da espécie. No entanto, quando apresentam resultados deletérios, podem aumentar a incidência de doenças hereditárias, câncer e teratogenicidade (OLIVEIRA *et al.*, 2013).

O teste de micronúcleo é um dos métodos mais utilizados para avaliar efeitos genotóxicos causados por agentes químicos, tem baixo custo, é minimamente invasivo e baseia-se na detecção de pequenas inclusões arredondadas, chamadas micronúcleos, sendo utilizado, preferencialmente, células de animais mamíferos devidamente tratados (UCHÔA; MAGALHÃES, 2020).

Os micronúcleos, que são facilmente visíveis sob microscopia de luz, são fragmentos cromossômicos acêntricos ou cromossomos inteiros que não são incorporados ao núcleo principal de uma célula filha durante a divisão nuclear (SANDOVAL- HERRERA *et al*., 2020; CANEDO *et al*., 2021). Sua formação se deve a alterações estruturais cromossômicas espontâneas ou decorrentes de fatores ambientais, ou ainda, a falhas no fuso mitótico, sendo excluído, portanto, do núcleo formado na telófase (CARRARD *et al*., 2007).

Neste ensaio são utilizadas, principalmente, células provenientes da linhagem hematopoiética, como linfócitos e hemácias. Alguns autores utilizam a medula óssea propriamente dita para análise de micronúcleo. Essa técnica tem desvantagem em relação a que utiliza sangue periférico por permitir somente uma coleta, enquanto na análise de sangue periférico podem ser realizadas várias coletas, analisando a quantidade de dano cromossômico em diferentes tempos de exposição (BRAGA *et al*., 2021).

O ensaio de micronúcleo em sangue periférico utiliza reticulócitos para avaliar a presença de danos cromossômicos. No processo natural de maturação dessas células, antes de se tornarem eritrócitos, os eritroblastos devem expelir seu núcleo e nesse momento, caso tenha ocorrido dano cromossômico, o dano persistirá no citoplasma da célula, podendo ser visualizado com uso de corantes fluorescentes (HAYASHI *et al*., 1990).

A técnica de micronúcleo consiste em depositar sobre uma lâmina coberta previamente com Alaranjado de Acridina uma gota de sangue periférico. Este corante possui a capacidade de se intercalar ao DNA e de se ligar ao RNA e, quando submetido à radiação ultravioleta, o DNA emite uma fluorescência verde/amarelada, marcando assim os micronúcleos fixados no citoplasma dos eritrócitos que por usa vez são ricos em RNA que emitem fluorescência vermelha quando sob radiação ultravioleta (figura 7). A coloração proveniente do corante ligado aos DNA/RNA nos permite excluir a possibilidade de artefatos gerados por coloração não específica (HAYASHI *et al*., 1990; OLIVEIRA *et al*., 2009; KANG *et al*., 2013).

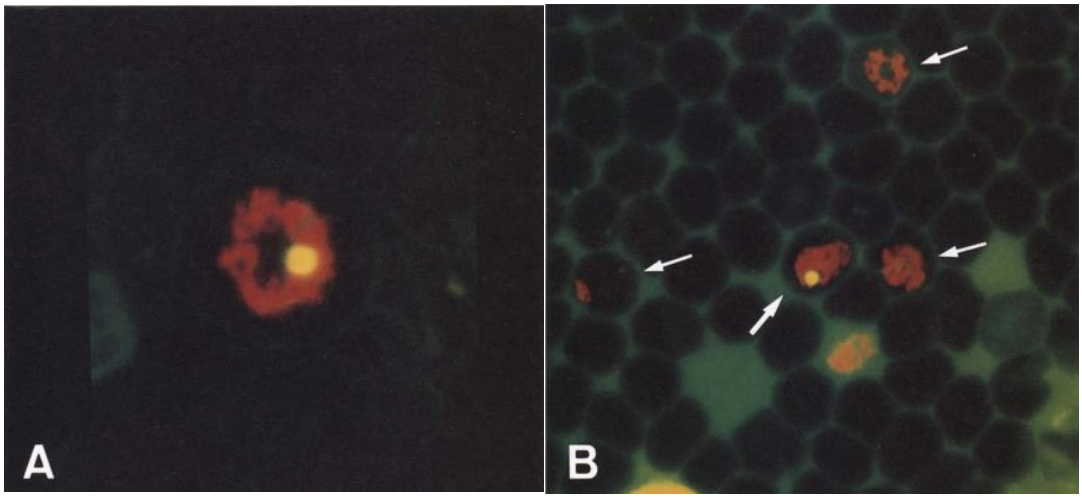


Figura 7: A) Um reticulócito micronucleado (MNRET); B) Três reticulócitos normais (seta fina) e 1 MNRET (seta grossa); Fonte: HAYASHI *et al.*, 1990.

O baço é o maior órgão linfoide secundário do corpo e, como tal, hospeda uma ampla gama de funções imunológicas. Juntamente com seus papéis na hematopoiese e na eliminação de glóbulos vermelhos, ele filtra o sangue de patógenos e células vermelhas anormais e facilita interações entre células sinalizadores de antígenos (APCs) e linfócitos cognatos. As APCs exclusivas do baço regulam a resposta das células T e B a esses alvos antigênicos no sangue (LEWIS; WILLIAMS; EISENBARTH, 2019).

A filtração do sangue é a função mais conhecida e muito importante do baço, pois assim pode remover células com danos no DNA da circulação sanguínea, inclusive células micro nucleadas, células envelhecidas e inclusões intraeritrocíticas (micronúcleos e parasitas intraeritrocíticos, como *Plasmodium falciparum*) (CARVALHO *et al*., 2015; MARTELLO *et al*., 2016). As considerações anteriores levaram Lima *et al*. (2013) a adaptar o protocolo desenvolvido por Hayashi *et al.* (1990) para medir a atividade fagocitária do baço, pois a ação antigenotóxica contra eritrócitos micronucleados no sangue periférico pode ser amplamente modulada pela atividade fagocítica esplênica que é aumentada quando animais são tratados com substâncias toxicogênicas.

Como o baço está diretamente envolvido nos processos de imunomodulação e a fagocitose esplênica é efetiva no sequestro de células anucleadas e/ou micronucleadas, mascarando possíveis lesões celulares, o ensaio da fagocitose esplênica favorece a precisão das análises de frequência de danos no DNA (CARVALHO *et al.*, 2015).

2.7 Teratologia

Os teratógenos são caracterizados como agentes ambientais, químicos, físicos e biológicos que podem causar anormalidades obstétricas e/ou embriofetais e depende de diversos fatores como o estágio de desenvolvimento do concepto, mecanismo patogênico específico de cada agente, tempo de exposição da gestante, dose do medicamento/substância, tempo de exposição a eles e da suscetibilidade genética do indivíduo (RENCUZOGULLARI; AYDIN, 2019; SHROUKH; STEINKE; WILLIS, 2020; PEREIRA *et al*., 2021).

A ciência que estuda as anomalias congênitas causadas por esses teratógenos e como afetam o embrião, o estudo de vias e mecanismos que podem ser causados ou alterados por eles, é conhecida como teratologia ("*teratos*" = monstro, "*logos*" = estudo) (WACHHOLZ *et al.*, 2021).

As anomalias congênitas são identificadas como defeitos congênitos, distúrbios congênitos ou malformações congênitas e adquiriram, atualmente, um papel importante na morbimortalidade, pois podem provocar abortos espontâneos, natimortos e incapacidade a longo prazo, afetando não apenas os indivíduos e suas famílias, mas também os sistemas nacionais de saúde (LUNAGÓMEZ *et al*., 2020). Tais anomalias podem ser identificadas no pré-natal, no nascimento ou mesmo mais tarde durante a infância, são encontradas em cerca de 3 a 6% dos nascimentos e são uma das principais causas de mortalidade neonatal e infantil em todo o mundo (FINELL *et al.*, 2021; FRAGA *et al.*, 2022).

A embriotoxidade se refere à perturbação no desenvolvimento embrionário ou fetal, às custas de dosagens que não afetam o organismo materno. A embrioletalidade observada após administração de doses tóxicas para a mãe deve ser considerada como efeitos tóxicos gerais da planta. O aborto é a interrupção da gravidez pela morte do embrião ou feto, junto com os anexos, e todos estes eventos podem estar relacionados ao uso de plantas medicinais (RODRIGUES *et al*., 2011).

Devido ao fato de que as anomalias congênitas podem causar morte, doenças e deficiências em bebês e crianças, é importante realizar uma investigação mais aprofundada de suas causas, fatores de risco e mecanismos, e tais investigações auxiliam diretamente no desenvolvimento de estratégias para prevenir novas ocorrências, intervenções terapêuticas e óbitos por anomalias congênitas (WACHHOLZ *et al.*, 2021). Esse tipo de conhecimento ainda é importante para o desenvolvimento de medicamentos e/ou outros produtos ingeridos por humanos e/ou utilizados em outras ações antropogênicas.

Estudos em teratogênese tornaram-se proeminentes depois que a talidomida foi descoberta como um teratógeno. Desde a década de 1960, estudos foram realizados não apenas para prevenir defeitos congênitos. Mas também, para identificar rapidamente um teratógeno, descrever os fenótipos malformativos associados a eles e investigar os mecanismos relacionados a essas exposições (FRAGA *et al.,* 2022).

Assim, para o bem-estar das populações, a investigação tem-se centrado nas lacunas de conhecimento sobre as plantas medicinais e em suas toxicidades potenciais, fortemente encorajadas por muitas organizações médicas e por investigadores em medicina complementar e alternativa (ASSOGBA *et al.,* 2021).

Os modelos de laboratório têm uma longa história de uso na avaliação do potencial de toxicidade de novos medicamentos e produtos químicos antes de entrarem na clínica ou no comércio. Eles também são os meios pelos quais os mecanismos de desenvolvimento anormal são elucidados e desempenham um papel importante em estabelecer ou refutar a plausibilidade biológica das associações observadas em humanos (DASTON, 2011).

Diante do exposto, optou-se por um modelo experimental que alia dados de desempenho reprodutivo de fêmeas e desenvolvimento embriofetal (teratogênese) para o desenvolvimento desse estudo. A esses modelos ainda foi associado um modelo de genética toxicológica para avaliar a integridade do DNA por meio do ensaio do micronúcleo.

3. OBJETIVOS

3.1 Objetivo Geral

Avaliar os efeitos do extrato etanólico de *Alternanthera littoralis, Salvia lachnostachys Benth* e *Serjania erecta* no desempenho reprodutivo, desenvolvimento embriofetal e integridade do DNA em camundongos *swiss.*

3.2 Objetivos Específicos

Avaliar a toxicidade materna e o desempenho reprodutivo de fêmeas Swiss tratadas com extrato etanólico de *Alternanthera littoralis, Salvia lachnostachys Benth* e de *Serjania erecta;*

Investigar o potencial teratogênico extrato etanólico de *Alternanthera littoralis, Salvia lachnostachys Benth* e de *Serjania erecta* sobre os embriões/fetos expostos intra útero, por meio de análises de malformações externas, viscerais e esqueléticas;

Investigar a atividade genotóxica do extrato etanólico de *Alternanthera littoralis, Salvia lachnostachys Benth* e de *Serjania erecta* por meio do ensaio de micronúcleo em sangue periférico.

Investigar a indução de fagocitose esplênica do extrato etanólico de *Alternanthera littoralis* por meio do ensaio de fagocitose esplênica;

4. METODOLOGIA

4.1 Material vegetal e preparação do extrato

4.1.1 Experimento 1

As partes aéreas de *A. littoralis* foram coletadas na Restinga de Maricá, Rio de Janeiro, Brasil, em dezembro de 2010 e identificadas pelo Prof. Dr. Josafá Carlos de Siqueira, Pontifícia Universidade Católica do Rio de Janeiro (PUC-RJ), Brasil. Um espécime de comprovante (SPFR-4758) foi depositado no herbário da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo (FFCLRP/USP).

As partes aéreas em pó secas ao ar (1,0 Kg) foram extraídas exaustivamente (maceração à temperatura ambiente) com etanol na proporção 1:2 (p/v) de massa de pó planta/solvente. A biomassa utilizada foi filtrada e os solventes foram removidos em evaporador rotativo a pressão reduzida e temperatura abaixo de 40°C, obtendo-se 75 g de extrato bruto etanólico da planta (EEAl).

4.1.2 Experimento 2

Folhas de *S. lachnostachys* foram coletadas em Curitiba, Paraná, Brasil (25°30'44,6"S, 49°18'7,13"W), de uma população natural em maio de 2010. A planta foi identificada por E. P. Santos, e um exemplar da espécie foi depositada no Herbário da Universidade Federal do Paraná (UPCB 85285). O código registrado no Sistema Nacional de Gestão do Patrimônio

Genético e Conhecimentos Tradicionais Associados (SISGEN) é o A19F875. Folhas secas e em pó (415,3 g) foram extraídas com hexano seguido de etanol (3 x 2 L, para cada solvente). Os solventes foram removidos sob pressão reduzida, obtendo-se os respectivos extratos em hexano (2,7%) e etanol (11,5%), e os testes farmacológicos foram realizados com extrato etanólico bruto de folhas de *S. lachnostachys* (EESl) diluído em solução salina.

4.1.3 Experimento 3

S. erecta foi coletada em Aquidauana (MS - Brasil) e um espécime comprovante (número do depósito: HMS: 8355) foi depositado no Herbário da Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) no Estado de Mato Grosso do Sul, Brasil. O código registrado no Sistema Nacional de Gestão do Patrimônio Genético e Conhecimentos Tradicionais Associados (SISGEN) é o A055721.

As folhas (600 g) de S. erecta foram extraídas sucessivamente com solventes hexano, acetato de etila e etanol à temperatura ambiente. Além disso, neste estudo foi utilizado apenas o extrato etanólico das folhas. O extrato foi fracionado por cromatografia em coluna de resina XAD-2 eluída com solventes de água, metanol e finalmente acetona. Além disso, a fração metanólica foi fracionada por cromatografia em coluna Sephadex LH-20 e eluída com solvente metanol. As fracções foram combinadas por cromatografia em camada fina (placas de gel de sílica, acetato de etilo/n-propanol/água, 120:8:70 em volume, fase superior). Os compostos obtidos foram ainda purificados por cromatografia em coluna repetida em polivinilpolipirrolidona eluída com metanol. Os compostos isolados foram analisados por espectros de RMN (Ressonância Magnética Nuclear) foram registrados em um espectrômetro Bruker DPX 300, o espectro IR (Infravermelho) foi realizado em um FT-IR-Nicolet Impact IMACT-400 e o espectro UV (Ultravioleta) foi realizado em um Espectrofotômetro Hitachi 110.

4.2. Animais

Os animais, camundongos (*Mus musculus*) da linhagem *Swiss,* foram cedidos pelo Biotério Central da Universidade Federal de Mato Grosso do Sul (UFMS), em um total de 45 camundongos para os experimentos 1 e 2 (EEAl e EESl – 30 fêmeas e 15 machos), e 60 camundongos para o experimento 3 (EESe – 40 fêmeas e 20 machos). Os animais de ambos os sexos em idade reprodutiva com fêmeas de peso médio de 30g e 20 machos com peso médio

de 35g. Todos os animais passaram por período de adaptação de 7 dias e foram alocados em mini-isoladores (Rack ventilada Alesco®), forrados com maravalha de *Pinus sp.*. Foram mantidos sob controle de temperatura (22 ± 2°C), luz por fotoperíodo (12h claro/ 12h escuro), umidade relativa de 55 ± 10% e acesso a ração e água *ad libitum*. A pesquisa foi realizada de acordo com os protocolos da Declaração Universal dos Direitos Animais e com aprovação da Comissão de Ética no Uso de Animais (CEUA) da UFMS, sob o parecer número 965/2018.

4.3 Delineamento Experimental

4.3.1 Experimento 1

Os animais foram acasalados durante a noite na proporção de 1 macho: 2 fêmeas. A detecção de *plugs* vaginais nas fêmeas determinou a ocorrência de gravidez e esse dia foi considerado o dia zero da gestação (OLIVEIRA *et al.*, 2009; OLIVEIRA *et al.*, 2015). As fêmeas prenhes foram divididas aleatoriamente em 3 grupos experimentais (n = 10): Grupo Controle - os animais receberam 0,1 mL/10 g de peso corpóreo (p.c.) de veículo (Tween 80 a 1%) por gavage (v.o.) ao longo da gestação (1º ao 18º dia gestacional); Grupos EEAl - os animais receberam EEAl por gavagem nas doses de 100 mg/kg (p.c., v.o.) (Grupo EEAl 100) e 1000 mg/kg (p.c., v.o.) (Grupo EEAl 1000) durante a gestação.

Segundo Aquino *et al*. (2015) a dose de 100mg/Kg tem efeito anti-hiperalgésico e anti-inflamatório. Logo, ela foi escolhida como a dose efetiva. Além dessa, optamos por testar uma dose de segurança que é 10x maior do que a dose efetiva de acordo com as recomendações da área (GOMES *et al*., 2012; ISHIKAWA, *et al.*, 2018; VANI *et al.,* 2018). Em relação ao período de tratamento nós optamos por avaliar durante todo o período gestacional visto que essa janela de tratamento poderia demonstrar efeitos mais severos. Essa possibilidade é destacada pelos guideline OECD 414 (2018), além de diferentes autores optarem pelo tratamento durante todo o período gestacional (MORAES-SOUZA *et al.*, 2017; PAULA *et al.* 2020; SALUSTRIANO *et al.*, 2022).

4.3.2 Experimento 2

As fêmeas prenhes foram divididas aleatoriamente em 3 grupos experimentais (n = 10): Grupo Controle - os animais receberam 0,1 mL/10 g de peso corpóreo (p.c.) de veículo (Tween 80 a 1%) por gavage (v.o.) ao longo da gestação (1º ao 18º dia gestacional); Grupos EESl - os

animais receberam EESl por gavagem nas doses de 100 mg/kg (p.c., v.o.) (Grupo EESl 100) e 1000 mg/kg (p.c., v.o.) (Grupo EESe 1000) durante a gestação. Tais doses foram escolhidas baseadas no estudo de Radai *et al*. (2018) e a partir das recomendações dos guidelines da área (ANVISA, 2013; OECD 421, 2016; OECD 443, 2018). Segundo Radai *et al.* (2018) a dose de 10 e 100mg/Kg tem efeito antiartrítico e ant-inflamatório. Assim, optamos por testar uma dose 10x maior do que cada uma delas o que é recomendado pela literatura da área para garantir segurança de uso por gestantes.

4.3.3 Experimento 3

As fêmeas prenhes foram divididas aleatoriamente em 4 grupos experimentais (n = 10): Grupo Controle - os animais receberam 0,1 mL/10 g de peso corpóreo (p.c.) de veículo (Tween 80 a 1%) por gavage (v.o.) ao longo da gestação (1º ao 18º dia gestacional); Grupos EESe - os animais receberam EESe por gavage nas doses de 500 mg/kg (p.c., v.o.) (Grupo EESe 500), 1000 mg/kg (p.c., v.o.) (Grupo EESe 1000) e 2000 mg/kg (p.c., v.o.) (Grupo EESe 2000) durante a gestação. A dose de 500mg/Kg foi escolhida a partir da dose efetiva descrita por Arruda *et al*. (2009) em modelo de úlcera gástrica induzida por etanol absoluto. A partir desta dose, foram definidas as doses 2x e 4x maiores (1000 e 2000mg/kg) do que aquela que tem a atividade desejada, seguindo a recomendação dos guidelines da área (ANVISA, 2013; OECD 421, 2016; OECD 443, 2018). Além disso, optamos pela maior dose (2000mg/Kg) visto que Arruda *et al.* (2009) classificou o extrato clorofórmico de *S. erecta* como praticamente não tóxico visto que a dose de 5.000mg/Kg não havia causado sinais de toxicidade aguda.

4.4 Ensaios Biológicos

Os ensaios biológicos foram executados de maneira semelhante nos três experimentos, com as coletas dos materiais para as avaliações toxicogenéticas feitas em 3 tempos distintos, sendo estes T1, T2 e T3 referentes ao 16º, 17º e 18º dias de gestação (d.g.), respectivamente. No 18º d.g., os animais foram submetidos à eutanásia seguida de laparotomia, histerectomia e onfalectomia para a coleta, pesagem e devido armazenamento dos órgãos (pulmão, coração, baço, fígado, rins, placenta) e fetos para análise posterior.

4.5 Parâmetros biométricos

Os parâmetros biométricos, em ambos os experimentos, foram calculados a partir dos registros de peso inicial (fêmeas pesadas no dia zero), peso final (fêmeas pesadas no 18º d.g.), ganho de peso (peso final - peso inicial), peso do útero, ganho de peso líquido (ganho de peso - peso do útero), pesos absolutos e relativos do coração, pulmão, baço, rins e fígado.

4.6 Desenvolvimento Embriofetal

Os fetos retirados foram pesados e passaram por análise sistemática de malformações externas e sexagem. Em seguida, foram distribuídos aleatoriamente em dois subgrupos, cada um com 50% da ninhada. Os fetos do primeiro grupo foram fixados em solução de Bodians por pelo menos sete dias e destinados à análise visceral por meio de microdissecção com cortes estratégicos, para o estudo de tórax e abdômen propostos por Barrow e Taylor (1969) e para estudo da cabeça segundo Wilson (1965), com alterações propostas por Wise *et al.* (1997), Oliveira *et al*. (2005), Damasceno *et al*. (2008), Oliveira *et al*. (2009) e Oliveira *et al*. (2015)

A classificação das alterações viscerais foi baseada, principalmente nos trabalhos de Taylor (1986), Manson e Kang (1994), Damasceno et al. (2008), Oliveira *et al.* (2009) e Oliveira *et al*. (2015). Os fetos do segundo subgrupo foram fixados em acetona pura por pelo menos sete dias e destinados à análise esquelética pela técnica descrita por Staples e Schnell (1964) e modificada por Oliveira *et al.* (2009). Após a fixação, os fetos foram eviscerados para o processo de diafanização em solução de KOH (0,8%). Em seguida, foram adicionadas quatro gotas de alizarina, e esta solução foi trocada a cada 24 horas durante quatro dias consecutivos. Depois dos fetos corados, a solução de KOH foi substituída pela solução clareadora (1 litro de glicerina : 1 litro de álcool etílico : 0,5 litro de álcool benzílico) e trocada a cada 24 horas, durante cinco dias. As classificações foram feitas segundo Taylor (1986), Manson e Kang (1994), Wise *et al.* (1997). Damasceno *et al*. (2008), Oliveira *et al*. (2009) e Oliveira *et al*. (2015).

As análises de vísceras e esqueletos foram realizadas em lupa estereomicroscópica (Nikon® – SMZ 745T) com aumento de 1,6 vezes.

A classificação do peso fetal segundo a idade gestacional (CPFIG) foi feita conforme proposto por Soulimane-Mokhtari *et al*. (2005). Após a coleta dos fetos dos cornos uterinos, eles foram pesados e de acordo com a média ± 1,7 x desvio padrão (DP) dos pesos corporais

obtidos no grupo controle, foram classificados em adequados para idade gestacional (AIG), pequenos para a idade gestacional (PIG) e grandes para a idade gestacional (GIG).

4.7 Desempenho Reprodutivo

Foram registrados, em ambos os experimentos, o número de sítios de implantação (nº de fetos vivos + nº de fetos mortos + nº de reabsorções) e reabsorção, além do número de fetos vivos, mortos e do peso fetal e placentário. Com base nestes dados, obteve-se: a viabilidade fetal (número de fetos vivos × 100 / número de implantações), taxa de perda pós-implantação [(número de implantações - número de reabsorções) × 100 / número de implantações], taxa de reabsorção (nº de reabsorções X 100 / nº de implantes), índice placentário (peso da placenta / peso fetal), eficiência placentária (peso fetal / peso placentário) e razão sexual (número de fetos machos / número de fetos fêmeas).

4.8 Ensaio do Micronúcleo em Sangue Periférico

O teste do micronúcleo no sangue periférico, em ambos os experimentos, foi realizado de acordo com o método descrito por Hayashi *et al.* (1990) e modificado por Oliveira *et al*. (2015). O sangue periférico, coletado por punção da veia caudal (cerca de 20μL), foi depositado em uma lâmina preparada com Alaranjado de Acridina (1mg/mL). Recobriu-se o sangue com uma lamínula e o material foi armazenado em freezer (-20ºC) por um período mínimo de sete dias. Um total de 2.000 reticulócitos foram examinados por animal por meio de um microscópio EVOS M7000 (Zeiss®) no aumento de 40x, com filtro de excitação 470nm ±22 e filtro de emissão 525nm ±50.

4.9 Avaliação da Fagocitose Esplênica

O baço dos animais tratados é cortado em pedaços e após, prensados em uma tela de aço inoxidável com 5 mL de tampão fosfato estéril, livre de Ca^{2+} e Mg^{+2}, pH 7,4. A pipetagem repetida com uma pipeta de Pasteur é utilizada para obter uma suspensão de células homogênea. Cem microlitros de suspensão de células são colocados imediatamente no centro de uma lâmina pré-revestida com Laranja de Acridina (1 mg/mL) e a lâmina foi então coberta com lamínula e são armazenadas em freezer até o momento da análise.

A fagocitose de eritrócitos com micronúcleos pela presença de coloração avermelhada junto das células do baço conforme é mostrada na figura 8.

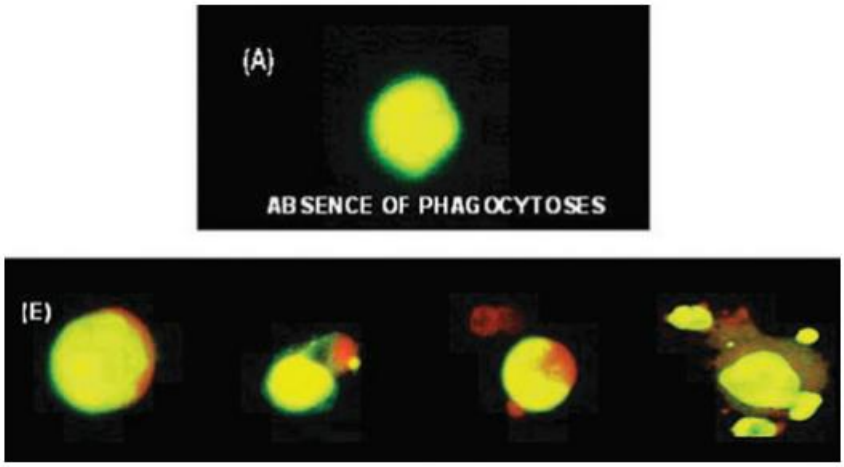


Figura 8: Ausência (A) de fagocitose (célula fluorescente intensamente verde sem qualquer resquício de RNA exógeno de cor vermelha). Fagocitose (E) evidente (qualquer célula fluorescente verde, que pode ser visualizada com um mínimo de RNA exógeno). Fonte: Lima *et al*., 2013.

4.10 Análise Estatística

Em todos os experimentos, para dados com distribuição normal foi utilizado o teste ANOVA de uma via com pós-teste de Tukey. Para os demais dados utilizou-se o teste de Kruskal-Wallis com pós-teste de Dunn. Para comparações de frequências utilizou-se o teste do Qui-quadrado. As análises foram realizadas pelo programa GraphPad Instat® (Versão 3.06, 2003). Os dados foram apresentados em média ± erro padrão da média ou média ± desvio padrão e o nível de significância estabelecido foi de $p<0,05$.

5. RESULTADOS, DISCUSSÃO E CONSIDERAÇÕES FINAIS

Os resultados, discussão e considerações finais serão apresentados na forma de artigo (Anexo 2, 3 e 4).

REFERÊNCIAS

AHMED, S. M.; NORDENG, H.; SUNDBY, J.; ARAGAW, Y. A.; BOER, H. J. The use of medicinal plants by pregnant women in Africa: A systematic review. **J Ethnopharmacol**., v. 224, p. 297-313, oct., 2018.

AHMED, S. M.; SUNDBY, J.; ARAGAW, Y. A.; NORDENG, H. Medicinal plants used among pregnant women in a tertiary teaching hospital in Jimma, Ethiopia: a cross-sectional study. **BMJ Open**., v. 11, n. 8, e046495, aug, 2021.

ANVISA - Agência Nacional de Vigilância Sanitária. **Guia para a condução de estudos não clínicos de segurança necessários ao desenvolvimento de medicamentos**. Versão 2, Brasília, 2013.

ANVISA - Agência Nacional de Vigilância Sanitária. Resolução-RE Nº 90. **Guia para a realização de estudos de toxicidade pré-clínica de fitoterápicos.** 2004. Disponível em: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2004/rdc0090_16_03_2004.html

AQUINO, D. F. S.; PICCINELLI, A. C.; SOARES, F. L.; ARENA, A. C.; SALVADOR M. J.; KASSUYA, C. A. Anti-hyperalgesic and Anti-inflammatory Activity of Alternanthera Maritima Extract and 2″-O-α-L-rhamnopyranosylvitexin in Mice. **Inflammation**, v. 38, n. 6, p. 2057-66, 2015.

ARAÚJO, C. R.; SANTIAGO, F. G.; PEIXOTO, M. I.; OLIVEIRA, J. O.; COUTINHO, M. D. E. S. Use of Medicinal Plants with Teratogenic and Abortive Effects by Pregnant Women in a City in Northeastern Brazil. **Rev Bras Ginecol Obstet**., v. 38, n. 3, p.127-31, mar., 2016.

ARRUDA, A. P. C. C. B. N.; COELHO, R.G.; HONDA, N.K.; FERRAZOLI, C.; POTT, A.; HIRUMA-LIMA, C.A. Gastroprotective Effect of Serjania erecta Radlk (Sapindaceae): Involvement of Sensory Neurons, Endogenous Nonprotein Sulfhydryls, and Nitric Oxide. **JOURNAL OF MEDICINAL FOOD,** v. 12, n. 6, p 1411–15, 2009.

ASSOGBA, P.; DOUGNON, V.; HOUNSA, E.; BADJABAISSI, P.; TARI, R. M.; KLOTOE, J. R.; BANKOLE, H.; DIALLO, A. Assessment of Larval Toxicity and the Teratogenic Effect of Three Medicinal Plants Used in the Traditional Treatment of Urinary Tract Infections in Benin. **Biomed Res Int**.,1401945, dec., 2021.

BALARASTAGHI, S.; DELIRRAD, M.; JAFARI, A.; MAJIDI, M.; SADEGHI, M.; ZARE-ZARDINI, H.; KARIMI, G.; GHORANI-AZAM, A. Potential benefits versus hazards of herbal therapy during pregnancy; a systematic review of available literature. **Phytother Res**., v. 36, n. 2, p. 824-41, feb, 2021.

BARROW M. V.; TAYLOR W. I. A rapid method for detecting malformation in rat fetuses. **J. Morphol.** v. 127, p 291-306, 1969.

BERNSTEIN, N.; AKRAM, M.; YANIV-BACHRACH, Z.; DANIYAL, M. Is it safe to consume traditional medicinal plants during pregnancy? **Phytotherapy Research**, v. 35, n. 4, p. 1908-24, 2020.

BOLSON, M.; HEFLER, S. M.; CHAVES, E. I. D.; GASPAROTTO JUNIOR, A.; CARDOZO JUNIOR, E. L. Ethno-medicinal study of plants used for treatment of human ailments, with residents of the surrounding region of forest fragments of Paraná, Brazil. **J Ethnopharmacol**, v. 161, p.1-10, 2015.

BRAGA, L. S. S. B.; DAMASCENO, M. R. A.; ALMEIDA, V. H. H.; DAMASCENO, J. E. A. Avaliação da frequência de micronúcleos em eritrócitos de peixes do médio Rio São Francisco em Januária/MG. **Brazilian Journal of Development**, v.7, n.6, p.59389-96, 2021.

BRANDT, C. T.; CASTRO, C. M. M. B.; LAVOR, S. M. L.; CASTRO, F. M. M. Fagocitose e viabilidade monocitária de pacientes com esquistossomose mansônica na forma hepatoesplênica submetidos à esplenectomia e ao auto-implante esplênico. **Revista da Sociedade Brasileira de Medicina Tropical,** v. 39, n. 5, p.439-45, 2006.

BROGGINI, L. S. C.; FERNANDES, R. S.; NOGUEIRA, T.; SUZANO, F. R.; CAETANO, A. L.; BUCK, H. S.; COUTO, L. B.; FRANÇA, S. C.; PEREIRA, P. S. Behavioral and enzymatic bioassays with Serjania erecta Radlk., Sapindaceae, correlated with cognitive dysfunctions. **Revista Brasileira de Farmacognosia**, v. 20, n. 4, p. 519-28, ago./set., 2010.

BRUNO, L. O.; SIMOES, R. S.; SIMOES, M. J.; GIRÃO, M. J. B. C.; GRUNDMANN, O. Pregnancy and herbal medicines: An unnecessary risk for women's health-A narrative review. **Phytother Res**., v. 32, n. 5, p. 796-810, may, 2018.

CANEDO, A.; JESUS, L. W. O.; BAILÃO, E. F. L. C.; ROCHA, T. L. Micronucleus test and nuclear abnormality assay in zebrafish (Danio rerio): Past, present, and future trends. **Environ Pollut**., v. 290, 118019, dec., 2021.

CARDOSO, B. S.; AMAARAL, V. C. S. O uso da fitoterapia durante a gestação: um panorama global. **Ciência & Saúde Coletiva**, n. 24, v. 4, p. 1439-50, 2019.

CARDOSO, C. A. L.; COELHO, R. G., HONDA, N. K.; POTT, A.; PAVAN, F. R.; LEITE, C. Q. F. Phenolic compounds and antioxidant, antimicrobial and antimycobacterial activities of Serjania erecta Radlk. (Sapindaceae). **Brazilian Journal of Pharmaceutical Sciences,** v. 49, n. 4, p. 775-82, 2013.

CARRARD, V. C.; FERREIRA, L. A.; LAUXEN, I. S.; COSTA, C. H.; RADOS, P. V. Teste dos micronúcleos: um biomarcador de dano genotóxico em células descamadas da mucosa bucal. **R. Fac Odontol. Porto Alegre**, v. 48, n. 1/3, p. 77-81, 2007.

CARVALHO, J. T. G.; BALDIVIA, D. S.; LEITE, D. F.; ARAÚJO, L. C. A.; ESPINDOLA, P. P. T.; ANTUNES, K. A.; ROCHA, P. S.; SOUZA, K. P.; SANTOS, E. L. Medicinal Plants from Brazilian Cerrado: Antioxidant and Anticancer Potential and Protection against Chemotherapy Toxicity. **Oxid Med Cell Longev**, v. 2019, 3685264, 2019.

CARVALHO, P. C.; SANTOS, E. A.; SCHNEIDER, B. U; MATUO, R.; PESARINI, J. R.; CUNHA-LAURA, A. L.; MONREAL, A. C.; LIMA, D. P.; ANTONIOLLI, A. C.; OLIVEIRA, R. J. Diaryl sulfide analogs of combretastatin A-4: Toxicogenetic, immunomodulatory and apoptotic evaluations and prospects for use as a new chemotherapeutic drug. **Environ Toxicol Pharmacol**. v. 40, n. 3, p. 715-21, 2015.

CARVALHO, A. C.; RAMALHO, L. S.; MARQUES, R. F.; PERFEITO, J. P. Regulation of herbal medicines in Brazil. **J Ethnopharmacol**, v. 158, p. 503-6, 2014.

CORDOVA, S. M., BENFATTI, C. S.; MAGINA, M. D. A.; GUEDES, A.; CORDOVA, C. M. M. Análise da atividade antimicrobiana de extratos isolados de plantas nativas da flora brasileira frente a Mycoplasma arginini, M. hominis e Ureaplasma urealyticum. **RBAC,** v. 42, n. 4, p. 241-44, 2010.

CORSO, C. R.; STIPP, M. C.; ADAMI, E. R.; SILVA, L. M.; MARIOTT, M.; ANDRADE, S. F.; RAMOS, E. A. S.; KLASSEN, G.; BELTRAME, O. C.; QUEIROZ-TELLES, J. E.; OLIVEIRA, C. S.; STEFANELLO, M. E. A.; ACCO, A. Salvia lachnostachys Benth has antitumor and chemopreventive efects against solid Ehrlich carcinoma. **Molecular Biology Reports**, v. 46, p. 4827-41, 2019.

CORSO, C. R.; STIPP, M. C.; RADULSKI, D. R.; SILVA, L. M.; MARIOTT, M.; SILVA, L. M.; RAMOS, E. A. S.; KLASSEN, G.; QUEIROZ-TELLES, J. E.; OLIVEIRA, C. S.; STEFANELLO, M. E. A.; VERHOEVEN, A. J.; ELFERINK, R. P. J. O.; ACCO, A. Fruticuline A, a chemically-defned diterpene, exerts antineoplastic efects in vitro and in vivo by multiple mechanisms. **Sci Rep**, v. 10, n. 1, p. 1-14, 2020.

COUTINHO, L. M. **Biomas brasileiros**. São Paulo: Oficina de Textos, 2016.

DAMASCENO, D. C.; KEMPINAS, W. G; VOLPATO, G. T.; CONSONI, M. Anomalias Congênitas: Estudos Experimentais. **Coopmed Editora Médica**, Belo Horizonte. 2008.

DASTON, G. P. Laboratory models and their role in assessing teratogenesis. **Am J Med Genet C Semin Med Genet**., v. 157C, n. 3, p. 183-7, 2011.

DOSOKY, N. S.; SETZER, W. N. Maternal Reproductive Toxicity of Some Essential Oils and Their Constituents. **Int J Mol Sci**., v. 22, n. 5, 2380, feb., 2021.

DUTRA, R. C.; CAMPOS, M. M.; SANTOS, A. R.; CALIXTO, J. B. Medicinal plants in Brazil: Pharmacological studies, drug discovery, challenges and perspectives. **Pharmacol Res.,** v. 112, p. 4-29, 2016.

EMRE, I.; KURSAT, M. Biochemical parameters and antioxidant property of three Salvia L. taxa endemic in Turkey. **Brazilian Journal of Biology**. e239539, vol. 82, p. 1-11, 2022.

FERNANDES, R. S.; COSTA, T. R.; MARCUSSI, S.; BERNARDES, C. P.; MENALDO, D. L.; RODRIGUÉZ, G. II, PEREIRA, P. S.; SOARES, A. M. Neutralization of pharmacological and toxic activities of Bothrops jararacussu snake venom and isolated myotoxins by Serjania erecta methanolic extract and its fractions. **The Journal of Venomous Animals and Toxins including Tropical Diseases**, v. 17, n. 1, p. 85-93, 2011.

FINNELL, R. H.; CAIAFFA, C. D.; KIM, S. E.; LEI, Y.; STEELE, J.; CAO, X.; TUKEMAN, G.; LIN, Y. L.; CABRERA, R. M.; WLODARCZYK, B. J. Gene Environment Interactions in the Etiology of Neural Tube Defects. **Front Genet**., v. 12, art. 659612, 2021.

FONTOURA, A.; AYRES, L. R.; MARTINS-NAGAI, M.; DEWULF, N. L. S.; SANTOS, V.; MATINEZ, E. Z.; PEREIRA, L. R. L. Prevalence of medication use among low risk pregnant

women: a drug utilization study. **African Journal of pharmacy and pharmacology**, v.*8*, n. 36, p. 883-92, 2014.

FRAGA, L.R.; VIANNA, F. S. L.; CAMPO, M.; SANSEVERINO, M. T. V.; SCHULER-FACCINI, L. Editorial: Teratogenesis: Experimental Models, Mechanisms and Clinical Findings in Humans. **Front Genet**., v.13, art. 901400, 2022.

GASPARETTO, A.; LAPINSKI, T. F.; ZAMUNER, S. R.; KHOURI, S.; ALVES, L. P.; MUNIN, E.; SALVADOR, M. J. Extracts from Alternanthera maritima as natural photosensitizers in photodynamic antimicrobial chemotherapy (PACT). **J Photochem Photobiol B,** v. 99, n. 1, p. 15-20, 2010.

GOMES, C.; LOURENÇO, E. L.; LIUTI, É. B.; DUQUE, A. O.; NIHI, F.; LOURENÇO, A. C.; MENDES, T. C.; GASPAROTTO JUNIOR, A.; DALSENTER, P. R. Evaluation of subchronic toxicity of the hydroethanolic extract of Tropaeolum majus in Wistar rats. **J Ethnopharmacol**., v. 142, n. 2, p. 481-7, 2012.

GOMIG, F.; PIETROVSKI, E. F.; GUEDES, A.; DALMARCO, E. D.; CALDERARI, M. T.; GUIMARÃES, C. L.; PINHEIRO R. M.; CABRINI, D. A.; OTUKI, M. F. Topical anti-inflammatory activity of Serjania erecta Radlk (Sapindaceae) extracts. **Journal of Ethnopharmacology,** v. 118, p. 220–24, 2008.

GORRIL, L. E.; JACOMASSI, E.; MELLA JUNIOR, S. E.; DALSENTER, P. R.; GASPAROTTO JUNIOR, A.; LOURENÇO, E. L. B. Risco das plantas medicinais na gestação: uma revisão dos dados de acesso livre em língua portuguesa. **Arq. Cienc. Saúde UNIPAR**, v. 20, n. 1, p, 67-72, jan./abr. 2016.

GUARIM-NETO, G.; SANTANA, S. R.; SILVA, J. V. B. NOTAS ETNOBOTÂNICAS DE ESPÉCIES DE SAPINDACEAE JUSSIEU. **Acta botânica brasileira**, v.14, n. 3, p. 327-34, 2000.

GUIMARÃES, C. C.; OLIVEIRA, D. D.; VALDEVITEA, M.; SALTORATTOA, A. L. F.; PEREIRA, S. I. V.; FRANÇA, S. C.; PEREIRA, A. M. S.; PEREIRA, P. S. The glycosylated flavonoids vitexin, isovitexin, and quercetrin isolated from Serjania erecta Radlk (Sapindaceae) leaves protect PC12 cells against amyloid-β25-35 peptide-induced toxicity. **Food and Chemical Toxicology**, v. 86, p. 88–94, 2015.

HAYASHI, M.; KODAMA, Y.; SOFUNI, T.; ISHIDATE JR., M. The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. **Mut Res**, v. 245, p. 245-49, 1990.

HYACIENTH, B. M. S.; SÁNCHEZ-ORTIZ, B. L.; PICANÇO, K. R. T.; PEREIRA, A. C. M.; HYACIENTH, D. C. S.; SOUZA, G. C.; SARQUIS, R. D. S. F. R.; ADUANGA, G. M. G.; NAVARRETE, A.; CARVALHO, J. C. T. Endopleura uchi (Huber) Cuatrec.: A medicinal plant for gynecological treatments - A reproductive toxicity assessment in zebrafish (Danio rerio). **J Ethnopharmacol**., v. 250, 112457, mar., 2020.

ISHIKAWA, R. B.; VANI, J. M.; NEVES, S. C. D.; RABACOW, A. P. M.; KASSUYA, C. A. L.; CRODA, J.; CARDOSO, C. A. L.; MONREAL, A. C. D. F.; ANTONIOLLI, A. C. M. B.; CUNHA-LAURA, A. L.; OLIVEIRA, R. J. The safe use of Doliocarpus dentatus in the

gestational period: Absence of changes in maternal reproductive performance, embryo-fetal development and DNA integrity. **Journal of Ethnopharmacology**, v. 217, p. 1-6, 2018.

JAMSHIDI-KIA, F.; LORIGOOINI, Z.; AMINI-KHOEI, H. Medicinal plants: Past history and future perspective. **J Herbmed Pharmacol**., v.7, n.1, p. 1-7, 2018.

KAM, P. C.; BARNETT, D. W.; DOUGLAS, I. D. Herbal medicines and pregnancy: A narrative review and anaesthetic considerations. **Anaesth Intensive Care**, v. 47, n. 3, p. 226-34, may., 2019.

KANDEMIR, S. I.; FIDAN, H. S.; YENER, I.; METE, N.; ERTAS, A.; TOPCU, G.; KOLAK, U. Investigation of cytotoxic and apoptotic effects of 63 compounds obtained from Salvia species: Promising anticancer agents. **J Food Biochem**, e14226, p. 1-17, 2022.

KASSUYA, R. M.; SANTOS, E.; BOSSO, F. H.; PEDROSO, T. F.; MARINHO, J. V. N.; SALVADOR, M. J.; GASPAROTTO JUNIOR, A. Anti-inflammatory Properties of Ethanolic Extract and 2 ″-O-β-D-Glucopyranosyl-Vitexin Obtained from Alternanthera Tenella Colla Whole Plant. **Inflammation**, v. 44, n. 4, p.1540-52, 2021.

KASSUYA, C. A. L.; WISNIEWSKI-JUNIOR, A.; SIMIONATTO, E. L.; SANTOS, E. P.; STEFANELLO, M. E. A. Composição dos óleos essenciais de Salvia lachnostachys e Salvia melissiflora (Laminaceae). **Lat. Am. J. Pharm**., v. 28, p. 919-21, 2009.

KOOLEN, H. H.; PRAL, E. M.; ALFIERI, S. C.; MARINHO, J. V.; SERAIN, A. F.; HERNÁNDEZ-TASCO, A. J.; ANDREAZZA, N. L.; SALVADOR, M. J. Antiprotozoal and antioxidant alkaloids from Alternanthera littoralis. **Phytochemistry**, v. 134, p. 106-13, 2017.

LEAL, L.; BIONDI, D. Potencial ornamental de espécies nativas. **Revista Científica Eletrônica de Engenharia Florestal**, v. 8, p. 1-16, 2006.

LEWIS SM, WILLIAMS A, EISENBARTH SC. Structure and function of the immune system in the spleen. **Sci Immunol**., v. 4, n. 33, eaau6085, 2019.

LI, L.; TANG, L. Y.; LIANG, B.; WANG, R.; SUN, Q.; LAU, C. B. S.; LEUNG, P. C.; FRITSCHE, E.; LIEBSCH, M.; WULCZYN, A. E. M. S.; SPIELMANN, H.; WANG, C. C. Evaluation of in vitro embryotoxicity tests for Chinese herbal medicines. **Reprod Toxicol**., v. 89, p. 45-53, oct., 2019.

LIMA, R. M.; GODINHO, J. L. P.; MAGALHÃES, E. I.S.; BRITO, A. B.; BRITTO, M. H. S. S.; ALVES, M. T. S. S. B.; SANTOS, A. M. Perfil do uso de medicamentos na gravidez: resultados do estudo BRISA, São Luís - MA, Brasil. **Pesquisa, Sociedade e Desenvolvimento,** v. 11, n. 2, e21811224986, 2022.

LIMA, P. L.; SUGUI, M. M.; PETRÍCIO, A. I.; VILELA, L. C.; PINTO, A. V.; MARTINS, P. R.; KANENO, R.; RIBEIRO, D. A.; SALVADORI, D. M.; RIBEIRO, L. R. Lentinula edodes (shiitake) modulates chemically induced mutagenesis by enhancing pitting. **J Med Food**, v. 16, n. 8, p. 733-9, 2013.

LUNAGÓMEZ, L. S.; SANTIAGO-ROQUE, I.; GHENO-HEREDIA, Y. A.; CORONA-MORALES, A. A.; BOLADO-GARCÍA. E. Teratogenic effects of Bocconia frutescens L. **J Dev Orig Health Dis**, v. 11, p. 415-418, 2020.

MANSON, J. M.; KANG, Y. J. Test Methods for Assessing Female Reproductive and Developmental Toxicology. In: **Principles and Methods of Toxicology** (Hayves AW, ed.). Raven Press, New York. 1994.

MARTELLO, M. D.; DAVID, N.; MATUO, R.; CARVALHO, P. C.; NAVARRO, S. D.; MONREAL, A. C.; CUNHA-LAURA, A. L.; CARDOSO, C. A.; KASSUYA, C. A.; OLIVEIRA, R. J; Campomanesia adamantium extract induces DNA damage, apoptosis, and affects cyclophosphamide metabolism. **Genet Mol Res**., v. 15, n. 2, p. 1-11, 2016.

MARTINS, J. L. R.; RODRIGUES, O. R. L.; SOUSA, F. B.; FAJEMIROYE, J. O.; GALDINO, P. M.; FLORENTINO, I. F.; COSTA, E. A. Medicinal species with gastroprotective activity found in the Brazilian Cerrado. **Fundamental & Clinical Pharmacology**, v. 29, p. 238–51, 2015.

MAZZARI, A. L.; PRIETO, J. M. Herbal medicines in Brazil: pharmacokinetic profile and potential herb-drug interactions. **Front Pharmacol**. v. 5, art. 162, p. 1-12, 2014.

MORAES-SOUZA, R. Q.; SOARES, T. S.; CARMO, N. O. L.; DAMASCENO, D. C.; CAMPOS, K. E.; VOLPATO, G. T. Adverse effects of Croton urucurana B. exposure during rat pregnancy. **J Etnofarmacol,** v. 199, p. 328-33, 2017.

NEGRELLE, R. R. B.; FORNAZZARI, K. R. C. Ethnobotanical study in two rural communites (Limeira and Ribeirão Grande) in Guaratuba (Paraná, Brazil). Rev. Bras. Pl. Med., v. 9, p. 36-54, 2007.

NETO, J. A. R.; TARÔCO, B. R. P., SANTOS, H. B., THOMÉ, R. G., WOLFRAMB, E.; RIBEIRO, R. I. M. A. Using the plants of Brazilian Cerrado for wound healing: From traditional use to scientific approach. **Journal of Ethnopharmacology,** v. 260, 112547, 2020.

OECD (2002), *Test No. 423:* ***Acute Oral toxicity - Acute Toxic Class Method,*** OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264071001-en.

OECD (2016), *Test No. 421:* ***Reproduction/Developmental Toxicity Screening Test,*** OECD Publishing, Paris, https://doi.org/10.1787/9789264070967-en.

OECD (2018), *Test No. 414:* ***Prenatal Developmental Toxicity Study,*** OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264070820-en.

OECD (2018), *Test No. 443: Extended One-Generation Reproductive Toxicity Study*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264185371-en.

OLIVEIRA, A. B.; ANTAR, G. M.; MOTA, M. C. A.; PASTORE, J. F. B. *Salvia*. In: **Flora e Funga do Brasil**. Jardim Botânico do Rio de Janeiro. 2022.

OLIVEIRA, C. S.; ALVAREZ, C. J.; CABRAL, M. R. P.; SARRAGIOTTO, M. H.; SALVADOR, M. J.; STEFANELLO, M. E. A. Three new diterpenoids from the leaves of Salvia lachnostachys. **Nat. Prod. Res**. 2021.

OLIVEIRA, C. S.; SALVADOR, M. J.; CARVALHO, J. E.; SANTOS, E. P.; BARISON, A.; STEFANELLO, M. E. A. Cytotoxic abietane-derivative diterpenoids of Salvia lachnostachys. **Phytochemistry Letters**, v. 17, p. 140-43, 2016.

OLIVEIRA, R.J.; KANNO, T.Y.N.; SALLES, M.J.S.; LOURENÃ, O.A.C.S.; RIBEIRO, L.R.; FREIRA, G.A.; MATIAZI, H.J.O.; MANTOVANI, M.S.; SILVA, A.F. Effects of the polysaccharide beta-glucan on clastogenicity and teratogenicity caused by acute exposure to cyclophosphamide in mice. **Regul. Toxicol. Pharmacol.** v. 53, n. 3, p. 164-73, 2009.

OLIVEIRA, R. J.; MANTOVANI, M. S., PESARINI, J. R., MAURO, M.O.; SILVA, A .F.; SOUZA, T. R.; RIBEIRO, L. R. 6-Dimethylaminopurine and cyclohexamide are mutagenic and alter reproductive performance and intrauterine development in vivo. **Genet Mol Res**. v. 14, p. 834-49, 2015.

OLIVEIRA, R. J.; OLIVA, S. U. ; DAROZ, G. A. ; RUBIO, E. M. . Fertility assessment and possible external structural defects on progeny from male rats chronically exposed to arsenic. **Rev. Bras. Toxicol.**, v. 18, n.1, 2005.

PALHARES RM, BARATTO LC, SCOPEL M, MÜGGE FLB, BRANDÃO MGL. Medicinal Plants and Herbal Products From Brazil: How Can We Improve Quality? **Front Pharmacol**., v. 11, art 606623, p. 1-4, 2021.

PAULA VG, CRUZ LL, SENE LB, GRATÃO TB, SOARES TS, MORAES-SOUZA RQ, DAMASCENO DC, VOLPATO GT. Maternal-fetal repercussions of Phyllanthus niruri L. treatment during rat pregnancy. **J Ethnopharmacol.,** v. 254, art. 112728, 2020.

PEREIRA, G.; SURITA, F. G.; FERRACINI, A. C.; MADEIRA, C. S.; OLIVEIRA, L. S.; MAZZOLA, P. G. Self-Medication Among Pregnant Women: Prevalence and Associated Factors. **Front Pharmacol**., v. 12, 659503, set., 2021. Erratum in: Front Pharmacol., dec. 2021.

PICCINELLI, A. C.; FIGUEIREDO, S. A. D.; MORATO, P. N.; KURAOKA-OLIVEIRA, Â. M.; STRAPASSON, R. L. B.; SANTOS, É. P.; KASSUYA, C. A. L. Anti-inflammatory and antihyperalgesic activities of ethanolic extract and fruticulin a from *salvia lachnostachys* leaves in mice. **Evidence-Based Complementary and Alternative Medicine**, v. 2014, 2014.

POTRICH, F. B.; BAGGIO, C. H.; FREITAS, C. S.; MAYER, B.; SANTOS, A. C.; TWARDOWSCHY, A.; GUEDES, A., MARQUES, M. C. A. Ação de extratos de plantas medicinais sobre a motilidade do trato gastrointestinal. **Rev. Bras. Pl. Med**., Campinas, v.16, n.3, supl. I, p.750-54, 2014.

POTT, A.; POTT, V. **Plantas do Pantanal**. Corumbá: Embrapa – SPI, 1994.

RADAI, J. A. S.; DA SILVA GRACIANI, F.; BERNO, C. R.; LEITÃO, M. M.; DOS SANTOS, E. P.; OLIVEIRA, R. J.; KASSUYA, C. A. L. Genetic toxicological assessment and

anti-arthritic effects of an ethanolic extract obtained from *Salvia lachnostachys* Benth leaves in mice. **Regulatory Toxicology and Pharmacology**, v. 92, p. 239-244, 2018.

RENCÜZOĞULLARI, E.; AYDIN, M. Genotoxic and mutagenic studies of teratogens in developing rat and mouse. **Drug Chem Toxicol**., v. 42, n. 4, p. 409-29, 2019.

ROCHA, T. A.; MOURA, D. F.; SILVA, M. M. D.; SOUZA, T. G. S.; LIRA, M. A. D. C.; BARROS, D. M.; SILVA, A. G.; XIMENES, R. M.; FALCÃO, E. P. D. S.; CHAGAS, C. A.; JÚNIOR, F. C. A. A.; SANTOS, N. P. D. S.; SILVA, M. V. D.; CORREIA, M. T. D. S. Evaluation of cytotoxic potential, oral toxicity, genotoxicity, and mutagenicity of organic extracts of Pityrocarpa moniliformis. **J Toxicol Environ Health A**. v. 82, n. 3, p. 216-31, 2019.

RODRIGUES, H. G.; MEIRELES, C. G.; LIMA, J. T. S.; TOLEDO, G. P.; CARDOSO, J. L.; GOMES, S. L. Efeito embriotóxico, teratogênico e abortivo de plantas medicinais. **Rev. Bras. Pl. Med.**, Botucatu, v.13, n.3, p.359-66, 2011.

SALUSTRIANO, F. R.; MONREAL, A. C. D.; NEVES, S. C.; OLIVEIRA, G. M.; OLIVEIRA, D. D. M.; VILELA, M. L. B.; NASCIMENTO, V. A.; MARTINS, A. C. F.; SAROJA, B.; KARUPPUSAMY, A.; COELHO, H. R. S.; KASSUYA, C. A. L.; GELVES, D. I. A.; SALVADOR, M. J.; OLIVEIRA, R. J; GOMES, R. D. S. The Ethanolic Extract of *Gomphrena celosioides* Mart. Does Not Alter Reproductive Performance or Embryo-Fetal Development, nor Does It Cause Chromosomal Damage. **Pharmaceutics**., v. 14, n. 11, art. 2369, 2022.

SALVADOR, M. J.; DIAS, D. A. Flavone C-glycosides from Alternanthera maritima (Mart.) St. Hil. (Amaranthaceae). **Biochemical Systematics and Ecology**, v. 32, p. 107-10, 2004.

SALVADOR, M. J.; PEREIRA, P. S.; FRANÇA, S. C.; CÂNDIDO, R. C.; ITO, I. Y.; DIAS, D. A. Comparative study of antibacterial and antifugal activity of callus culture and adult plants extracts from Alternanthera maritima (Amaranthaceae). **Brazilian Journal of Microbiology**, v. 35, p. 131-36, 2004a.

SALVADOR, M. J.; ZUCCHI, O. L. A. D.; CANDIDO, R. C.; ITO, I. Y.; DIAS, D. A. In vitro antimicrobial activity of crude extracts and isolated constituents of Alternanthera maritima. **Pharmaceutical Biology**, v. 42, n. 2, p. 138-48, 2004b.

SANDOVAL-HERRERA, N.; CASTILLO, J. P.; MONTALVO, L. G. H.; WELCH, K. C. JR. Micronucleus Test Reveals Genotoxic Effects in Bats Associated with Agricultural Activity. **Environ Toxicol Chem.**, v. 40, n. 1, p. 202-7, 2021.

SANTOS J. A.; PICCINELLI, A. C.; FORMAGIO, M. D.; OLIVEIRA, C. S.; SANTOS, E. P.; STEFANELLO, M. É. A.; LANZA JUNIOR, U.; OLIVEIRA, R. J.; SUGIZAKI, M. M.; KASSUYA, C. A. Antidepressive and antinociceptive effects of ethanolic extract and fruticuline A from Salvia lachnostachys Benth leaves on rodents. **PLoS One**, v.12, n. 2, e0172151, 2017.

SENNA, L. R. Alternanthera in Flora do Brasil. **Jardim Botânico do Rio de Janeiro**, 2020.

SHROUKH, W. A.; STEINKE, D. T.; WILLIS, S. C. Risk management of teratogenic medicines: A systematic review. **Birth Defects Res**., v. 112, n. 20, p.1755-86, 2020.

SINGLA, R. K.; DHIR, V.; MADAAN, R.; KUMAR, D.; BOLA, S. S.; BANSAL, M.; SHEN, B. The Genus Alternanthera: Phytochemical and Ethnopharmacological Perspectives. **Frontiers in Pharmacology**, v. 13, art 769111, 2022.

SOULIMANE-MOKHTARI, N. A.; GUERMOUCHE, B.; YESSOUFOU, A.; SAKER, M.; MOUTAIROU, K.; HICHAMI, A.; MERZOUK, H.; KHAN, N. A. Modulation of lipid metabolism by n-3 polyunsaturated fatty acids in gestational diabetic rats and their macrosomic offspring. **Clin Sci (Lond).,** v. 109, n. 3, p. 287-95, 2005.

SPIESS, D.; WINKER, M.; CHAUVEAU, A.; ABEGG, V. F.; POTTERAT, O.; HAMBURGER, M.; GRÜNDEMANN, C.; SIMÕES-WÜST, A. P. Medicinal Plants for the Treatment of Mental Diseases in Pregnancy: An In Vitro Safety Assessment. **Planta Med**. oct., 2021.

SOUZA, J. G.; TOMEI, R. R.; KANASHIRO, A.; KABEYA, L. M.; AZZOLINI, A. E.; DIAS, D. A.; SALVADOR, M. J.; LUCISANO-VALIM, Y. M. Ethanolic crude extract and flavonoids isolated from Alternanthera maritima: neutrophil chemiluminescence inhibition and free radical scavenging activity. **Z Naturforsch C J Biosci**., v.62, n. 5-6, p. 339-47, 2007.

SPONCHIADO. G.; ADAM, M. L.; SILVA, C. D.; SOLEY, B. S.; MELLO-SAMPAYO, C.; CABRINI, D. A.; CORRER, C. J.; OTUKI, M. F. Quantitative genotoxicity assays for analysis of medicinal plants: A systematic review. **J Ethnopharmacol**. v. 178, p. 289-96, 2016.

STAPLES, R. E.; SCHNELL, V. L. Refinements in rapid clearing technic in the koh-alizarin red s method for fetal bone. **Stain Technol**, v. 39, p. 61-63, 1964.

TAYLOR, P. Pratical Teratology. **Academic Press**, New York. 1986.

TRIBESS, B.; PINTARELLI, G. M.; BINI, L. A.; CAMARGO, A.; FUNEZ, L. A.; GASPER, A. L.; ZENI, A. L. Ethnobotanical study of plants used for therapeutic purposes in the Atlantic Forest region, Southern Brazil. **J Ethnopharmacol**. v. 164, p. 136-46, 2015.

UCHÔA, I. S.; MAGALHÃES, M. A. V. Teste de micronúcleo um importante biomarcador celular. **Braz. J. of Develop**., v. 6, n.8, p. 61601-606, 2020.

USTULIN, M.; FIGUEIREDO, B. B.; TREMEA, C.; POTT, A.; POTT, V. J.; BUENO, N. R.; CASTILHO, R. O. Plantas medicinais comercializadas no Mercado Municipal de Campo Grande-MS. **Rev. bras. Farmacogn.,** v. 19, n. 3, p. 805-13, jul.-set. 2009.

VANI, J. M.; MONREAL, M. T. F. D.; AUHAREK, S. A.; CUNHA-LAURA, A. L.; ARRUDA, E. J.; LIMA, A. R.; SILVA, C. M.; ANTONIOLLI-SILVA, A. C. M. B.; LIMA, D. P.; BEATRIZ, A.; OLIVEIRA, R. J. The mixture of cashew nut shell liquid and castor oil results in an efficient larvicide against Aedes aegypti that does not alter embryo-fetal development, reproductive performance or DNA integrity. **PLoS One**., v.13, n. 3, e0193509, 2018.

WILSON J. G. Methods for administering agents and detecting malformation in experimental animal. In: Wilson J. C., Warkany J. **Teratology principles and techniques.** Chicago: Univ of Chicago Press; p. 262-77, 1965.

WISE, L. D.; BECK, S. L.; BELTRAME, D.; BEYER, B. K.; CHAHOUD, I.; CLARK, R. L.; CLARK, R.; DRUGA, A. M.; FEUSTON, M. H.; GUITTIN, P.; HENWOOD, S.M.; KIMMEL, C.A.; LINDSTROM, P.; PALMER, A.K.; PETRERE, J.A.; SOLOMON, H. M.; YASUDA, M.; YORK, R. G. 1997. Terminology of developmental abnormalities in common laboratory mammals (version 1). **Teratol.**, v. 55, p. 249-92, 1997.

WACHHOLZ, G. E.; RENGEL, B. D.; VARGESSON, N.; FRAGA, L. R. From the Farm to the Lab: How Chicken Embryos Contribute to the Field of Teratology. **Front Genet**., v. 12, art. 666726, 2021.

YAZBEK, P. B.; TEZOTO, J.; CASSAS, F.; RODRIGUES, E. Plants used during maternity, menstrual cycle and other women's health conditions among Brazilian cultures. **J Ethnopharmacol.,** v. 179, p. 310-31, feb., 2016.

ANEXOS

Anexo 1 – Certificado de Aprovação do CEUA.

Serviço Público Federal
Ministério da Educação
Fundação Universidade Federal de Mato Grosso do Sul

UFMS

C E R T I F I C A D O

Certificamos que a proposta intitulada "Avaliação dos efeitos tóxico-reprodutivos, teratogênicos e (anti)genotóxicos dos extratos etanólicos de plantas com possíveis efeitos medicinais em camundongos Swiss", registrada com o nº 965/2018, sob a responsabilidade de **Rodrigo Juliano Oliveira** - que envolve a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata, para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS/CEUA DA UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL/UFMS, na 6ª reunião ordinária do dia 07/08/2018.

FINALIDADE	( ) Ensino (x) Pesquisa Científica
Vigência da autorização	1º/10/2018 a 30/03/2020
Espécie/Linhagem/Raça	*Mus musculus* / Swiss
Nº de animais	Machos 620 / Fêmeas 520= 1.140
Peso/Idade	30g / 8 - 10 semanas
Sexo	Machos e Fêmeas
Origem	Biotério - UT/INBIO/UFMS

Joice Stein
Coordenadora da CEUA/UFMS
Campo Grande, 08 de agosto de 2018.

Comissão de Ética no Uso de Animais/CEUA
http://www.propp.ufms.br/ceua
ceua.propp@ufms.br
fone (67) 3345-7925

Anexo 2 – Manuscrito 1: Artigo Publicado no *Journal of Toxicology and Environmental Health*

JOURNAL OF TOXICOLOGY AND ENVIRONMENTAL HEALTH, PART A
https://doi.org/10.1080/15287394.2023.2223624

Absence of maternal-fetal adverse effects of *Alternanthera littoralis P.* Beauv. following treatment during pregnancy in mice

Giovana Corbucci Danti Rezende[a,b#], Renata Coelho Rodrigues Noronha[c#], Hudman Cunha Ortiz[a,b#], Luís Adriano Santos do Nascimento[d], Silvia Cordeiro das Neves[a,b], Yasmin Lany Ventura Said[a,b], Adauto Lima Cardoso[c,d,e], Vanessa Albuquerque de Mescouto[d], Marcelo Luiz Brandão Vilela[f], Valter Aragão do Nascimento[b], Henrique Rodrigues Scherer Coelho[a], Candida Aparecida Leite Kassuya[g], Taise Fonseca Pedroso[h], Marcos José Salvador[h], and Rodrigo Juliano Oliveira[a,b]

[a]Centro de Estudos em Células-Tronco, Terapia Celular e Genética Toxicológica (CeTroGen), Faculdade de Medicina (FAMED), Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brasil; [b]Programa de Pós-Graduação em Saúde e Desenvolvimento na Região Centro-Oeste, Faculdade de Medicina (FAMED), Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brasil; [c]Laboratório de Citogenética, Centro de Estudos Avançados da Biodiversidade, Instituto de Ciências Biológicas (ICB), Universidade Federal Do Pará (UFPA), Belém, Brasil; [d]Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Pará (UFPA), Belém, Brasil; [e]Laboratório Genômica Integrativa, Departamento de Biologia Estrutural e Funcional, Instituto de Biociências de Botucatu (IBB), Universidade Estadual Paulista (UNESP), Botucatu, São Paulo, Brasil; [f]Faculdade de Medicina (FAMED), Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brasil; [g]Faculdade de Ciências da Saúde (FCS), Universidade Federal da Grande Dourados (UFGD), Dourados, Mato Grosso do Sul, Brasil; [h]Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), São Paulo, Brasil

ABSTRACT

Alternanthera littoralis P. Beauv is a plant native to Brazil that exhibits various beneficial activities including antioxidant, antibacterial, antifungal, antiprotozoal, anti-hyperalgesic, and anti-inflammatory properties. The aim of this study was to assess the impact of the ethanol extract of *Alternanthera littoralis* (EEAl) on reproductive outcomes, embryofetal development, and DNA integrity of pregnant female mice. Pregnant Swiss female mice were randomly assigned to three experimental groups ($n = 10$): controls were administered either 1% Tween 80 (vehicle), EEAl 100 mg/kg or EEAl 1000 mg/kg. Treatment was administered through gavage during the gestational period until day 18. On gestational days 16, 17, and 18, a peripheral blood sample from the tail vein was obtained for DNA integrity analysis (micronucleus test). After the last collection, animals were euthanized by cervical dislocation. Maternal organs and fetuses were collected, weighed, and subsequently analyzed. Reproductive outcome parameters were assessed by measurement of number of implants, live fetuses, and resorptions. Embryonic development was determined by adequacy of weight for gestational age as well as determination of external, visceral, and skeletal malformations. Data demonstrated that EEAl did not produce maternal toxicity at either dose associated with no marked alterations in any of the reproductive outcome parameters including implantation sites, live/dead fetuses ratio, fetal viability, post-implantation losses, resorptions, and resorption rate. However, EEAl 1000 group reduced embryofetal development by lowering placental weight. In addition, there was an increase in the frequency of external and skeletal malformations in the EEAl 1000 group, which could not be attributed to extract exposure as these values were within control levels. Based upon our findings, evidence indicates that the EEAl at the concentrations employed in our study may be considered safe for use during pregnancy and extracts of this plant show potential for development of phytomedicines to be used in pregnancy.

KEYWORDS
teratogenesis; mutagenesis; DNA damage; medicinal plants

Introduction

The Amaranthaceae family includes approximately 65 genera and 1,000 species of annual and perennial herbaceous plants, shrubs, and some trees found in tropical, subtropical, and temperate regions (Souza et al. 2007). This family is commonly used in nutrition and traditional medicine (Salvador et al. 2004a; Souza et al. 2007). According to Singla et al. (2022), the *Alternanthera* genus includes 139 species, among which 14 are

CONTACT Rodrigo Juliano Oliveira rodrigo.oliveira@ufms.br Centro de Estudos em Células-Tronco, Terapia Celular e Genética Toxicológica (CeTroGen), Faculdade de Medicina (FAMED), Universidade Federal de Mato Grosso do Sul (UFMS), Cidade Universitária, Avenida Costa e Silva - Pioneiros, Campo Grande, Mato Grosso do Sul 79070-900, Brasil
[#]Same contribution as first author.

traditionally used for treating various ailments such as hypertension, pain, inflammation, diabetes, cancer, microbial infections, and mental disorders. However, Aquino et al. (2015) noted that this genus has only 80 species, 30 of which are found in Brazil and are typically located on sandy beaches of the east coast.

Alternanthera littoralis P. Beauv. possesses botanical synonyms (heterotypic) and might therefore be found in literature as *Alternanthera littoralis* var. maritima (Mart.) Pedersen, *Alternanthera maritima* Mart., or *Telanthera maritima* (Mart.) Moq (Koolen et al. 2017; Senna 2020; Senna et al. 2020). Despite the synonyms, the classification *Alternanthera littoralis* P. Beauv. is the most current and therefore used in this study.

A. littoralis has been used in Brazilian folk medicine for treating inflammatory and infectious diseases (Souza et al. 2007). This plant is a source of secondary metabolites, including vitexin (A14), 2″-O-α-L-rhamnopyranosyl-vitexin (A18), and 2″-O-β-D-glucopyranosyl-vitexin (A19), which are present in the enriched fraction of glycosylated flavones obtained from its ethanolic extracts (Kassuya et al. 2021; Salvador and Dias 2004). These fractions or compounds may be responsible for the antioxidant, antibacterial, antifungal, antiprotozoal, anti-hyperalgesic, and anti-inflammatory activity observed in previous assays using hexane and ethanolic extracts of *A. littoralis* (Aquino et al. 2015; Gasparetto et al. 2010; Koolen et al. 2017; Salvador et al. 2004a, 2004b; Souza et al. 2007).

The Brazilian Ministry of Health has a National List of Medicinal Plants of Interest to the Unified Health System (Renisus), which are cataloged according to traditional use and proven efficacy and safety through review studies, analysis and systematization of scientific information and publications in the area of strategic medicines and inputs for the Unified Health System (SUS). This list is still under construction (Brasil 2009) and up to the present moment *A. littoralis* has not been included even though it is a commonly used medicinal plant

Although no apparent information on the adverse effects of *A. littoralis* was found in the literature, this plant extract is frequently used for treating infections and inflammation (Souza et al. 2007). Due to its popularity and potential use by individuals of different ages and stages of life, pregnant women may also consider extracts of this plant as an alternative to allopathic medications which are perceive to be more harmful than natural products (Araújo et al. 2016; Hyacinth et al. 2020; Maciel et al. 2019).

Although no apparent publication states that this ethanolic extract of *Alternanthera littoralis* (EEAl) tea is used by pregnant women, it is frequently mentioned by vendors of medicinal plants at the Municipal Market of Campo Grande, MS, Brazil that this substance is consumed. Based upon these reports the present study was designed with the aim of investigating the impact of the ethanolic extract of *Alternanthera littoralis* (EEAl) on reproductive parameters, embryofetal development, and DNA integrity of pregnant female mice.

Material and methods

Vegetal material and preparation of ethanolic extract of *Alternanthera littoralis*

The aerial parts of *A. littoralis* were collected from Restinga de Maricá, Rio de Janeiro, Brazil, in December 2010, and identified by Prof. Dr. Josafá Carlos de Siqueira of Pontifical Catholic University of Rio de Janeiro (PUC-RJ), Brazil. A voucher specimen (SPFR–4758) was deposited in the herbarium of the Faculty of Philosophy, Sciences, and Letters of Ribeirão Preto, University of São Paulo (FFCLRP/USP).

The air-dried aerial parts of *A. littoralis* (1 kg) were extracted using ethanol at a ratio of 1:2 (w/v) of plant powder to solvent through maceration at room temperature. The plant material was then filtered, and the solvents evaporated under reduced pressure and temperature below 40°C using a rotary evaporator. This process yielded 75 g of crude ethanolic extract of the plant (EEAl). The extract was dissolved in Tween 80 and subsequently diluted in distilled water. The final concentration of Tween 80 in the solutions was 1%.

Animals

A total of 45 Swiss mice of both genders at reproductive age were obtained from the Central Animal Facility of the Federal University of Mato Grosso do Sul, consisting of 30 females weighing an

average of 30 g and 15 males weighing an average of 35 g. After a 7-day acclimation period, mice were housed in ventilated racks (Alesco®) lined with Pinus sp. shavings. Males were housed individually while females were housed in pairs. The animals were kept in controlled conditions of temperature (22 ± 2°C), photoperiod (12 hr light/dark), relative humidity (55 ± 10%), with *ad libitum* access to food and water. The study was conducted according to the protocols of the Universal Declaration of Animal Rights and approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Mato Grosso do Sul (UFMS) under protocol number 965/2018.

Design of the experiment

The animals were mated at a ratio of 1 male to 2 females overnight, and pregnancy was confirmed by the detection of vaginal plugs, which was considered day zero of gestation (Oliveira et al. 2009, 2015). The pregnant females were then randomly assigned to one of three experimental groups ($n = 10$): control, which received 0.1 ml/10 g of body weight (b.w.) of vehicle (1% Tween 80) by gavage (p.o.) throughout gestation (1st to 18th gestational day); EEAl 100, which received EEAl by gavage at a dose of 100 mg/kg (b.w., p.o.) during gestation; and EEAl 1000, which received EEAl by gavage at a dose of 1000 mg/kg (b.w., p.o.) during gestation (Figure 1).

The gestation period of a mouse lasts approximately 21–22 days. However, in this study, gestation was terminated on the 18th gestational day. Thus, females were pregnant for 18 consecutive days (Gonçalves et al. 2014; Ishikawa et al. 2018; Nunes et al. 2023; Ortiz et al. 2023; Salustriano et al. 2022; Vani et al. 2018).

The dose of 100 mg/kg was selected as this quantity was found to exhibit anti-hyperalgesic and anti-inflammatory effects (Aquino et al. 2015). To test for safety, a dose 10-fold higher was employed, following recommendations by Gomes et al (2012). Treatment was continued throughout the gestational period, as more severe effects might arise during gestation which was suggested by the OECD 414 guidelines (2018). This approach has been employed by several investigators (Khalki et al. 2010; Roman et al. 2014; Gonçalves et al. 2014; Moraes-Souza et al. 2017; Paula et al. 2020; Salustriano et al. 2022; Soares et al. 2015). Further, Ishikawa et al. (2018) and Vani et al. (2018) recommended testing the effective dose and a safety dose 10-fold higher throughout the gestational period.

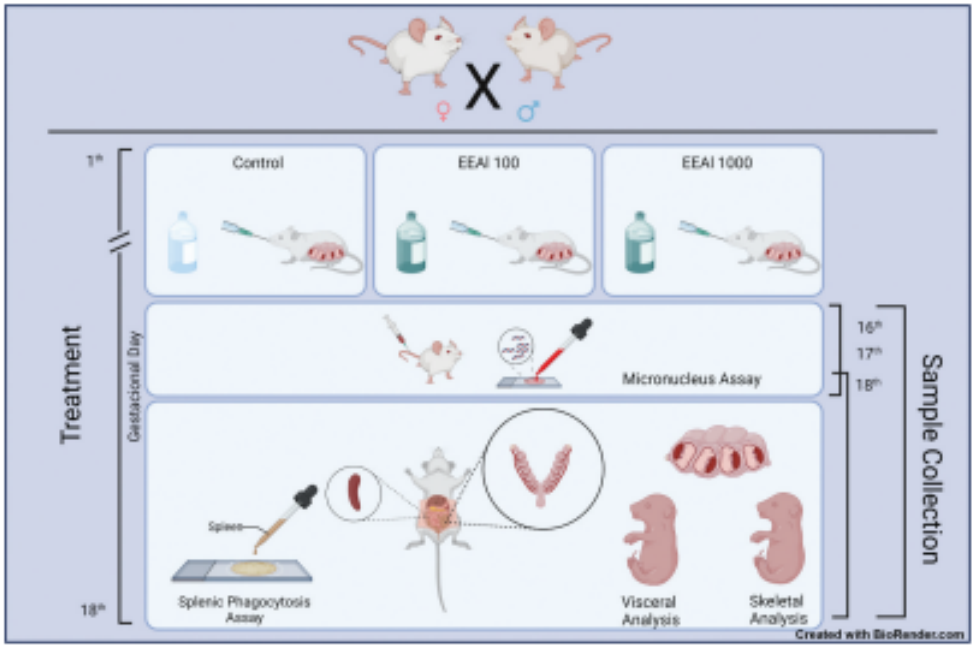


Figure 1. Experimental design and biological assays. Legend: EEAl 100 and EEAl 1000 - ethanol extract of *Alternanthera littoralis* (EEAl) at doses of 100 and 1000 mg/Kg, respectively.

Biological assays

Peripheral blood samples were collected from the mouse tail vein on the 16th, 17th, and 18th days of gestation. After final blood collection, pregnant mice were euthanized by cervical dislocation, and then subjected to laparotomy and hysterectomy to collect, weigh, and store organs, fetuses, and placentas (Figure 1).

The fetuses underwent external malformation analysis, gender determination and randomly divided into two subgroups. The first subgroup was fixed in Bouin's solution, consisting of distilled water (142 mL), acetic acid (50 mL), formaldehyde (50 ml), and 95% alcohol (758 ml), for at least 7 days. Subsequently, samples were subjected to incision/microdissection using strategic cuts according to Barrow and Taylor (1969) for the thorax and abdomen, and Wilson (1965), modified by Oliveira et al. (2009), for the head. The classification of visceral alterations was based upon Taylor (1986), Manson and Kang (1994), Wise et al. (1997), Damasceno et al. (2008), and Oliveira et al. (2009).

The second subgroup, intended for skeletal analysis, was fixed in absolute acetone for at least 7 days, then immersed in 0.8% potassium hydroxide (KOH) and stained with Alizarin Red for diaphanization according to Staples and Schnell (1964) with modifications by Oliveira et al. (2015a). After staining, the KOH solution was replaced with a cleaning solution (1 L glycerin: 1 L ethyl alcohol: 0.5 L benzyl alcohol) which was changed daily for 5 days. Fetal visceral and skeletal analyses were conducted using a stereomicroscope (Nikon® - SMZ 745T) with 1.6× magnification. Malformations were classified based upon data of Taylor (1986), Manson and Kang (1994), Wise et al. (1997), Damasceno et al. (2008), and Oliveira et al. (2009).

Biometric parameters

Biometric parameters were calculated using initial weight (weight of females on day zero), final weight (weight of females on the 18th gestational day), weight gain (final weight minus initial weight), uterine weight, net weight gain (weight gain minus uterine weight), absolute and relative weights of heart, lungs, spleen, kidneys, and liver.

Reproductive parameters and embryofetal development

The study recorded the number of implantation sites, which included live fetuses, dead fetuses, and resorptions, as well as number of live and dead fetuses, and weight of the fetuses and placenta. Using these data, the study calculated fetal viability as (number of live fetuses × 100/number of implantations), post-implantation loss rate as [(number of implantations – number of resorptions) × 100/number of implantations], resorption rate as (number of resorptions × 100/number of implants), placental index as (placental weight/fetal weight), and head-tail and anogenital distance.

The fetal weight classification according to gestational age (FWCGA) was conducted according to the method of Soulimane-Mokhtari et al. (2005). The fetuses were weighed after being collected from the uterine horns and then categorized as appropriate for gestational age (AGA), small for gestational age (SGA), or large for gestational age (LGA) based upon the mean ±1.7 × standard deviation (SD) of the body weights obtained in the controls.

Micronucleus (MN) levels in peripheral blood

The peripheral blood micronucleus (MN) test (Figure 1) was conducted according to the method of Hayashi et al. (1990) with modifications by Oliveira et al. (2015b). Approximately 20 µl peripheral blood was collected from the tail vein and deposited on a slide prepared with 10 µl Acridine Orange (1 mg/ml). The blood was then covered with a coverslip and stored at −20°C for at least 7 days. Using a fluorescence microscope (Motic®; Model BA 410) at 400× magnification, with an excitation filter of 420–490 nm and a barrier filter of 520 nm, 2,000 reticulocytes were examined per animal. The number of MN was counted in 2,000 cells/animal, as described by Carvalho et al. (2015).

Splenic phagocytosis

The spleen was macerated in physiological solution to obtain a homogeneous cell suspension, from which 100 µl was placed on a slide stained with 10 µl Acridine Orange (1 mg/ml), covered with

a coverslip, and stored at −20°C until analysis. Phagocytosis was determined using a fluorescence microscope (Zeiss®) at 40× magnification with a 420–490 nm filter and a 520 nm barrier filter. The presence or absence of phagocytosis (Figure 1) was based upon methods of Hayashi et al. (1990) and Carvalho et al. (2015), with 100 cells per animal examined.

Statistical analysis

Data with normal distribution was measured using a one-way ANOVA test followed by Tukey's posttest, while the Kruskal-Wallis' test with Dunn's posttest was used for non-normally distributed data. Frequency comparisons were assessed using the chi-square test. Statistical analyses were conducted using GraphPad Instat® software (Version 3.06, 2003). The results are presented as either mean ± standard error of the mean or mean ± standard deviation. The criterion for significance was set at $p < 0.05$.

Results

Biometric parameters

No significant differences were observed in initial weight, final weight, weight gain, net weight gain, uterine weight, and absolute and relative weights of organs between the experimental groups (data not shown)

Reproductive parameters

The number of implantations, live and dead fetuses, resorptions, fetal viability, resorption rate, and post-implantation loss rate did not differ significantly among experimental groups as shown in Table 1.

Embryofetal development and placental efficiency

There were no significant differences in fetal weight, placental index, weight adequacy for gestational age, distance between head and tail, and urogenital distance of males and females among the experimental groups. However, placental weight in the EEAl 1000 group was significantly reduced compared to control and EEAl 100 (Table 2).

Table 1. Reproductive parameters of females treated with the ethanolic extract of *Alternanthera littoralis* (EEAl).

Reproductive Performance			
Experimental Groups (mg/kg)			
Parameters	Control	EEAl 100	EEAl 1000
Implants[1]	8.80 ± 1.340[a]	10.00 ± 1.000[a]	9.40 ± 0.819[a]
Live Fetuses[1]	6.40 ± 1.284[a]	8.10 ± 1.038[a]	7.50 ± 0.719[a]
Dead Fetuses[2]	0.10 ± 0.100[a]	0.20 ± 0.133[a]	0.10 ± 0.100[a]
Fetal Viability[1]	71.29 ± 7.533[a]	80.35 ± 6.726[a]	79.96 ± 3.796[a]
PILR[1]	28.71 ± 7.533[a]	19.65 ± 6.726[a]	20.04 ± 3.796[a]
Reabsorption[1]	2.30 ± 0.700[a]	1.70 ± 0.597[a]	1.80 ± 0.359[a]
Reabsorption Rate[1]	28.05 ± 7.517[a]	17.45 ± 6.982[a]	19.33 ± 3.525[a]

Legend: PILR – post-implantation loss rate; Equal letters indicate no statistically significant differences. Mean ± Standard Error of the mean ([1]Anova/Tukey's test; [2]Kruskal-Wallis/Dunn, $p > 0.05$).

External malformations detected included hyperextension of the forelimbs (unilateral and bilateral), hyperflexion of the hindlimb (unilateral), curly tail and gastroschisis. Only the % malformations in forelimbs and hindlimbs was significantly elevated in the EEAl 100 group (Table 3; Figure 2).

The observed visceral malformations were hydrocephaly and hydronephrosis. There were no marked differences in the occurrence among the different experimental groups (Table 4; Figure 3).

Skeletal malformations noted were femur agenesis and reduced ossification of skull bones, including parietal, interparietal, presphenoid, palate, and vomer. The frequency and % malformations for skull bone alterations rose significantly in animals from the EEAl 1000 group (Table 5; Figure 4).

Toxicogenetic evaluation

The frequency of MN was not markedly altered for any of the tested doses or at any of the sampling times in response to EEAl treatment (Data not shown). Further, the EEAl did not significantly affect the frequency of splenic phagocytosis (Data not shown).

Discussion

Pregnant women tend to consume medicinal plants more frequently during the first trimester, increasing the risk of malformations in their children (Luna-Gómez et al. 2020). However, at present there is lack of comprehensive knowledge

Table 2. Parameters of embryofetal development and placental efficiency of females treated with the ethanolic extract of *Alternanthera littoralis* (EEAl).

Embryo Development and Placental Efficiency			
Experimental groups (mg/kg)			
Parameter	Control	EEAl 100	EEAl 1000
Fetal weight (g)[2]	0.74 ± 0.015[a]	0.73 ± 0.013[a]	0.71 ± 0.017[a]
Placental weight (g)[2]	0.07 ± 0.003[a]	0.07 ± 0.002[a]	0.06 ± 0.002[b]
Placental index[2]	0.10 ± 0.005[a]	0.09 ± 0.003[a]	0.08 ± 0.003[a]
% AGA[3]	21.88	19.75	21.33
% SGA[3]	34.38	32.10	37.33
% LGA[3]	43.75	48.15	41.33
Head-to-tail distance (mm)[2]	22.89 ± 0.232[a]	23.28 ± 0.182[a]	23.07 ± 0.208[a]
Male fetuses anogenital distance (mm)[1]	1.47 ± 0.054[a]	1.47 ± 0.048[a]	1.58 ± 0.030[a]
Female fetuses anogenital distance (mm)[2]	0.91 ± 0.019[a]	1.01 ± 0.038[a]	0.91 ± 0.012[a]

Legend: g – grams; AGA – adequate for gestational age, SGA – small for gestational age, LGA – large for gestational age; Statistical test: [1]Anova/Tukey – Mean ± Standard error of the mean: different letters indicate statistically significant differences; [2]Kruskal-Wallis/Dunn – Mean ± Standard error of the mean: different letters indicate statistically significant differences; [3]Chi-square test - *indicates statistically significant difference compared to the control group; p*0.05.

Table 3. External malformations found in the offspring of females treated with the ethanolic extract of *Alternanthera littoralis* (EEAl).

	Experimental Groups		
Parameters	Control	EEAl 100	EEAl 1000
	Anterior and Posterior paw		
Analyzed fetuses	64	81	75
Normal fetuses	62	70	71
UAHE	1	5	3
BAHE	0	2	0
UPHF	1	4	1
Malf. Freq.	2	11	4
% M.F.	3.13	13.58*	5.33
		Tail	
Normal fetuses	62	78	74
Curved Tail	2	3	1
Malf. Freq.	2	3	1
% M.F.	3.13	3.70	1.33
		Abdomen	
Normal fetuses	64	78	75
Gastroschisis	0	3	0
Malf. Freq.	0	3	0
% M.F.	0.00	3.70	0.00

Legend: UAHE – Unilateral anterior paw hyperextension; BAHE – bilateral anterior paw hyperextension; UPHF – Unilateral posterior paw hyperflexion; Malf. Freq. - Malformations Frequency; % M.F. - percentage of malformed fetuses. Statistical test: Chi-square; *indicates statistically significant difference compared to the Control group ($p < 0.05$).

regarding herbal medicine usage during pregnancy and there is a misconception that natural products are safe for them and the fetus (Ahmed et al. 2018; Bernstein et al. 2021; Costa, Coelho, and Santos 2017; Kan, Barnett, and Douglas 2019; Maciel et al. 2019). It should be noted that data regarding the effects of *A littoralis* natural products on DNA stability are not available.

Our results do not detect any signs of maternal toxicity. Classic symptoms such as diarrhea, tremors, excessive salivation, convulsions, hypoactivity, ataxia, lethargy, tail curvature, piloerection, or reduced food and water intake (Corso et al. 2019) were not observed.

The absence of apparent maternal toxicity is supported by lack of changes in biometric parameters such as final weight, weight gain, uterine weight, net weight, and absolute and relative organ weights, which are typically associated with toxic effects during pregnancy (Teng et al. 2019). In addition, the absence of MN induction and splenic phagocytosis activation further confirms the absence of EEAl-mediated maternal toxicity. The MN assay is used to evaluate genotoxic effects induced by chemical agents that result in cytogenetic damage (Canedo et al. 2021). Similarly, the phagocytosis assay is employed to confirm that cells with DNA damage are not being underestimated due to an increase in spleen phagocytic activity. Combining these two pieces of information (MN rate + splenic phagocytosis frequency) provides a more reliable assessment of the organism's response to medicinal extract exposure. Thus, non-genotoxic compounds do not affect MN frequency or phagocytosis (Gonçalves et al. 2014; Ishikawa et al. 2018; Salustriano et al. 2022, Stein et al. 2022; Santos Radai et al. 2018). However, if an elevation in splenic phagocytosis occurs despite the absence of a change in MN frequency, this may indicate an increase in cells with DNA damage (including MN) that need to be removed from the bloodstream in. In this case, the spleen is responsible for this process (Araújo et al. 2017; Navarro et al. 2014, 2018, Oliveira et al. 2015b; Oliveira et al. 2018, 2018, 2019; Schneider et al. 2016).

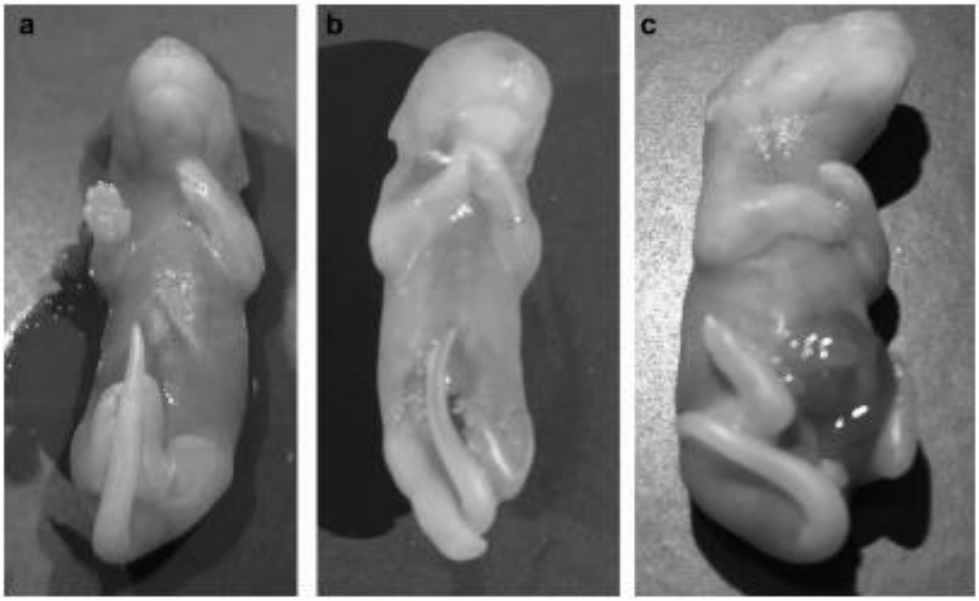


Figure 2. Aspects of fetuses with external malformations: (a) Appearance of a normal fetus; (b) fetus with unilateral hyperextension of the hind limb; and (c) gastroschisis. The arrows indicate the malformations.

Table 4. Visceral malformations found in the offspring of females treated with the ethanolic extract of *Alternanthera littoralis* (EEAl).

		Experimental Groups		
Parameters		Control	EEAl 100	EEAl 1000
	Brain			
Analyzed fetuses		27	35	32
Normal fetuses		24	32	27
Hydrocephaly	Moderated	3	3	5
Malf. Freq.		3	3	5
% M.F.		11.11	8.57	15.63
	Urogenital Tract			
Normal fetuses		24	30	27
Hydronephrosis	Moderated	3	5	5
Malf. Freq.		3	5	5
% M. F.		11.11	14.29	15.63

Legend: Malf. Freq. - Malformations Frequency, % M.F – percentage of fetuses with malformation.
*Statistically significant difference (Statistical test: Chi-square; $p < 0.05$).

No apparent toxicity studies utilizing *A. littoralis* were found, but investigations on plants of the same genus were reported. Samudrala et al. (2015) reported that the ethyl acetate extract of *Alternanthera brasiliana* did not induce acute toxicity in Swiss mice up to a dose of 2,000 mg/kg. Similarly, Khandker et al. (2022) evaluated the subacute toxicity of the methanolic extract of *Alternanthera philoxeroides* and found that it exerted no marked adverse effects on female Swiss mice. Therefore, these studies on plants of the same genus support our findings.

Our results show that EEAl did not significantly alter any parameters related to reproductive parameters as evidenced by no effect in the number of implants, no difference in number of live and dead fetuses, no reabsorption and/or post-implantation losses, and fetal viability was not affected. These findings indicate the safety of the extract suggesting no apparent toxic-reproductive and/or maternal-toxic damage occurrences. This observation is similar to findings of other investigators examining plant extracts in pregnancy (Ishikawa et al. 2018; Paula et al. 2020; Pessatto et al. 2017; Salustriano et al. 2022, Stein et al. 2022; Santos Radai et al. 2018; Soares et al. 2015; Vani et al. 2018; Volpato et al. 2015).

The decrease in placental weight may be linked to fetal malnutrition, reduced growth, low birth

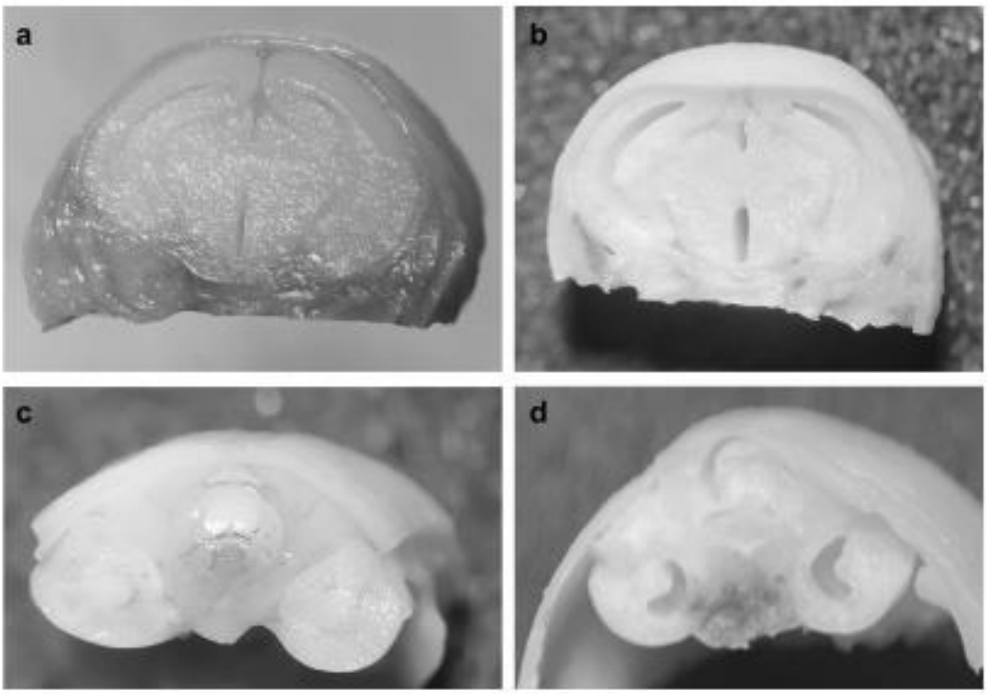


Figure 3. Microdissection sections demonstrating hydrocephalus and hydronephrosis (examples of visceral malformations): Hydrocephalus - (a) appearance of a normal brain; (b) mild hydrocephalus – dilation of the cerebral ventricles; and Hydronephrosis; (c) appearance of normal kidneys; (d) moderate hydronephrosis – dilation of the renal pelvis and hypoplasia of the renal papilla.

Table 5. Skeletal malformations found in the offspring of females treated with the ethanolic extract of *Alternanthera littoralis* (EEAl).

		Experimental Groups		
Parameters		Control	EEAl 100	EEAl 1000
		Members		
Analyzed fetuses		29	39	35
Normal fetuses		29	38	35
Femur	Absent	0	1	0
Malf. Freq.		0	1	0
% M.F.		0.00	2.56	0.00
	Cranium			
Normal fetuses		28	35	29
Parietal	R.O.	0	0	1
Interparietal	R.O.	0	1	4
Presphenoid	R.O.	1	4	5
Palatine	R.O.	0	1	1
Vomer	R.O.	0	1	1
Malf. Freq.		1	7	12*
% M.F.		3.45	17.95*	34.29*

Legend:R.O. - Reduced Ossification; Malf. Freq. - Malformations Frequency; % F.M – percentage of fetuses with malformation. *Statistically significant difference (Statistical test: Chi-square; $p < 0.05$).

weight, and a lower placental index. These changes might lead to a decrease in fetal weight (Moraes-Souza et al. 2017) as well as insufficient weight for gestational age (Soulimane-Mokhtari et al. 2005). However, in our study, only a diminished placental weight was detected in the EEAl 1000 group, which may be considered an isolated finding without biological significance since there were no changes in placental index, and no alterations in inadequacy of weight for gestational age.

Regarding external malformations, the only change identified was a difference in % malformations in the EEAl 100 group related to the mispositioning of anterior and posterior paws. However, these findings were also detected in controls, and

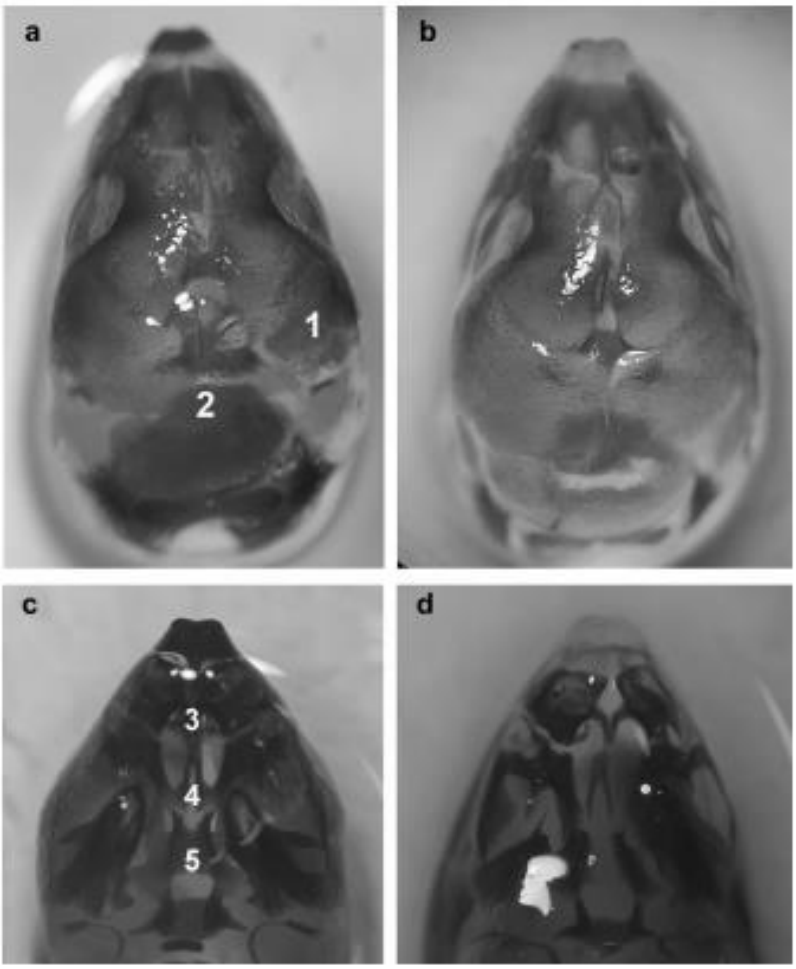


Figure 4. Cuts and microdissections of diaphanized fetuses (examples of skeletal malformations): Normal fetus appearance (a) - Head ossification: 1 - Parietal; 2 - Interparietal; (b) Reduced ossification of parietal and interparietal. Normal fetus appearance (c) - Normal ossification of Presphenoid, Palatine and Vomer; (d) Reduced ossification of Presphenoid, Palatine and Vomer.

did not appear endanger fetal survival. Both external and visceral malformations may be considered variants of normality where Taylor (1986) defined variants of normality as changes that may regress with progression of gestation or at birth, as fetuses complete their development. This interpretation is also cited by Damasceno et al. (2002), Gonçalves et al. (2013, 2014), Volpato et al. (2015), Pessatto et al. (2017), Vani et al. (2018), and Salustriano et al. (2022).

Skeletal malformations displayed an overall increase in frequency in the EEAl 1,000 group, as well as % malformed fetuses in both treated groups. The most frequently found malformations were reduced ossification of skull bones, which are small bones that complete their ossification process if the pregnancy was not prematurely interrupted, as suggested by Taylor (1986) and Gonçalves et al. (2013). Therefore, this cannot be attributed to treatment with the plant extract. Delayed ossifications are considered skeletal variations (Kimmel and Wilson 1973; Sartori et al. 2020; Tyl, Chernoff, and Rogers 2007), and this inference is also evident in studies by Gonçalves et al. (2013, 2014), Pessatto et al. (2017), Vani et al. (2018), and Salustriano et al. (2022).

In Brazil, medicinal plants are categorized according to their importance to the Unified Health System (SUS) (Brasil 2009, Silva et al 2020; Marmitt et al. 2021; Nunes et al. 2023; Ferreira, Arcanjo, and Peron 2023) given that part of the population lacks adequate access to healthcare and medication (Macedo 2009; Nunes et al. 2023), medicinal plants may be their only treatment option. In this situation, individuals use both categorized and non- categorized plants (Nunes et al.

2023). Although *A. Littorallis* is commonly used it not listed in the Renisus relationship. Thus, our findings on its efficacy and toxicology might assist in integrating this species into the relationship in the future.

Conclusions

Based upon the results presented, it may be concluded that the ethanolic extract of *Alternanthera littoralis* is safe for use in pregnancy for maternal and fetal health, as this plant did not exhibit maternotoxic effects at the doses used as evidenced by no marked effects on reproductive parameters, and absence of teratogenic alterations. Data suggest that the plant extract exhibits the potential to be explored as a source of phytomedicines that may be safely used by women during pregnancy.

Acknowledgments

The authors acknowledge the CNPq, FUNDECT, CAPES, FAPESPA and UFMS for providing various research grants and to Prof. Dr. Josafá Carlos de Siqueira for identifying the plant material. We are also grateful to FAPESP and FAEPEX-UNICAMP (Fundo de Apoio ao Ensino, à Pesquisa e à Extensão) and PROPESP/UFPA.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was funded by FUNDECT, Coordination for the Improvement of Higher Education Personnel - Brazil (Capes) - Code Funding 001, Support Foundation for the Development of Education, Science and Technology of Mato Grosso do Sul, UFMS, FAPESP (21/09693-5; 15/03726-8 and 06/06079-4), FAEPEX-UNICAMP (Fundo de Apoio ao Ensino, à Pesquisa e à Extensão), CNPQ (316246/2021-2, Grant Term 1089700081951461, 315279/2021-4 and 313798/2022-2) and FAPESPA (2022/1437745).

Data availability statement

The data that support the findings of this study are available from the corresponding author (RJO) upon reasonable request.

References

Ahmed, S. M. H. N., J. Sundby, Y. S. Aragaw, and H. J. de Boer. 2018. The use of medicinal plants by pregnant women in Africa: A systematic review. *J. Ethnopharmacol.* 224:297–313. doi:10.1016/j.jep.2018.05.032.

Aquino, D. F. S., A. C. Piccinelli, F. L. Soares, A. C. Arena, M. J. Salvador, and C. A. L. Kassuya. 2015. Anti-hyperalgesic and anti-inflammatory activity of *Alternanthera maritima* extract and 2″-O-α-L-rhamnopyranosylvitexin in mice. *Inflammation* 38 (6):2057–66. doi:10.1007/s10753-015-0187-0.

Araújo, F. H. S., D. R. Figueiredo, S. A. Auharek, J. R. Pesarini, A. Meza, R. S. Gomes, A. C. D. Monreal, A. C. M. B. Antoniolli-Silva, D. P. Lima, C. A. L. Kassuya, et al. 2017. *In vivo* chemotherapeutic insight of a novel isocoumarin (3-hexyl-5,7-dimethoxy-isochromen-1-one): Genotoxicity, cell death induction, leukometry and phagocytic evaluation. *Genet. Mol. Biol.* 40(3):665–75. doi:10.1590/1678-4685-GMB-2016-0316.

Araújo, C. R., F. G. Santiago, M. I. Peixoto, J. O. Oliveira, and M. D. E. Coutinho. 2016. Use of medicinal plants with teratogenic and abortive effects by pregnant women in a city in Northeastern Brazil. *Rev. Bras. Ginecol. Obstet.* 38 (3):127–31. doi:10.1055/s-0036-1580714.

Barrow, M. V., and W. I. Taylor. 1969. A rapid method for detecting malformation in rat fetuses. *J. Morphol.* 127 (3):291–306. doi:10.1002/jmor.1051270303.

Bernstein, N., M. Akram, Z. Yaniv-Bachrach, and M. Daniyal. 2021. Is it safe to consume traditional medicinal plants during pregnancy? *Phytother. Res.* 35 (4):1908–24. doi:10.1002/ptr.6935.

Brasil. 2009. Ministério Da Saúde. Portal Da Saúde: Programa Nacional De Plantas Medicinais E Fitoterápicos. Accessed on 22 May 2023. https://bvsms.saude.gov.br/bvs/publicacoes/programa_nacional_plantas_medicinais_fitoterapicos.pdf

Canedo, A., L. W. O. Jesus, E. F. L. C. Bailão, and T. L. Rocha. 2021. Micronucleus test and nuclear abnormality assay in zebrafish (*Danio rerio*): Past, present, and future trends. *Environ. Pollut.* 290:118019. doi:10.1016/j.envpol.2021.118019.

Carvalho, P. C., E. A. Santos, B. U. C. Schneider, R. Matuo, J. R. Pesarini, A. L. Cunha-Laura, A. C. D. Monreal, D. P. Lima, A. C. M. B. Antoniolli, and R. J. Oliveira. 2015. Diaryl sulfide analogs of combretastatin A-4: Toxicogenetic, immunomodulatory and apoptotic evaluations and prospects for use as a new chemotherapeutic drug. *Environ. Toxicol. Pharmacol.* 40 (3):715–21. doi:10.1016/j.etap.2015.08.028.

Corso, C. R., M. C. Stipp, E. R. Adami, L. M. Silva, M. Mariott, S. F. Andrade, E. A. S. Ramos, G. Klassen, O. C. Beltrame, J. E. Queiroz-Telles, et al. 2019. *Salvia lachnostachys* Benth has antitumor and chemopreventive effects against solid Ehrlich carcinoma. *Mol. Biol. Rep.* 46 (5):4827–41. doi:10.1007/s11033-019-04931-3.

Costa, D. B., H. L. L. C. Coelho, and D. B. Santos. 2017. Utilização de medicamentos antes e durante a gestação: prevalência e fatores associados [Use of medication before and during pregnancy: prevalence and associated factors]. *Cad. Saude Publica.* 33 (2):e00126215. doi:10.1590/0102-311x00126215.

Damasceno, D. C., W. G. Kempinas, G. T. Volpato, and M. Consoni. 2008. Anomalias Congênitas: Estudos Experimentais *[Congenital Anomalies: Experimental Studies]*. Belo Horizonte, Minas Gerais, Brazil: Coopmed Editora Médica.

Damasceno, D. C., G. T. Volpato, O. C. Person, A. Yoshida, M. V. C. Rudge, and I. M. P. Calderon. 2002. Efeito do ácido acetilsalicílico na performance reprodutiva e na prole de ratas wistar [Effect of acetylsalicylic acid on reproductive performance and offspring of wistar rats]. *Rev. Assoc. Med. Bras.* 48 (4):312–16. doi:10.1590/S0104-42302002000400036.

Ferreira, P. M. P., D. D. R. Arcanjo, and S. P. Peron. 2023. Drug development, Brazilian diversity and political choices: Where are we heading? *J. Toxicol. Environ. Health B* 26 (5):257–74. doi:10.1080/10937404.2023.2193762.

Gasparetto, A., T. F. Lapinski, S. R. Zamuner, S. Khouri, L. P. Alves, E. Munin, and M. J. Salvador. 2010. Extracts from *Alternanthera maritima* as natural photosensitizers in photodynamic antimicrobial chemotherapy (PACT). *J. Photochem. Photobiol. B, Biol.* 99 (1):15–20. doi:10.1016/j.jphotobiol.2010.01.009.

Gomes, C., E. L. Lourenço, É. B. Liuti, A. O. Duque, F. Nihi, A. C. Lourenço, T. C. Mendes, A. Gasparotto Junior, and P. R. Dalsenter. 2012. Evaluation of subchronic toxicity of the hydroethanolic extract of Tropaeolum majus in Wistar rats. *J. Ethnopharmacol* 142:481–87. doi:10.1016/j.jep.2012.05.023.

Gonçalves, C. A., N. L. Silva, M. O. Mauro, N. David, A. L. Cunha-Laura, S. A. Auharek, A. C. Monreal, M. C. Vieira, D. B. Silva, F. J. Santos, et al. 2014. Evaluation of mutagenic, teratogenic, and immunomodulatory effects of Annona nutans hydromethanolic fraction on pregnant mice. *Genet. Mol. Res.* 13:4392–405. PMID: 25036345. doi:10.4238/2014.June.11.3.

Gonçalves, C. A., J. M. Siqueira, C. A. Carollo, M. O. Mauro, N. D. Davi, A. L. Cunha-Laura, A. C. D. Monreal, A. H. Castro, L. Fernandes, R. R. Chagas, et al. 2013. Gestational exposure to *Byrsonima verbascifolia*: Teratogenicity, mutagenicity and immunomodulation evaluation in female Swiss mice. *J. Ethnopharmacol.* 150 (3):843–50. doi:10.1016/j.jep.2013.09.012.

Hayashi, M., T. Kodama, Y. Sofuni, M. Ishidate Jr., and M. Ishidate. 1990. The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. *Mutat. Res. Lett* 245 (4):245–49. doi:10.1016/0165-7992(90)90153-B.

Hyacinth, B. M. S., B. L. Sánchez-Ortiz, K. R. T. Picanço, A. C. M. Pereira, D. C. S. Hyacinth, G. C. Souza, R. D. S. F. R. Sarquis, G. M. G. Aduanga, A. Navarrete, and J. C. T. Carvalho. 2020. *Endopleura uchi* (Huber) Cuatrec.: A medicinal plant for gynecological treatments - a reproductive toxicity assessment in zebrafish *Danio rerio*. *J. Ethnopharmacol.* 250:112457. doi:10.1016/j.jep.2019.112457.

Ishikawa, R. B., J. M. Vani, S. C. D. Neves, A. P. M. Rabacow, C. A. L. Kassuya, J. Croda, C. A. L. Cardoso, A. C. D. F. Monreal, A. C. M. B. Antoniolli, A. L. Cunha-Laura, et al. 2018. The safe use of *Doliocarpus dentatus* in the gestational period: Absence of changes in maternal reproductive performance, embryo-fetal development and DNA integrity. *J. Ethnopharmacol.* 217:1–6. doi:10.1016/j.jep.2018.01.034.

Kan, P. C., D. W. Barnett, and I. D. Douglas. 2019. Herbal medicines and pregnancy; a narrative review and anaesthetic considerations. *Anaesth. Intensive Care* 47 (3):226–34. doi:10.1177/0310057X19845786.

Kassuya, R. M., E. Santos, F. H. Bosso, T. F. Pedroso, J. V. N. Marinho, M. J. Salvador, A. Gasparotto-Junior, and A. Gasparotto Junior. 2021. Anti-inflammatory properties of ethanolic extract and 2 ″-O-β-D-glucopyranosyl-vitexin obtained from *Alternanthera tenella colla* whole plant. *Inflammation* 44 (4):1540–52. doi:10.1007/s10753-021-01438-7.

Khalki, L., S. B. M'Hamed, M. Bennis, A. Chait, and Z. Sokar. 2010. Evaluation of the developmental toxicity of the aqueous extract from Trigonella foenum-graecum (L.) in mice. *J. Ethnopharmacol* 131:321–25. doi:10.1016/j.jep.2010.06.033.

Khandker, S. S., M. Alam, F. Uddin, U. M. Shapla, N. Lubna, T. A. Mazumder, M. S. Hossen, M. Mondal, M. I. Khalil, and N. Karim. 2022. Subchronic toxicity study of *Alternanthera philoxeroides* in Swiss albino mice having antioxidant and anticoagulant activities. *J. Toxicol.* 2022:1–12. doi:10.1155/2022/8152820.

Kimmel, C. A., and J. G. Wilson. 1973. Skeletal deviations in rats: Malformations or variations? *Teratology* 8 (3):309–15. doi:10.1002/tera.1420080311.

Koolen, H. H., E. M. Pral, S. C. Alfieri, J. V. Marinho, A. F. Serain, A. J. Hernández-Tasco, N. L. Andreazza, and M. J. Salvador. 2017. Antiprotozoal and antioxidant alkaloids from *Alternanthera littoralis*. *Phytochemistry* 134:106–13. doi:10.1016/j.phytochem.2016.11.008.

Luna-Gómez, L. S., I. Santiago-Roque, Y. A. Gheno-Heredia, A. A. Corona-Morales, and V. E. Bolado-García. 2020. Teratogenic effects of *Bocconia frutescens* L. *J. Dev. Orig. Health Dis.* 11 (4):415–18. doi:10.1017/S2040174419000461.

Macedo, E. V. 2009. Gemal AL A produção de fitomedicamentos e a Política Nacional de Plantas Medicinais e Fitoterápicos. *Rev. Bras. Fazenda* 90:290–97.

Maciel, M. D., L. C. L. Inocencio, M. S. Rechsteiner, B. C. Jorge, P. S. Balin, R. M. Kassuya, S. C. Heredia-Vieira, C. A. L. Cardoso, M. C. Vieira, C. A. L. Kassuya, et al. 2019. Effects of exposure to ethanolic extract from *Achyrocline satureioides* (Lam.) D.C. flowers on reproductive and developmental parameters in Wistar rats.

J. Toxicol. Environ. Health Part A 82 (5):321–30. doi:10.1080/15287394.2019.1593904.

Manson, J. M., and Y. J. Kang. 1994. Test methods for assessing female reproductive and developmental toxicology. In *Principles and Methods of Toxicology*, ed. A. W. Hayes, pp. 679–89. 3rd ed. New York: Raven Press.

Marmitt, D. J., S. Bitencourt, G. R. da Silva, C. Rempel, and M. I. Goettert. 2021. Traditional plants with antioxidant properties in clinical trials—A systematic review. *Phytother. Res.* 35(10):5647–67. doi:10.1002/ptr.7202. Epub 2021 Jun 24. PMID: 34165846.

Moraes-Souza, R. Q., T. S. Soares, N. O. L. Carmo, D. C. Damasceno, K. E. Campos, and G. T. Volpato. 2017. Adverse effects of *Croton urucurana* B. exposure during rat pregnancy. *J. Ethnopharmacol.* 199:328–33. doi:10.1016/j.jep.2016.10.061.

Navarro, S. D., A. Beatriz, A. Meza, J. R. Pesarini, R. D. A. S. Gomes, C. B. Karaziack, A. L. Cunha-Laura, A. C. Monreal, W. Romão, V. L. Júnior, et al. 2014. A new synthetic resorcinolic lipid 3-heptyl-3,4,6-trimethoxy-3H-isobenzofuran-1-one: Evaluation of toxicology and ability to potentiate the mutagenic and apoptotic effects of cyclophosphamide. *Eur. J. Med. Chem.* 75:132–42. doi:10.1016/j.ejmech.2014.01.057.

Navarro, S. D., L. R. Pessatto, A. Meza, E. J. T. Oliveira, S. A. Auharek, L. C. Vilela, D. P. Lima, R. B. Azevedo, C. A. L. Kassuya, O. I. A. Cáceres, et al. 2018. Resorcinolic lipid 3-heptyl-3,4,6-trimethoxy-3H-isobenzofuran-1-one is a strategy for melanoma treatment. *Life Sci.* 15:300–12. doi:10.1016/j.lfs.2018.08.022.

Nunes, R. C. S., S. C. D. Neves, F. R. Salustriano, M. L. B. Vilela, V. A. D. Nascimento, K. Arunachalam, R. D. S. Gomes, C. A. L. Kassuya, J. D. S. Mota, and R. J. Oliveira. 2023. The ethanolic extract of *Piper glabratum* Kunth is teratogenic and interferes with the ossification process of Swiss mice fetuses. *Pharmaceuticals.* 16 (3):430. doi:10.3390/ph16030430. PMID: 36986529; PMCID: PMC10051201.

OECD. 2018. Test No. 414: Prenatal developmental toxicity study. Accessed on 27 May 2023. https://www.oecd.org/env/ehs/testing/E414_2001.PDF

Oliveira, R. J., T. Y. N. Kanno, M. J. S. Salles, O. A. C. S. Lourenã, L. R. Ribeiro, G. A. Freira, H. J. O. Matiazi, M. S. Mantovani, and A. F. Silva. 2009. Effects of the polysaccharide β-glucan on clastogenicity and teratogenicity caused by acute exposure to cyclophosphamide in mice. *Regul. Toxicol. Pharmacol.* 53 (3):164–73. doi:10.1016/j.yrtph.2008.12.007.

Oliveira, R. J., M. S. Mantovani, J. R. Pesarini, M. O. Mauro, A. F. Silva, T. R. Souza, and L. R. Ribeiro. 2015a. 6-Dimethylaminopurine and cyclohexamide are mutagenic and alter reproductive performance and intrauterine development *in vivo*. *Genet. Mol. Res.* 14 (1):834–49. doi:10.4238/2015.February.2.8.

Oliveira, R. J., S. D. Navarro, D. P. Lima, A. Meza, J. R. Pesarini, R. S. Gomes, C. B. Karaziack, M. M. Oliveira, A. L. Cunha-Laura, A. C. D. Monreal, et al. 2015b. A novel cytosporone 3-Heptyl-4,6-dihydroxy-3H-isobenzofuran-1-one: Synthesis; toxicological, apoptotic and immunomodulatory properties; and potentiation of mutagenic damage. *BMC Cancer.* 15(1):1–15. doi:10.1186/s12885-015-1532-2.

Oliveira, R. J., F. P. A. N. Pereira, I. O. M. F. D. Silveira, R. V. Lima, C. R. Berno, J. R. Pesarini, A. C. M. B. Antoniolli-Silva, A. C. D. Monreal, B. Adilson, D. P. Lima, et al. 2019. Assessment of the toxicogenic effects and cell death potential of the ester (Z)-methyl4-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino)-4-oxobut-2-anoate in combination with cisplatin, cyclophosphamide and doxorubicin. *Genet. Mol. Res.* 42(2):399–410. doi:10.1590/1678-4685-GMB-2017-0279.

Oliveira, E. J. T., L. R. Pessatto, R. O. N. Freitas, B. I. Pelizaro, A. P. M. Rabacow, J. M. Vani, A. C. D. Monreal, M. S. Mantovani, R. B. Azevedo, A. C. M. B. Antoniolli-Silva, et al. 2018. New Bis copper complex (Z)-4-((4-chlorophenyl)amino)-4-oxobut-2-enoyl)oxy): Cytotoxicity in 4T1 cells and their toxicogenic potential in Swiss mice. *Toxicol. App. l Pharmacol* 356:127–38. doi:10.1016/j.taap.2018.08.004.

Oliveira, R. J., N. C. L. Santos, J. R. Pesarini, B. C. Oliveira, C. R. Berno, F. H. S. Araújo, I. O. M. F. Silveira, R. O. Nascimento, A. C. M. B. Antoniolli-Silva, A. C. D. Monreal, et al. 2018. Assessment of genetic integrity, splenic phagocytosis and cell death potential of Z)-4-((1,5-dimethyl-3-oxo-2-phenyl-2,3dihydro-1H-pyrazol-4-yl) amino)-4-oxobut-2-enoic acid and its effect when combined with commercial chemotherapeutics. *Genet. Mol. Biol.* 41(1):154–66. doi:10.1590/1678-4685-GMB-2017-0091.

Ortiz, H. C., S. C. Das Neves, C. A. L. Kassuya, H. R. S. Coelho, A. C. F. Martins, M. L. B. Vilela, V. A. Do Nascimento, A. Karuppusamy, M. É. A. Stefanello, R. J. Oliveira, et al. 2023. The ethanolic extract of *Salvia lachnostachys* Benth is not maternotoxic, does not alter reproductive performance, but has teratogenic potential. *BMC Complement Med. Ther* 23 (1):145. doi:10.1186/s12906-023-03953-6.

Paula, V., G. L. L. Cruz, L. B. Sena, T. B. Gratao, T. S. Soares, R. Q. Damasceno, G. T. Volpato, and G. T. Volpato. 2020. Maternal-fetal reprecussions of *Phyllanthus nirini L.* treatment during rat pregnancy. *J. Ethanopharmacol.* 254:112728. doi:10.1016/j.jep.2020.112728.

Pessatto, L. R., S. A. Auharek, C. A. Gonçalves, N. David, A. C. Monreal, C. A. Kassuya, A. C. Antoniolli-Silva, M. É. Stefanello, and R. J. Oliveira. 2017. Effects of dichloromethane and butanol fractions of *Gochnatia polymorpha floccosa* in maternal reproductive outcome, embryo-fetal development and DNA integrity in mice. *J. Ethnopharmacol.* 200:205–08. doi:10.1016/j.jep.2017.02.042.

Roman, S., A. Dambros, A. Rosset, M. Pistore, A. Schimid, and R. Cansian. 2014. Toxicidade materna e reprodutiva de

extrato hidroalcoólico de Sonchus oleraceus L. em ratos wistar. *Rev. Perspect.* 38:69–77.

Salustriano, F. R., A. C. D. Monreal, S. C. Das Neves, G. M. de Oliveira, D. D. M. de Oliveira, M. L. B. Vilela, V. A. Do Nascimento, A. C. F. Martins, B. Saroja, A. Karuppusamy, et al. 2022. The ethanolic extract of *Gomphrena celosioides* Mart. does not alter reproductive performance or embryo-fetal development, nor does it cause chromosomal damage. *Pharmaceutics* 14 (11):2369. doi:10.3390/pharmaceutics14112369.

Salvador, M. J., and D. A. Dias. 2004. Flavone C-glycosides from *Alternanthera maritima* (Mart.) St. Hil. (Amaranthaceae). *Biochem. Syst. Ecol.* 32 (1):107–10. doi:10.1016/s0305-1978(03)00180-7.

Salvador, M. J., P. S. Pereira, S. C. França, R. C. Candido, I. Y. Ito, and D. A. Dias. 2004a. Comparative study of antibacterial and antifungal activity of callus culture and adult plants extracts from *Alternanthera maritima* (Amaranthaceae). *Braz. J. Microbiol.* 35 (1–2):131–36. doi:10.1590/S1517-83822004000100022.

Salvador, M. J., O. L. A. D. Zucchi, R. C. Candido, I. Y. Ito, and D. A. Dias. 2004b. In vitro antimicrobial activity of crude extracts and isolated constituents of *Alternanthera maritima*. *Pharm Biol.* 42 (2):138–48. doi:10.1080/13880200490511954.

Samudrala, P. K., B. B. Augustine, E. R. Kasala, L. N. Bodduluru, C. Barua, and M. Lahkar. 2015. Evaluation of antitumor activity and antioxidant status of *Alternanthera brasiliana* against Ehrlich ascites carcinoma in Swiss albino mice. *Pharmacognosy Res.* 7 (1):66–73. doi:10.4103/0974-8490.147211.

Santos Radai, J. A., F. Silva Graciani, C. R. Berno, M. M. Leitao, E. P. Dos Santos, R. J. Oliveira, and C. A. L. Kassuya. 2018. Genetic toxicological assessment and anti-arthritic effects of an ethanolic extract obtained from *Saliva lachnostachys* Bentha leaves in mice. *Regul. Toxicol. Pharmacol.* 92:239–44. doi:10.1016/j.yrtph.2017.12.006.

Sartori, A. S., C. B. Hollenbach, L. H. Jardim, P. Silva, F. B. Mello, and J. R. B. Mello. 2020. Avaliação da toxicidade pré-natal: estudo de teratogenicidade do inseticida piriproxifeno em ratos Wistar [Assessment of prenatal toxicity: teratogenicity study of the insecticide pyriproxyfen in Wistar rats]. *Arq. Bras. Med. Vet. Zootec* 72 (3):719–28. doi:10.1590/1678-4162-11251.

Schneider, B. U. C., A. Meza, A. Beatriz, J. R. Pesarini, P. C. D. Carvalho, M. D. O. Mauro, C. B. Karaziack, A. L. Cunha-Laura, A. C. D. Monreal, R. Matuo, et al. 2016. Cardanol: Toxicogenetic assessment and its effects when combined with cyclophosphamide. *Genet. Mol. Biol.* 39(2):279–89. doi:10.1590/1678-4685-GMB-2015-0170.

Senna, L. R. 2020. Alternanthera in Flora do Brasil. Jardim Botânico do Rio de Janeiro. Accessed on 17 June 2023. https://floradobrasil2020.jbrj.gov.br/FB15401.

Senna, L. R., N. B. N. Monção, R. R. S. de Farias, A. M. D. G. L. Citó, M. H. Chaves, M. R. S. D. Araújo, D. J. B. Lima, C. Pessoa, A. D. Lima, and E. C. D. C. Araújo. 2020. Toxicological, chemopreventive and cytotoxic potentialities of rare vegetal species and supporting findings for the Brazilian Unified Health System (SUS). *J. Toxicol. Environ. Health Part A* 83 (13–14):525–45. doi:10.1080/15287394.2020.1780658.

Singla, R. K., V. Dhir, R. Madaan, D. Kumar, S. S. Bola, M. Bansal, S. Kumar, A. K. Dubey, S. Singla, and B. Shen. 2022. The genus Alternanthera: Phytochemical and ethnopharmacological perspectives. *Front Pharmacol.* 13:769111. doi:10.3389/fphar.2022.769111.

Soares, T. S., D. C. Damasceno, W. D. E. G. Kempinas, F. M. Resende, M. A. C. Santos, C. A. Hiruma-Lima, and G. T. Volpato. 2015. Effect of *Himatanthus sucuuba* in maternal reproductive outcome and fetal anomaly frequency in rats. *Birth Defects Res. B Dev. Reprod. Toxicol.* 104 (5):190–95. doi:10.1002/bdrb.21152.

Soulimane-Mokhtari, N. A., B. Guermouche, A. Yessoufou, M. Saker, K. Moutairou, A. Hichami, H. Merzouk, and N. A. Khan. 2005. Modulation of lipid metabolism byn–3 polyunsaturated fatty acids in gestational diabetic rats and their macrosomic offspring. *Clin. Sci.* 109 (3):287–95. doi:10.1042/CS20050028.

Souza, J. G., R. T. Tomei, A. Kanashiro, L. M. Kabeya, A. E. Azzolini, D. A. Dias, M. J. Salvador, and Y. M. Lucisano-Valim. 2007. Ethanolic crude extract and flavonoids isolated from *Alternanthera maritima*: Neutrophil chemiluminescence inhibition and free radical scavenging activity. *Z. Naturforsch C.J. Bioscience* 62 (5–6):339–47. doi:10.1515/znc-2007-5-604.

Staples, R. E., and V. L. Schnell. 1964. Refinements in rapid clearing technic in the koh-alizarin red s method for fetal bone. *Stain Technol.* 39:61–63.

Stein, J., B. C. Jorge, A. C. Casali Reis, J. A. Santos Radai, S. da Silva Moreira, T. L. Fraga, J. da Silva Mota, R. J. Oliveira, C. A. L. Kassuya, and A. C. Arena. 2022. Evaluation of the safety of ethanolic extract from Piper amalago L. (Piperaceae) leaves in vivo: Subacute toxicity and genotoxicity studies. *Regul. Toxicol. Pharmacol.* 129:105118. doi:10.1016/j.yrtph.2022.105118.

Taylor, P. 1986. *Practical teratology*. London: Academic Press.

Teng, C., J. Jia, Z. Wang, V. K. Sharma, and B. Yan. 2019. Size-dependent maternal-fetal transfer and fetal developmental toxicity of ZnO nanoparticles after oral exposures in pregnant mice. *Ecotoxicol. Environ. Saf* 182:109439. doi:10.1016/j.ecoenv.2019.109439.

Tyl, R. W., N. Chernoff, and J. M. Rogers. 2007. Altered axial skeletal development. *Birth Defects Res. B Dev. Reprod. Toxicol.* 80:451–72. doi:10.1002/bdrb.20134.

Vani, J. M., M. F. T. D. Monreal, S. A. Auharek, A. L. Cunha-Laura, E. J. de Arruda, A. R. Limsa, C. M. da Silva, A. C. M. B. Antonolli-Silva, D. P. Lima, A. Beatriz, et al. 2018. The mixture of cashew nut shell liquid and castor oil

results in an efficient larvicide against *Aedes aegyppti* that does not alter embryo-fetal development, reproductive performance or DNA integrity. *PLos One* 13 (3):e0193509. doi:10.1371/journal.pone.0193509.

Volpato, G. T., L. A. Francia-Farje, D. C. Damasceno, R. V. Oliveira, C. A. Hiruma-Lima, and W. G. Kempinas. 2015. Effect of essential oil from *Citrus aurantium* in maternal reproductive outcome and fetal anomaly frequency in rats. *An. Acad. Bras. Cienc.* 87 (1):407–15. doi:10.1590/0001-3765201520140354.

Wilson, J. G. 1965. Methods for administering agents and detecting malformation in experimental animal. In *Teratology: Principles and techniques*, ed. J. C. Wilson and J. Warkany, pp. 262–77. Chicago: University of Chicago Press.

Wise, L. D., S. L. Beck, D. Beltrame, B. K. Beyer, I. Chahoud, R. L. Clark, R. Clark, A. M. Druga, M. H. Feuston, P. Guittin, et al. 1997. Terminology of developmental abnormalities in common laboratory mammals version 1. *Teratology*. 55(4):249–92. doi:10.1002/(SICI)1096-9926(199704)55:4<249:AID-TERA5>3.0.CO;2-W.

Anexo 3 – Manuscrito 2 – Artigo publicado na *BMC Complementary Medicine and Therapies*

Ortiz *et al.*
BMC Complementary Medicine and Therapies (2023) 23:145
https://doi.org/10.1186/s12906-023-03953-6

BMC Complementary Medicine and Therapies

RESEARCH Open Access

The ethanolic extract of *Salvia lachnostachys* Benth is not maternotoxic, does not alter reproductive performance, but has teratogenic potential

Hudman Cunha Ortiz[1], Silvia Cordeiro das Neves[1,2], Cândida Aparecida Leite Kassuya[3], Henrique Rodrigues Scherer Coelho[1,2], Allana C. F. Martins[4], Marcelo Luiz Brandão Vilela[5], Valter Aragão do Nascimento[2,6], Arunachalam Karuppusamy[1,2], Maria Élida Alves Stefanello[7], Rodrigo Juliano Oliveira[1,2*] and Roberto da Silva Gomes[4*]

Abstract

Salvia lachnostachys Benth is native to Brazil and has anti-inflammatory, anti-arthritic, cytotoxic, antitumor, and antihyperalgesic activities. The population, including pregnant women, consume this plant to treat pain, inflammation, flu, spasms, insomnia, and depression, mainly. There are no safety reports on the use of this plant during pregnancy. The present study aimed to evaluate the effects of *S. lachnostachys* ethanolic extract (EESl) on reproductive performance, embryofetal development, and DNA integrity of pregnant female mice. Pregnant females were randomly divided into three experimental groups ($n = 10$): The Control group was treated with a vehicle, and treatment groups were administered with EESl at 100 and 1000 mg/kg, respectively. Treatment occurred by gavage throughout the gestational period until day 18. Afterward, reproductive performance, embryofetal development, and DNA integrity parameters were evaluated. The results indicated that EESl did not alter any reproductive performance parameters. However, it changed embryofetal outcome through reduced placental weight (EESl 100 mg/kg), decreased fetal weight (EESl 100 and 1000 mg/kg), and increased frequency of small for gestational age fetuses (EESl 1000 mg/kg). In addition, EES1 increased the frequency of external, visceral, and skeletal malformations. Because of the above, it is considered that EESl is not maternotoxic, does not alter reproductive performance, but does alter embryofetal development. Its use in the gestational period is not indicated due to its teratogenic potential.

Keywords Teratogenesis, Malformation, Genotoxicity, Medicinal plants

*Correspondence:
Rodrigo Juliano Oliveira
rodrigo.oliveira@ufms.br
Roberto da Silva Gomes
roberto.gomes@ndsu.edu
Full list of author information is available at the end of the article

Introduction

The species, *Salvia lachnostachys* Benth, is an endemic perennial herb of Brazil, occurring wild only in the South and Southeast regions of the country [1]. It is considered an ornamental species [2] with the possibility of being distributed to other parts of the country. This plant is known as "melissa" and has been used for treating spasms, flu, and insomnia in popular medicine [3].

The essential oil from the flowers and leaves of *S. lachnostachys* has saturated aliphatic compounds and a sesquiterpene fraction [4]. Diterpenes, triterpenes, and rosmarinic acid were isolated from the ethanolic extract of the leaves [5, 6]. The chromatographic profile of the ethanolic extract also was obtained by HPLC [7].

The ethanolic extract of *S. lachnostachys* has already been described with anti-inflammatory, cytotoxic, antiarthritic, analgesic, antidepressant, and antinociceptive activities. Fructiculin A (a diterpene isolated or derived from *S. lachnostachys*) is believed to be one of the compounds that assist in these properties, and is cited as anti-inflammatory, antihyperalgesic, antidepressant and antinociceptive [5, 8-10]. Furthermore, in the literature, *S. lachnostachys* extract was characterized as chemopreventive and antitumor in Ehrlich models [7] and fructiculin A as antineoplastic in vitro and in vivo models [11]. Four diterpenes (fructiculine A, demethylfruticuline A, fruticuline B, and demethylfruticuline B) showed antioxidant activity [6].

In a recent publication, we reported the characterization of *S. lachnostachys* extract. We identified the presence of three new diterpenoids: demethylfruticuline B, 20-hydroxyfruticuline B, and 6-hydroxyisofruticuline A, isolated from the leaves of *S. lachnostachys*. We also reported the presence of five known compounds: fruticuline B, fruticuline A, demethylfruticuline A, heterobetulinic acid, and maslinic acid [6].

Considering the above, it appears that *S. lachnostachys* extract can primarily be used to treat inflammatory processes, pain, and depression. Pregnant women are affected by all these conditions. Anti-inflammatory and analgesic drugs are widely used during pregnancy, as pointed out by studies conducted by Fontoura et al. [12] and Lima et al. [13]. Depression is also common during pregnancy and the use of antidepressants in this period has increased significantly in the last 20 years [14]. As an example, we can mention duloxetine, which can lead to an increase in the number of abortions [15], and selective serotonin reuptake inhibitors, which increase the risk of congenital heart diseases, neonatal maladaptive syndrome, or persistent pulmonary hypertension. This is because serotonin is essential for developing all embryonic cells and organogenesis [14, 16-18]. In view of the above, we consider that allopathic drugs can harm embryonic and fetal development. In this way, many pregnant women seek natural alternatives for the treatment of pain, inflammation, flu, spasms, insomnia and depression, mainly through the use of *S. lachnostachys* tea. There are no eport regarding the safety on the use of this plant during pregnancy. However, reports of people who are using this tea are growing. Thus, there is a need to evaluate the safety of using this product during the gestational period, since in locations distant from large centers, where populations make use of teas for the primary treatment of these conditions, exposure of pregnant women to *S. lachnostachys* tea would not be uncommon. Thus, the present research aimed to evaluate the effects of the ethanolic extract of *S. lachnostachys* (EESl) on reproductive performance, embryofetal development, and DNA integrity of pregnant female mice.

Materials and methods

Plant material and extract preparation

Leaves of *S. lachnostachys* were collected in Curitiba, Paraná, Brazil (25°30′44,6"S, 49°18′7,13"W) from a natural population in May 2010. E. P. Santos identified the plant, and a voucher specimen was deposited in the Herbarium of the Universidade Federal do Paraná (UPCB 85,285). The collection followed the Brazilian National guidelines. The approved code registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN/Brazil) is A19F875. Leaves were dried at 40° in an oven for seven days. Then, the dried material (415.3 g) was powdered in a mill, and extracted with hexane followed by ethanol (3 × 2 L, for each solvent). The solvents were removed under reduced pressure, yielding the respective extracts in hexane (2.7%) and ethanol (EESl, 11.5%). The preliminary extraction with hexane removes long-chain aliphatic compounds ("waxes") that are abundant in leaves, in order to facilitate the isolation of secondary metabolites. Waxes do not present biological activities. The ethanolic extract was maintened in a freezer (at -4° C) until the tests were performed. Thin layer chromatography (TLC) analyses showed no alteration after the time of storage. The ethanol extract has a high content of rosmarinic acid [7], which is an antioxidant and contribute for its stability. Pharmacological tests were performed with crude ethanolic extract of S. lachnostachys leaves (EESl) diluted in saline.

Animals

The animals were provided by the Central Animal Facility of the Federal University of Mato Grosso do Sul in a total of 60 mice (*Mus musculus*) of the *Swiss* strain of both sexes at reproductive age: 30 females with an average weight of 30 g and 15 males with an average weight

of 35 g. All animals underwent a 7-day adaptation period and were placed in mini-isolators (Alesco® ventilated rack) lined with sawdust (*Pinus* sp.). The males were isolated, and the females were in pairs. The animals were kept under temperature control (22 ± 2 °C), light by photoperiod (12 h light/ 12 h dark), relative humidity of 55 ± 10%, and access to feed and water ad libitum. The research was conducted according to the Universal Declaration of Animal Rights protocols and with approval from the Ethics Committee on Animal Use (CEUA) of the Federal University of Mato Grosso do Sul (UFMS) under opinion number 965/2018.

Experimental design

The animals were mated during the night at a ratio of 1 male: to 2 females. Females were not subjected to estrous cycle synchronization. All females were placed in mini-isolators in contact with males every day at 6 pm and were removed the following day at 6 am for pregnancy assessment. The detection of vaginal plugs in females determined pregnancy occurrence, and this day was considered day zero of gestation [19-21]. Pregnant females did not return to the mini-isolators with the males. However, non-pregnant females went through new matings until all females became pregnant (this period did not exceed 2 weeks [21]. Pregnant females were randomly divided into three experimental groups ($n = 10$): Control Group—animals received 0.1 mL/10 g body weight (b.w.) of vehicle (in saline – 0.9% NaCl) by gavage (p.o.) throughout gestation (gestational day 1 to 18); EESl Groups—animals received EESl by gavage at doses of 100 and 1000 mg/kg (b.w., p.o.) during gestation. Such doses were chosen based on the study by Radai et al. [10] and from the recommendations of guidelines in the field [21-23]. According to Radai et al. [10], the dose of 50 and 100 mg/kg has anti-arthritic and anti-inflammatory effects. Thus, it was decided to test the highest effective dose and a dose ten times greater, as recommended by the literature in the area, to ensure the safety of use by pregnant women. According to Wilson and Warkanny (1965) the pre-implantation period is from the 1st to the 5th gestational day [24]. Thus, we also wanted to evaluate possible pre-implantation losses, the treatments were started on the 1st gestational day.

Biological assays

Peripheral blood was collected by tail vein puncture on the 16th, 17th, and 18th gestational days (g.d.). After the last blood collection, the females were euthanized by cervical dislocation, followed by laparotomy, omphalectomy, and hysterectomy for collection, weighing, and proper storage of organs, fetuses, and placentas.

The fetuses underwent systematic analysis for external malformations and sexing and were then randomly distributed into two subgroups. The first subgroup was destined for visceral analysis. It was fixed in Bodians' solution, composed of distilled water (142 mL), acetic acid (50 mL), formaldehyde (50 mL), and 95% alcohol (758 mL), for at least seven days. They were then submitted to incision/microdissection with strategic cuts proposed by Barrow and Taylor [25] for the study of the thorax and abdomen and by Wilson [26], modified by Oliveira et al. [19] for the study of the head. The classification of visceral changes was based on the experiments described by Taylor [27], Manson and Kang [28], Wise et al. [29], Damasceno et al. [30], and Oliveira et al. [19]. Hydrocephalus and hydronephrosis were classified as mild, moderate or severe according to the pattern shown in Figs. 1 and 2. The second subgroup, intended for skeletal analysis, was fixed in absolute acetone for at least seven days. After removal of the viscera, the fetuses were immersed in KOH (0.8%) and stained with Alizarin Red during the diaphanization process, as proposed by Straples and Schenell [31] with modifications by Oliveira et al. [20]. After the fetuses were stained, the KOH solution was replaced with the bleaching solution (1 L glycerin: 1 L ethyl alcohol: 0.5 L benzyl alcohol), which was changed every 24 h for five days. The fetal viscera and skeletons analyses were performed under a stereomicroscopic magnifying glass (Nikon®—SMZ 745 T) at 1.6 × magnification.

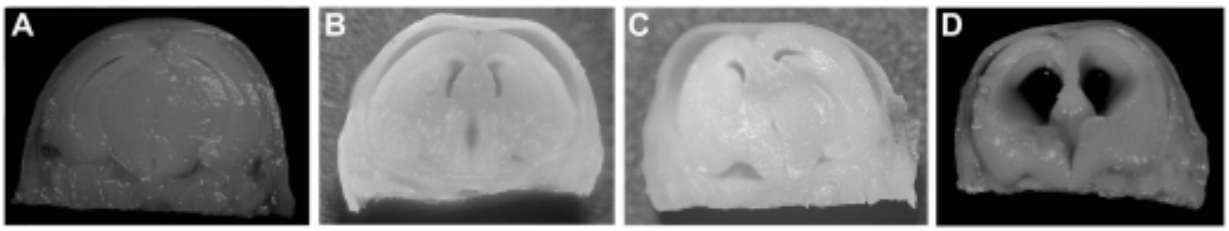


Fig. 1 Photomicrograph of brain microdissections. **A** Normal brain (aspect of the lateral ventricles and the third and fourth ventricles). **B**-**D** Hydrocephalus—excessive cerebrospinal fluid within the sckull: **B**—mild grade; **C** – moderate grade and **D** – severe grade

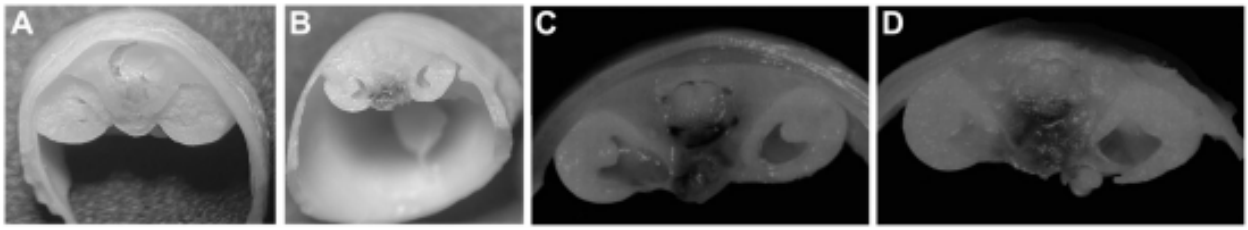


Fig. 2 Photomicrograph of rim microdissections. **A** Rim (aspect of renal papilla and renal pelvis). **B**-**D** Hydronephrosis – marked dilatation of the renal pelvis and calyces secondary to obstruction of urine flow, usually combined with destruction of the renal parenchyma: **B** – gravity; **C** – moderate grain and **D** – severe grain

Evaluation of biological parameters

The biological parameters were calculated from the records of initial weight (females weighed at day zero), final weight (females weighed at 18 g.d.), weight gain (final weight—initial weight), uterus weight, net weight gain (weight gain—uterus weight), absolute and relative weights of heart, lung, spleen, kidneys, and liver.

Reproductive performance and embryofetal development

The absolute number of implantation (number of live fetuses + number of dead fetuses + number of resorptions), resorption of embryos or fetuses in the uterus, live and dead fetuses was registered. The fetal and placental weigth (g) were mensured on a precision scale. Based on these data, we obtained: fetal viability (number of live fetuses × 100 / number of implantations), post-implantation loss rate [(number of implantations—number of resorptions) × 100 / number of implantations], resorption rate (number of resorptions X 100 / number of implantations), placental index (placental weight / fetal weight), placental efficiency (fetal weight / placental weight) and sex ratio (number of male fetuses/numbers of female fetuses).

The classification of fetal weight according to gestational age (CFWGA). After collecting fetuses from the uterine horns, they were weighed, and according to the mean ± 1.7 × standard deviation (SD) of the body weights obtained in the control group, they were classified as adequate for gestational age (AGA), small for gestational age (SGA) and large for gestational age (LGA).

Evaluation of micronucleus in peripheral blood

Micronucleus testing in peripheral blood was performed according to the method described by Hayashi et al. [32] and modified by Oliveira et al. [20]. Peripheral blood, collected by tail vein puncture (about 20µL), was deposited on a slide prepared with Acridine Orange (1 mg/mL). The blood was covered with a coverslip, and the material was stored in a freezer (-20 °C) for a minimum of seven days. A total of 2,000 reticulocytes were examined per animal using an EVOS M7000 microscope (Zeiss®) at 40 × magnification with an excitation filter of 470 nm ± 22 and an emission filter of 525 nm ± 50. The frequency of micronuclei was calculated by the sum of micronuclei observed in 2,000 reticulocytes/animal divided by the sample n ($n = 10$ animals/group).

Statistical analysis

Data distribution was evaluated using the Kolmogorov–Smirnov test. The normal distribution data for the one-way ANOVA test with Tukey post-test was used. For other data, the Kruskal–Wallis test with the Dunn post-test was used. For frequency comparisons, the Chi-square test was used. The analyses were performed using the GraphPad Instat® program (Version 3.06, 2003). Data were presented as mean ± standard error of the mean or mean ± standard deviation, and the established significance level was $p < 0.05$.

Results

The initial weight, final weight, uterine weight, net weight gain, the absolute weight of the heart, lung, spleen, and kidneys, and the relative weight of the heart, lung, spleen, kidneys, and liver showed no statistically significant differences between the experimental groups ($p > 0.05$). However, there was a reduction ($p < 0.05$) in weight gain and a reduction in absolute liver weight by EESl at a dose of 100 mg/kg when compared to the control group (Table 1).

Treatment with EESl, at both doses, caused no statistically significant changes ($p < 0.05$) in the number of implants, live fetuses, dead fetuses, the average number of fetuses per litter, fetal viability, post-implantation loss rate, resorption, resorption rate, and sex ratio (Table 2).

A reduction ($p < 0.05$) in placental weight was observed in the EESl at a dose of 100 mg/kg and fetal weight reduction in the treatment of EESl at doses 100 and 1000 mg/kg groups. There was also an increase ($p < 0.05$) in the frequency of small for gestational age (SGA) fetuses in the EES1 1000 mg/kg group. The placental index did not differ from the control, but there was a reduction in the

Table 1 Biological parameters, absolute weight, and relative weight of organs of females treated with the ethanolic extract of *S. lachnostachys* (EESl)

Biological parameters					
Experimental groups (mg/kg)	**Initial weight[2]**	**Final weight[1]**	**Weight gain[1]**	**Uterus Weight[1]**	**Net Weight gain[1]**
Absolute Weight (g)					
Control	29.26 ± 0.57[a]	53.94 ± 0.95[a]	24.67 ± 0.84[a]	19.71 ± 0.65[a]	4.97 ± 0.64[a]
EESl 100	29.46 ± 0.38[a]	49.66 ± 1.23[a]	20.20 ± 1.07[b]	16.97 ± 0.85[a]	3.23 ± 0.43[a]
EESl 1000	29.15 ± 0.73[a]	51.69 ± 1.66[a]	22.53 ± 1.36[ab]	18.40 ± 1.05[a]	4.13 ± 0.49[a]
Absolute Weight of organs (g)					
	Heart[2]	Lungs[1]	Spleen[2]	Kidney[2]	Liver[1]
Control	0.18 ± 0.01[a]	0.23 ± 0.01[a]	0.15 ± 0.01[a]	0.40 ± 0.02[a]	2.49 ± 0.05[a]
EESl 100	0.16 ± 0.00[a]	0.21 ± 0.01[a]	0.13 ± 0.01[a]	0.36 ± 0.02[a]	2.15 ± 0.08[b]
EESl 1000	0.17 ± 0.01[a]	0.23 ± 0.01[a]	0.14 ± 0.00[a]	0.38 ± 0.01[a]	2.42 ± 0.11[ab]
Relative Weight of organs					
	Heart[2]	Lungs[1]	Spleen[2]	Kidney[2]	Liver[1]
Control	0.003 ± 0.00[a]	0.004 ± 0.00[a]	0.003 ± 0.00[a]	0.01 ± 0.00[a]	0.05 ± 0.00[a]
EESl 100	0.003 ± 0.00[a]	0.004 ± 0.00[a]	0.003 ± 0.00[a]	0.01 ± 0.00[a]	0.04 ± 0.00[a]
EESl 1000	0.003 ± 0.00[a]	0.004 ± 0.00[a]	0.003 ± 0.00[a]	0.01 ± 0.00[a]	0.05 ± 0.00[a]

Legend: *g* grams. Equal letters indicate no statistically significant differences. Mean ± Standard error of the mean (1—Anova/Tukey's test; 2—Kruskal–Wallis/Dunn, $p > 0.05$)

Table 2 Reproductive parameters of females treated with the ethanolic extract of *S. lachnostachys* (EESl)

Reproductive performance			
Experimental Groups (mg/kg)			
Parameters	**Control**	**EESl 100**	**EESl 1000**
Implants[1]	13.50 ± 0.43[a]	12.30 ± 0.60[a]	13.89 ± 0.63[a]
Live Fetuses[1]	13.10 ± 0.41[a]	11.80 ± 0.61[a]	13.00 ± 0.71[a]
Dead Fetuses	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Average no. of fetus[1]	13.10 ± 0.41[a]	11.80 ± 0.61[a]	13.00 ± 0.71[a]
Fetal Viability[2]	97.12 ± 1.18[a]	96.02 ± 1.75[a]	93.42 ± 1.84[a]
PILR[2]	02.88 ± 1.18[a]	03.97 ± 1.75[a]	06.58 ± 1.84[a]
Reabsorption[2]	0.40 ± 0.24[a]	0.50 ± 0.45[a]	2.12 ± 0.44[a]
Reabsorption Rate[2]	04.55 ± 0.16[a]	09.12 ± 0.22[a]	0.89 ± 0.26[a]
Sexual Reason[1]	161.54 ± 28.77[a]	141.00 ± 22.51[a]	123.48 ± 15.16[a]

Legend: *PILR* post-implantation loss rate; Equal letters indicate no statistically significant differences. Mean ± Standard Error of the mean (1—Anova/Tukey's test; 2—Kruskal–Wallis/Dunn, $p > 0.05$)

Table 3 Parameters of embryofetal development and placental efficiency of females treated with the ethanolic extract of *S. lachnostachys* (EESl)

Embryo development and placental efficiency			
Experimental groups (mg/kg)			
Parameter	**Control**	**EESl 100**	**EESl 1000**
Placental Weight (g)[1]	0.0916 ± 0.00[a]	0.0816 ± 0.00[b]	0.0918 ± 0.01[a]
Placental Index[1]	0.0736 ± 0.00[ab]	0.0717 ± 0.00[a]	0.0776 ± 0.00[b]
Placental Efficiency[1]	14.3326 ± 0.29[ab]	14.9859 ± 0.36[a]	13.5319 ± 0.27[b]
Fetal Weight (g)[1]	1.2572 ± 0.01[a]	1.1584 ± 0.01[b]	1.1903 ± 0.01[c]
% SGA[2]	7.6923	16.2393	18.2608*
% AGA[2]	89.2307	83.7606	80.8695
%LGA[2]	3.0769	00.00	0.8695

Legend: *g* grams, *SGA* small for gestational age, *AGA* adequate for gestational age, *LGA* large for gestational age; Statistical test: 1 Anova/Tukey—Mean ± Standard error of the mean: different letters indicate statistically significant differences; 2 Chi-square test; *indicates statistically significant difference compared to the control group; pˆ0.05

treatment of ESSl ($p < 0.05$) at a dose of 100 mg/kg when compared to the amount of 1000 mg/kg (Table 3).

The external malformations observed were curved tail, posterior paw hyperflexion (uni and bilateral), posterior paw hyperextension (uni and bilateral), unilateral anterior paw hyperextension and hyperflexion, gastroschisis, hyperlordosis, scoliosis, hematoma, and nasal cavity obstruction. An increase ($p > 0.05$) in the frequency of curved tail and hyperlordosis was observed in the EESl 1000 group and scoliosis in the EESl 100 and EESl 1000 groups. The frequency of malformations and the percentage of fetuses with malformations increased ($p < 0.05$) in the treatment of EESl at doses 100 and 1000 mg/kg groups (Table 4).

Concerning visceral malformations, mild and moderate grades hydrocephalus (excessive cerebrospinal fluid

Table 4 External malformations found in the offspring of females treated with the ethanolic extract of *S. lachnostachys* (EESl)

External malformation			
Experimental groups (mg/kg)			
Parameters	**Control**	**EESl 100**	**EESl 1000**
Analyzed Fetuses	131	118	117
Normal Fetuses	116	86	73
Curved Tail	0	3	5*
UPPH	0	3	1
BPPH	2	0	0
UPHPH	5	11	13
BPHP	1	3	0
UAHP	2	0	1
UAHPH	1	0	0
Gastroschisis	1	0	0
Hyperlordosis	0	2	10*
Scoliosis	2	7*	7*
Hematoma	0	1	3
Nasal Septum Obstruction	1	0	0
Frequency of Malformation	15	30*	40*
%M.F	11.45%	25.42%*	34.19%*

Legend: *UPPH* Unilateral posterior hind paw hyperflexion, *BPPH* bilateral posterior hind paw hyperextension, *UPHPH* Unilateral posterior hind paw hyperextension, *BPHP* bilateral posterior of hyperextension of hind paw, *UAHP* unilateral anterior hyperextension of the hind paw, *UAHPH* Unilateral anterior hind paw hyperflexion; *% M.F.* the percentage of malformed fetuses

* Statistically significant difference (Statistical test: Chi-square; *indicates statistically significant difference compared to the Control group; $p < 0.05$)

Table 5 Visceral malformations found in the offspring of females treated with the ethanolic extract of S. lachnostachys (EESl)

Visceral Malformation			
Experimental groups (mg/kg)			
Parameters	**Control**	**EESl 100**	**EESl 1000**
Analyzed fetuses	55	53	53
Normal Fetuses	44	35	45
Brain—Hydrocephalus			
Mild Hydrocephalus	10	7	6
Moderate Hydrocephalus	1	2	0
Frequency of Malformations	11	9	6
% M.F	20	16.98	11.32
Urogenital Region—Hydronephrosis			
Mild Hydronephrosis	0	8*	1
Absence of Right Kidney	0	1	0
Frequency of Malformations	0	9*	1
% M.F	0	16.98*	1.88

Legend: % M. F—the percentage of fetuses with malformation

* Statistical difference (Statistical test: Chi-square; $p < 0.05$)

within the sckull), mild hydronephrosis (marked dilatation of renal pelvis anda calices secondary ti obstruction of urine flow, usually combined with destruction of the renal parenchyma), and absence of right kidney were identified. An increase ($p < 0.05$) in the occurrence of mild hydronephrosis was observed in the EES1 100 group, which determined an increase ($p < 0.05$) in the frequency of malformations and the percentage of fetuses with a malformation in the EESl 100 group compared to the control group (Table 5).

The skeletal malformations found were the absence and reduced number of anterior and posterior phalanges, reduced number of metacarpal and metatarsal bones, decreased ossification and absence of the last sternal center, lack of the sternum, reduced ossification of the nasal, parietal, interparietal, supraoccipital, volar, palate and frontal bones, and absence of the basiphoid and hamulus bones. There was an increase ($p < 0.05$) in the percentage of malformed fetuses in the treatment of EES1 at doses 100 and 1000 mg/kg when the sternum and skull were evaluated. There was also an increase ($p < 0.05$) in the frequency of malformation for both groups when the skull was assessed (Table 6).

The frequency of chromosomal damage did not vary among the different experimental groups, indicating that EESl does not alter the frequency of micronuclei ($p > 0.05$). There were also no significant variations between the various analysis times, meaning that EESl has no cumulative effect ($p > 0.05$) (Fig. 3).

Discussion

According to the literature, no data on maternal toxicity, reproductive performance, and embryofetal development were found for *S. lachnostachys*. This demonstrates the importance, uniqueness, and pioneering nature of this study. Our findings will contribute to the ethnopharmacological indication and safety of the use of this plant since there are few studies of this species that deal with the biology of the plant and its medicinal properties [7-11].

Our results show no signs of maternotoxicity, such as diarrhea, tremors, excessive salivation, convulsions, hypoactivity, ataxia, lethargy, tail curvature and hair raising [7]. However, for the EES1 100 group, a significant decrease in weight gain and absolute liver weight was observed. According to Teng et al. [33], weight gain and organ weight variations can indicate toxicity. However, these findings in the present study do not appear to be associated with maternotoxicity, as there was no reduction in net weight gain. However, altered embryonic development and placental efficiency are suggested.

Table 6 Skeletal malformations found in the offspring of females treated with the ethanolic extract of *S. lachnostachys* (EESI)

Skeletal Malformation				
Experimental groups (mg/kg)				
Parameters		**Control**	**EESI 100**	**EESI 1000**
Analyzed fetuses		45	44	55
Normal Fetuses		18	11	20
Members				
Anterior phalanges	Absence	0	2	0
	Reduced number	4	2	2
Posterior phalanges	Absence	0	2	0
	Reduced number	1	2	3
Freq.Malf		5	8	5
% M.F		11.11	18.18	9.10
MTC	Reduced number	0	1	0
MTT	Reduced number	0	1	0
Freq.Malf		0	2	0
%M.F		0	4.55	0
Sternum				
Sternum Bones	O.R. Last Center Sternal	19	22	33
	Absence of the Last Sternal Center	1	6	3
Absence		0	1	0
Freq.Malf		20	29	36
%M.F		44.44	65.90*	65.45*
Cranium				
Nasal	R.O	0	3	4
Parietal	R.O	0	3	3
Interparietal	R.O	0	3	3
Supraoccipital	R.O	0	1	3
Volmer	R.O	3	8	0
Palate	R.O	1	5	1
Basisphenoid	Absence	0	1	0
Hamate	Absence	0	1	0
Frontal	R.O	0	1	4
Freq.Malf		4	26*	18*
% M.F		8.89	59.09*	32.73*

Legend: *MTC* metacarpal, *MTT* metatarsal, *R.O* Reduced Ossification, *Freq.Malf* Frequency of Malformations, % F.M— the percentage of fetuses with malformation

* Statistical difference (Statistical test: Chi-square; $p < 0.05$)

The animals started the experiments with similar weights; even EES1 at a dose of 100 mg/kg had the highest average. However, during gestation, this group showed the lowest weight gain. This fact was not only attributed to the lower number of fetuses since the difference in fetus frequency between the two EES1 treated groups was only one fetus. Additionally, the average fetal weight of EES1 at a dose of 100 mg/kg was the lowest of all the groups. Thus, these two facts may contribute to understanding weight gain reduction.

Therefore, it is assumed that these animals had a lower biometric development (lower maternal weight gain) without the influence of the treatment, i.e., without maternotoxicity. In this context, the weight of the organs (of the liver) may vary according to the animal's size (large animals have heavier livers than small animals). This inference can be corroborated by the absence of a significant difference in the relative weight of this same organ. The relative weight corrects distortions of this type since it normalizes the data by making a ratio between the organ's weight and the animal's final weight. Thus, as there are no significant differences in the relative weight, it is inferred that such data is an isolated occurrence and is not due to the treatment. It is also noteworthy that,

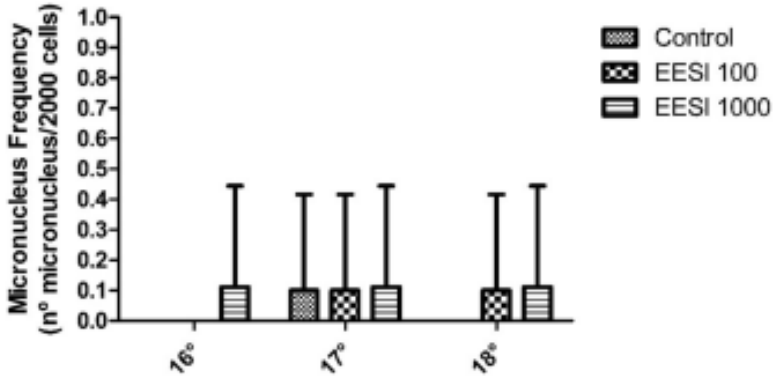


Fig. 3 Frequency of micronuclei in females treated with the ethanolic extract *S. lachnostachys* (EESl). Statistical test: ANOVA/Tukey ($p > 0.05$); Mean ± Standard Deviation

in general, xenobiotics that cause damage to the body lead mainly to an increase in the liver and kidneys and not their decrease [34]. Therefore, our results suggest no maternal toxicity at 100 and 1000 mg/kg doses according to the present design.

The absence of maternotoxicity is the absence of genotoxic effects evaluated by the micronucleus test. Regulatory agencies internationally accept this test for chromosomal damage assessment [34, 35]. In our study, we used the collection of three peripheral blood samples over 72 h to verify whether EESl had a cumulativeeffect. Yet, there was an absence of chromosomal damage at all of the analyzed times, and thus no cumulative effects. These factsreinforce the idea that EESl does not cause maternal toxicity or genotoxicity at 100 and 1000 mg/kg doses.

This safety of use is corroborated by the studies of Radai et al. [10] and Corso et al. [7]. According to Radai et al. [10], EESl has no genotoxic effect in male Swiss mice treated with a dose of up to 1,000 mg/kg. However, this author reported the need for subacute and chronic toxicity testing, providing complementary responses to genotoxicity. Corso et al. [7] noted that EES1 extract showed no acute toxicity up to a dose of 2,000 mg/kg in Swiss females.

Evaluation of parameters related to reproductive performance indicated that there were no differences ($p < 0.05$) between the control and EESl-treated groups for the number of implantations, number of resorptions, resorption rate, number of live and dead fetuses, the mean number of fetuses, post-implantation loss rate, fetal viability, or sex ratio. These findings suggest that EESl does not interfere with female reproductive performance and does not cause any embryofetal lethality.

According to David et al. [36] and Gonçalves et al. [37], the absence of change in these parameters indicates the safety of the extract and no interference with reproductive performance. When evaluating embryofetal development, a reduction in fetal weight was observed for the groups treated with EES1. However, the placental index and efficiency did not vary compared to the control group. It was also observed that the lowest placental index occurred in EESl (100 mg/kg) and the lowest placental efficiency in the EESl 1000 (mg/kg) group. Given these results, it is suggested that the EESl (100 mg/kg) group had the lowest placental average and the lowest placental index (even though it was not different from the control group). The lowest fetal weight averages were observed when determining the lowest embryofetal growth for this group. This fact occurred even in the presence of the higher placental efficiency (which did not differ from the control group but was higher than the placental efficiency observed for the EESl (1000 mg/kg).

Thus, it is considered that even in the face of reorganization of the organism to increase placental efficiency, the compensation of the EES1 (100 mg/kg) was insufficient to guarantee the same embryofetal growth in the mean fetal weight observed in the other experimental groups. However, when weight adequacy for gestational age was performed, it was observed that there were no significant differences between the fetuses of the treatment of EESl at a dose of 100 mg/kg and the control group, i.e., the occurrence of SGA, LGA, and AGA fetuses did not differ between these groups. But, when evaluating a higher dose at 1000 mg/kg group, an increase in the frequency of SGA was observed in the control group even though there was no change in placental weight, placental index, and placental efficiency.

Reduced placental weight can be associated with fetal malnutrition, reduced growth, low weight gain, and lower placental index. The outcome of these changes can be reduced fetal weight [38]. Reduced fetal weight can also correlate to altered placental efficiency.

Assessments of placental function and the effects of gestational insults often use the ratio of birth weight to placental weight as a measure of placental efficiency [39]. This ratio has been suggested to reflect placental exchange surface area, nutrient transport rates, and blood flow, potentially reflecting placental development and function adjustments to meet fetal demand [40, 41]. This parameter, a variable between species, translates the maternal–fetal relationship established during the gestational period and is a determinant of intrauterine growth since it is responsible for the nutritional and hormonal supply of the fetus [42]. Therefore, placental efficiency is influenced by the size, morphology, blood flow, and transport efficiency that can occur by simple diffusion and active transport [43].

Regarding the occurrence of malformations, it was observed that EESl, at doses of 100 and 1,000 mg/kg, increased the frequency of external and skeletal malformations. However, visceral malformations increased only for the EESl (100 mg/kg) group. Among the external malformations, the ones that occurred the most were curved tail, hyperlordosis, and scoliosis, which are spinal alterations.

Giampietro et al. [44] report that congenital vertebral malformations in the human population are etiologically heterogeneous with poorly understood environmental and genetic factors. Their prevalence is estimated to be between 0.13–0.51/1,000 live births. Among congenital spinal malformations are kyphosis and scoliosis, for example. According to Wang et al. [45], congenital scoliosis is a curvature of the spine resulting from abnormal vertebral development, with an incidence in the general population of approximately 1/1,000 to 1/2,000, also resulting from environmental and genetic factors. Among the environmental factors, one can also consider the consumption of drugs and teas, for example. These clinical findings can also be reproduced in experimental models.

Li et al. [46] observed that vitamin A deficiency causes congenital vertebral defects in rats. In their study with rats, Welch et al. [47] demonstrated that anabasine, a nicotinic receptor agonist substance extracted from N. glauca, leads to higher numbers of fetuses with scoliosis in the treated groups than in the control group. Medeiros et al. [48] and Gardner et al. [49] used a rat model to study congenital disabilities caused by the plant Mimosa tenuiflora and its compounds and identified bone malformations, including scoliosis, lordosis, and microcephaly.

The primary visceral malformations identified were hydrocephalus and hydronephrosis. Hydrocephalus did not present significant differences between the groups. Therefore, they are considered variants of normality. Hydronephrosis increased significantly in the EESl (100 mg/kg) group. However, these changes may regress at birth since the fetuses were collected on gestational day 18, and the end of natural gestation would only occur on gestational day 21. Thus, these alterations could be correctable. However, this data requires attention.

Some studies in the field [27, 50] describe variants of normality as changes that may regress as gestation progresses or at birth since the fetuses would have completed their development.

Regarding skeletal malformations, it was observed that EESl increased the frequency of reduced ossification or absence of ossification, especially in the skull and sternum. This change may occur due to the early removal of the fetus (18^{th} gestational day). Thus these observations would be transient, with less impact on the survival or health of the individual. Delayed ossifications are therefore included in skeletal variations [51]. However, as there was a statistical increase in frequency, we infer that this data requires attention, although the procedure of early collection of fetuses is protocol and indicated by the specialized literature of the area [26, 52].

Evaluation of skeletal development is routinely performed in embryofetal development studies to support the development of new therapeutic agents because ossification patterns in humans and rodents are similar, with bones originating from intramembranous or endochondral ossification and processes controlled by genetic and environmental factors [53]. Therefore, this is a crucial endpoint to evaluate since the toxic effects of xenobiotics on fetal bone development can result in skeletal malformations, delayed skeletal ossification, and skeletal variants [54].

Conclusions

Given the above, it is considered that the ethanolic extract of *Salvia lachnostachys* Benth is not maternotoxic and does not alter the reproductive performance of females according to the present design. However, it does change embryofetal development despite having low teratogenic potential. Thus, we don't recommend using this extract during the gestational period.

Acknowledgements

The authors acknowledge Carolina Calomeno (Design Department, UFPR), the CNPq, FUNDECT, CAPES and UFMS for providing various research grants.

Authors' contributions

Hudman Cunha Ortiz and Silvia Cordeiro das Neves: Biometric and analysis of biological materials: Cândida Aparecida Leite Kassuya, Henrique Rodrigues Scherer Coelho: scientific review and statistical analysis; Allana C. F. Martins: scientific review and statistical analysis; Marcelo Luiz Brandão Vilela and Valter Aragão do Nascimento: scientific review and statistical analysis; Arunachalam Karuppusamy: scientific revision and translation of the article; Roberto da Silva Gomes; scientific review and statistical analysis: Maria Élida Alves Stefanello: provision of ethanolic extract and scientific review; Rodrigo Juliano Oliveira: study guidance, data analysis and writing of the manuscript. The author(s) read and approved the final manuscript.

Funding
The authors acknowledge to the CNPq, FUNDECT, CAPES and UFMS for providing various research grants. ACFM and RSG acknowledge NIH-COBRE (1P20GM109024), NDEPSCoR (FAR0021960, FAR0034259 and FAR0035377) and NDSU Start-up.

Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
The animal study protocol followed the ARRIVE guidelines and was approved by the Institutional Review Board (or Ethics Committee) of Federal University of Mato Grosso do Sul, Brazil (protocol code 966/2018 and date of approval: 08/07/2018) for studies involving animals.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Centro de Estudos Em Células TroncoTerapia Celular E Genética Toxicológica (CeTroGen), Faculdade de Medicina (FAMED), Federal University of Mato Grosso Do Sul (UFMS), Campo Grande, Mato Grosso Do Sul, Brazil. [2]Programa de Pós-Graduação Em Saúde E Desenvolvimento Na Região Centro-Oeste-Faculdade de Medicina (FAMED), Federal University of Mato Grosso Do Sul (UFMS), Campo Grande, Mato Grosso Do Sul, Brazil. [3]Faculdade de Ciências da Saúde (FCS), Universidade Federal da Grande Dourados (UFGD), Dourados, Mato Grosso Do Sul, Brazil. [4]Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58102, USA. [5]Faculdade de Medicina (FAMED), Federal University of Mato Grosso Do Sul (UFMS), Campo Grande, Mato Grosso Do Sul, Brazil. [6]Group of Spectroscopy and Bioinformatics Applied to Biodiversity and Health (GEBABS), Graduate Program in Health and Development in the Central-West Region of Brazil, School of Medicine, Federal University of Mato Grosso do Sul, Campo Grande, Brazil. [7]Departamento de Química, Universidade Federal Do Paraná (UFPR), Curitiba, Paraná, Brazil.

Received: 8 November 2022 Accepted: 10 April 2023
Published online: 04 May 2023

References

1. Oliveira AB, Antar GM, Mota MCA, Pastore, J.F.B. *Salvia in*: Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro. 2022. Available from: https://floradobrasil.jbrj.gov.br/FB8326.
2. Leal L, Biondi D. Potencial ornamental de espécies Nativas. Rev Científica Eletrônica Engenharia Florestal. 2006;4(8):1–6.
3. Negrelle RRB, Fornazzari KCR. Ethnobotanical study in two rural communities (Limeira and Riberião Grande) in Guaratuba (Paraná, Brazil). Rev Bras Plant Med. 2007;9(2):36–54.
4. Kassuya CAL, Wisniewski A Jr, Simionatto EL, Santos EP, Stefanello MEA. Composição dos Óleos Essenciais de Salvia lachnostachys e S. melissiflora (Lamiaceae). Latin Am J Pharm. 2009;28(6):919–21.
5. Oliveira CS, Salvador MJ, de Carvalho JE, Santos ÉP, Barison A, Stefanello MÉA. Cytotoxic abietane-derivative diterpenoids of *Salvia lachnostachys*. Phytochem Lett. 2016;17:140–3.
6. Santos Oliveira C, Alvarez CJ, Pereira Cabral MR, Sarragiotto MH, Salvador MJ, Alves SM. Three new diterpenoids from the leaves of. Nat Prod Res. 2022;36(21):5600–5.
7. Corso CR, Stipp MC, Adami ER, da Silva LM, Mariott M, de Andrade SF, et al. Salvia lachnostachys Benth has antitumor and chemopreventive effects against solid Ehrlich carcinoma. Mol Biol Rep. 2019;46:4827–41.
8. Piccinelli AC, Figueiredo de Santana Aquino D, Morato PN, Kuraoka-Oliveira AM, Strapasson RL, Dos Santo EP, et al. Anti-Inflammatory and Antihyperalgesic Activities of Ethanolic Extract and Fruticulin A from Salvia lachnostachys Leaves in Mice. Evid Based Complement Alternat Med. 2014;2014:835914.
9. Santos JA, Piccinelli AC, Formagio MD, Oliveira CS, Santos EP, Alves Stefanello M, et al. Antidepressive and antinociceptive effects of ethanolic extract and fruticuline A from Salvia lachnostachys Benth leaves on rodents. PLoS One. 2017;12(2):e0172151.
10. Santos Radai JA, da Silva GF, Berno CR, Leitão MM, Dos Santos EP, Oliveira RJ, et al. Genetic toxicological assessment and anti-arthritic effects of an ethanolic extract obtained from Salvia lachnostachys Benth leaves in mice. Regul Toxicol Pharmacol. 2018;92:239–44.
11. Corso CR, Stipp MC, Radulski DR, Mariott M, da Silva LM, de Souza Ramos EA, et al. Fruticuline A, a chemically-defined diterpene, exerts antineoplastic effects in vitro and in vivo by multiple mechanisms. Sci Rep. 2020;10(1):16477.
12. Fontoura A, Ayres LR, Martins-Nagai M, Dewulf NLS, dos Santos V, Martinez EZ, et al. Prevalence of medication use among low risk pregnant women: a drug utilization study. Afr J Pharm Pharmacol. 2014;8(36):883–92.
13. Lima RM, Godinho JLP, Magalhães EIDS, Brito AB, Britto MHSSD, Alves MTSSDB, et al. Profile of medication use during pregnancy: Results of the BRISA study, São Luís - MA, Brazil. Res Soc Dev. 2022;11(2):e21811224986.
14. Bérard A, Zhao JP, Sheehy O. Antidepressant use during pregnancy and the risk of major congenital malformations in a cohort of depressed pregnant women: an updated analysis of the Quebec Pregnancy Cohort. BMJ Open. 2017;7(1):e013372.
15. Aragão FFd, Tobias AF. Pharmacological treatment of pain in pregnancy. Brazil J Pain. 2019;2(4):374–80.
16. Bałkowiec-Iskra E, Mirowska-Guzel DM, Wielgoś M. Effect of antidepressants use in pregnancy on foetus development and adverse effects in newborns. Ginekol Pol. 2017;88(1):36–42.
17. Gao SY, Wu QJ, Sun C, Zhang TN, Shen ZQ, Liu CX, et al. Selective serotonin reuptake inhibitor use during early pregnancy and congenital malformations: a systematic review and meta-analysis of cohort studies of more than 9 million births. BMC Med. 2018;16(1):205.
18. Hong F, Qiu J, Zhang S, Zhang L. Fetal congenital cardiac and vascular disorders associated with sertraline treatment during pregnancy: analysis of FAERS data. Biomed Res Int. 2022;2022:9914931.
19. Oliveira RJ, Salles MJ, da Silva AF, Kanno TY, Lourenço AC, Freiria GA, et al. Effects of the polysaccharide beta-glucan on clastogenicity and teratogenicity caused by acute exposure to cyclophosphamide in mice. Regul Toxicol Pharmacol. 2009;53(3):164–73.
20. Oliveira RJ, Mantovani MS, Pesarini JR, Mauro MO, da Silva AF, Souza TR, et al. 6-Dimethylaminopurine and cyclohexamide are mutagenic and alter reproductive performance and intrauterine development in vivo. Genet Mol Res. 2015;14(1):834–49.
21. OECD. Test No. 443: Extended One-Generation Reproductive Toxicity Study. OECD Guidelines for the Testing of Chemicals, Section 42018.
22. Sanitária A-ANdV. Guia para a condução de estudos não clínicos de segurança necessários ao desenvolvimento de medicamentos. 2013.
23. OECD. Test No. 421: Reproduction/Developmental Toxicity Screening Test. OECD Guidelines for the Testing of Chemicals, Section 42016.
24. Wilson JC, Warkany J. Teratology: Principles and Techniques. Chicago: Univ. of Chicago Press; 1965.
25. Barrow MV, Taylor WJ. A rapid method for detecting malformations in rat fetuses. J Morphol. 1969;127(3):291–305.
26. Wilson JG. Methods for administering agents and detecting malformations in experimental animals. Teratology. 1965;262:277.
27. Taylor P. Practical teratology. London: Academic Press; 1986.
28. Manson JC, Clarke AR, Hooper ML, Aitchison L, McConnell I, Hope J. 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. Mol Neurobiol. 1994;8:121–7.
29. Wise LD, Beck SL, Beltrame D, Beyer BK, Chahoud I, Clark RL, et al. Terminology of developmental abnormalities in common laboratory mammals (version 1). Teratology. 1997;55(4):249–92.
30. Damasceno DC. Anomalias congênitas: estudos experimentais. COOPMED; 2008.
31. Staples RE, Schnell VL. Refinements in rapid clearing technic in the koh-alizarin red s method for fetal bone. Stain Technol. 1964;39:61–3.

32. Hayashi M, Morita T, Kodama Y, Sofuni T, Ishidate M Jr. The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. Mut Res Lett. 1990;245(4):245–9.
33. Teng C, Jia J, Wang Z, Sharma VK, Yan B. Size-dependent maternal-fetal transfer and fetal developmental toxicity of ZnO nanoparticles after oral exposures in pregnant mice. Ecotoxicol Environ Saf. 2019;182:109439.
34. Vani JM, Monreal MTFD, Auharek SA, Cunha-Laura AL, de Arruda EJ, Lima AR, et al. The mixture of cashew nut shell liquid and castor oil results in an efficient larvicide against Aedes aegypti that does not alter embryo-fetal development, reproductive performance or DNA integrity. PLoS One. 2018;13(3):e0193509.
35. Oliveira RJ, da Cruz Leite Santos N, Pesarini JR, de Oliveira BC, Berno CR, de Araújo FHS, et al. Assessment of genetic integrity, splenic phagocytosis and cell death potential of (Z)-4-((1,5-dimethyl-3-oxo-2-phenyl-2,3dihydro-1H-pyrazol-4-yl) amino)-4-oxobut-2-enoic acid and its effect when combined with commercial chemotherapeutics. Genet Mol Biol. 2018;41(1):54–166.
36. David N, Mauro MeO, Gonçalves CA, Pesarini JR, Strapasson RL, Kassuya CA, et al. Gochnatia polymorpha ssp. floccosa: bioprospecting of an anti-inflammatory phytotherapy for use during pregnancy. J Ethnopharmacol. 2014;154(2):370–9.
37. Gonçalves CA, Silva NL, Mauro MO, David N, Cunha-Laura AL, Auharek SA, et al. Evaluation of mutagenic, teratogenic, and immunomodulatory effects of Annona nutans hydromethanolic fraction on pregnant mice. Genet Mol Res. 2014;13(2):4392–405.
38. Moraes-Souza RQ, Soares TS, Carmo NO, Damasceno DC, Campos KE, Volpato GT. Adverse effects of Croton urucurana B. exposure during rat pregnancy. J Ethnopharmacol. 2017;199:328–33.
39. Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M, Burton GJ. Placental efficiency and adaptation: endocrine regulation. J Physiol. 2009;587(Pt 14):3459–72.
40. Burton GJ, Fowden AL, Thornburg KL. Placental Origins of Chronic Disease. Physiol Rev. 2016;96(4):1509–65.
41. Christians JK, Grynspan D, Greenwood SL, Dilworth MR. The problem with using the birthweight:placental weight ratio as a measure of placental efficiency. Placenta. 2018;68:52–8.
42. Guimarães CF, Meirelles MG, Fernandes CB. Placental efficiency in the equine species: which factors may be related? Braz J Vet Res Animal Sci. 2015;52(2):98–105.
43. Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A, Adamo KB. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. Int J Mol Sci. 2014;15(9):16153–85.
44. Giampietro PF, Raggio CL, Blank RD, McCarty C, Broeckel U, Pickart MA. Clinical, genetic and environmental factors associated with congenital vertebral malformations. Mol Syndromol. 2013;4(1–2):94–105.
45. Wang X, Yu Y, Yang N, Xia L. Incidence of intraspinal abnormalities in congenital scoliosis: a systematic review and meta-analysis. J Orthop Surg Res. 2020;15(1):485.
46. Li Z, Shen J, Wu WK, Wang X, Liang J, Qiu G, et al. Vitamin A deficiency induces congenital spinal deformities in rats. PLoS One. 2012;7(10):e46565.
47. Welch KD, Lee ST, Panter KE, Gardner DR, Knoppel EL, Green BT, Hammond CK, Hammond ZJ, Pfister JA. Studies on the teratogenicity of anabasine in a rat model. Toxicon. 2014;87:32–7.
48. Medeiros RM, de Figueiredo AP, Benício TM, Dantas FP, Riet-Correa F. Teratogenicity of Mimosa tenuiflora seeds to pregnant rats. Toxicon. 2008;51(2):316–9.
49. Gardner D, Riet-Correa F, Lemos D, Welch K, Pfister J, Panter K. Teratogenic effects of Mimosa tenuiflora in a rat model and possible role of N-methyl- and N,N-dimethyltryptamine. J Agric Food Chem. 2014;62(30):7398–401.
50. Ishikawa RB, Vani JM, das Neves SC, Rabacow APM, Kassuya CAL, Croda J, et al. The safe use of Doliocarpus dentatus in the gestational period: absence of changes in maternal reproductive performance, embryo-fetal development and DNA integrity. J Ethnopharmacol. 2018;217:1–6.
51. Sartori AS, Hollenbach CB, Jardim LH, Silva P, Mello FB, Mello JRB. Prenatal toxicity assessment: teratogenicity study of the insecticide pyriproxyfen in Wistar rats. Arq Bras Med Vet Zootec. 2020;72:719–28.
52. Gonçalves CA, Siqueira JM, Carollo CA, Mauro MeO, de Davi N, Cunha-Laura AL, et al. Gestational exposure to Byrsonima verbascifolia: teratogenicity, mutagenicity and immunomodulation evaluation in female Swiss mice. J Ethnopharmacol. 2013;150(3):843–50.
53. Gómez T, García M, Bequer L, Freire C, Vila MA, Clapés S. Skeletal malformations and growth disturbances in fetuses of mild diabetic rats. Biomedica. 2021;41(3):493–503.
54. De Schaepdrijver L, Delille P, Geys H, Boehringer-Shahidi C, Vanhove C. In vivo longitudinal micro-CT study of bent long limb bones in rat offspring. Reprod Toxicol. 2014;46:91–7.

Publisher's Note

Anexo 4 – Manuscrito 3 – Submetido à *Evidence-Based Complementary and Alternative Medicine*

The ethanolic extract of *Serjania erecta* Radlk. has low teratogenic potential, but is embryofetotoxic

Hudman Cunha Ortiz[a,b], Silvia Cordeiro das Neves[a,b], Cândida Aparecida Leite Kassuya[c], Henrique Rodrigues Scherer Coelho[a,b], Marcelo Luiz Brandão Vilela[d], Valter Aragão do Nascimento[b], Arunachalam Karuppusamy[a,b], Roberto da Silva Gomes[e], Cláudia Andrea Lima Cardoso[f], Rodrigo Juliano Oliveira[a,b]

[a]Centro de Estudos em Células Tronco, Terapia Celular e Genética Toxicológica (CeTroGen), Faculdade de Medicina (FAMED), Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brasil.

[b]Programa de Pós-graduação em Saúde e Desenvolvimento na Região Centro-Oeste, Faculdade de Medicina Dr. Hélio Mandetta (FAMED), Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brasil.

[c]Faculdade de Ciências da Saúde, Universidade Federal da Grande Dourados (UFGD), Dourados, Mato Grosso do Sul, Brasil.

[d]Faculdade de Medicina Dr. Hélio Mandetta (FAMED), Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brasil.

[e]Department of Pharmaceutical Sciences, North Dakota State University, Fargo, North Dakota, 58102, United States.

[f]Centro de Estudos em Recursos Naturais, Universidade Estadual de Mato Grosso do Sul (UEMS), Dourados, Mato Grosso do Sul, Brasil.

Abstract

Ethnopharmacological relevance: The leaf tea of *Serjania erecta* Radlk., is popularly use in Brazil against ulcers and its roots against hypertension. There is also an indication of this plant against stomach disorders.

Aim of the study: The present study aimed to evaluate the effects of ethanolic extract of *Serjania erecta* (EESe) on the reproductive performance, embryofetal development and DNA integrity of pregnant female mice.

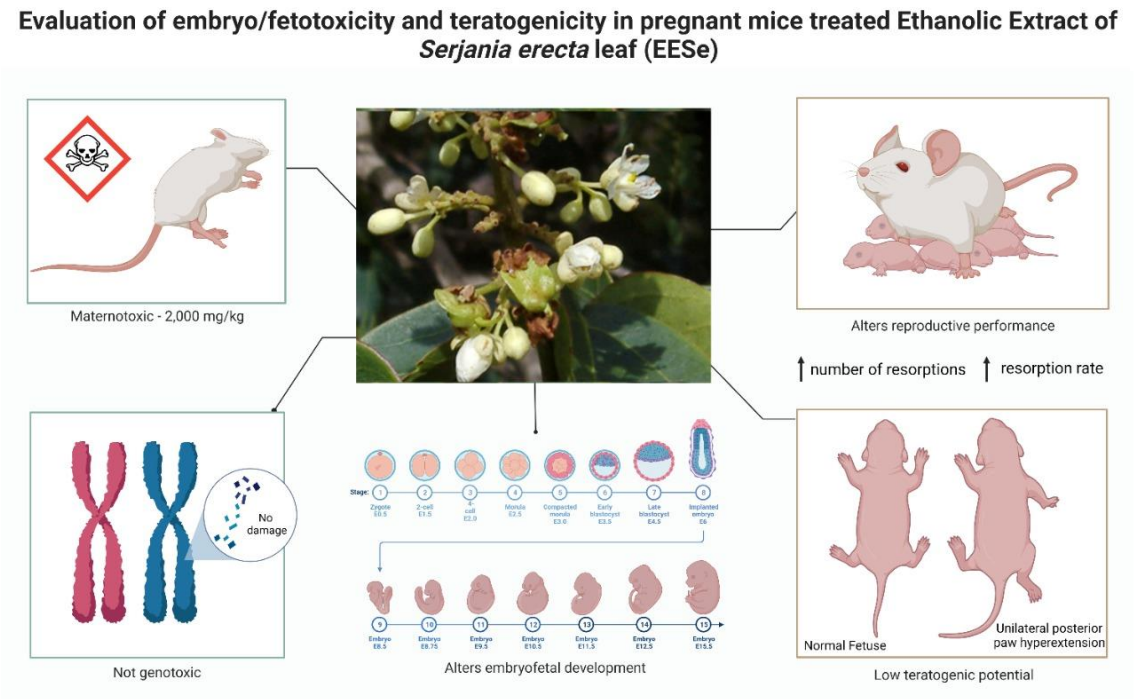
Material and methods: The leaf powder of *S. erecta* were extracted successively with hexane, ethyl acetate and ethanol solvents, whereas ethanol extract was further fractionated, isolated (Sephadex LH-20, column chromatography) and compound identified by nuclear magnetic resonance (NMR) spectroscopy. Pregnant females were randomLy divided into 4 experimental groups (n = 10): control group treated with vehicle and treatment groups as EESe (500, 1000 and 2000 mg/kg, p.o) respectively. The treatment occurred by gavage during the entire gestational period (from day 1 to day 18). Afterwards, parameters related to reproductive performance, embryofetal development and DNA integrity were evaluated.

Result: Statistically significant changes were found in biometric parameters of organs such as heart and lung. An increase in the number and rate of resorptions were observed. In addition, a slight increase in external, visceral and bone malformations were also observed.

Conclusion: The phytochemical analysis of ethanolic extract of leaves fractionated resulting in the isolation and identification of the kaempferol-3-O-a-l-rhamnopyranoside. EESe was maternotoxic at a dose of 2,000 mg/kg. At lower doses were not shown maternotoxicity or genotoxicity. EESe is embryofetotoxic at higher dose, since it increased the number and rate of resorption. Furthermore, it has a low teratogenic potential. Therefore, its consumption in the gestational period is contraindicated.

Keywords: Maternotoxicity, Genotoxicity, Medicinal Plants, Anti-inflammatory, hypertension

Graphic Abstract



1. Introduction

Serjania erecta Radlk is a medicinal plant belonging to the Sapindaceae family and to the Sapindales order. It is widely distributed in tropical regions of the world and some species are found in Brazil mainly in the states of Mato Grosso, Mato Grosso do Sul, Tocantins, Goias, and Distrito Federal. This plant popularly known as *Cinco Folhas* or *Cipó-cinco-folhas*. According to Pott and Pott (1994), the leaves of *S. erecta* have popular indication against ulcer and the roots against hypertension. Guarim-Neto; Santana and Silva (2000) reinforce this same popular indication and indicate the use in the form of tea. Martins et al. (2015) describe the popular use of *S. erecta* against stomach disorders.

Pharmacological studies indicate that *S. erecta* extract and/or some of its isolated compounds have topical anti-inflammatory potential (Gomig et al., 2008), in stomach pain, ulcerative diseases, hypertension (Arruda et al., 2009), antimicrobial activity (Cordova et al., 2010; Cardoso et al., 2013). Similarly acts directly on the central nervous system by increasing the rates of antioxidant activity with fighting Alzheimer's disease (Broggini et al., 2010; Guimarães et al., 2015). The literature also reported that *S. erecta* does not influence gastric motility (Potrich et al., 2014).

Fernandes et al. (2011) observed in their study that this plant presents antiphidic activity due to the presence of compounds that are able to inhibit the toxic activities of *Bothrops jararacussu* venom because they inhibit the action of enzymes involved in blood coagulation disorders responsible for hemorrhagic processes.

Despite the previous descriptions, no additional data on the effects of the ethanolic extract of *S. erecta* (EESe) in the gestational period were found in the literature, nor studies that guarantee the safety of its use in this period. This safety of use is essential because of the possible damage to the health of the pregnant woman and her fetus. Thus, the present study aimed to evaluate the effects of EESe on the reproductive performance, embryofetal development and DNA integrity of pregnant female mice.

2. Materials and methods

2.1 Plant material of Serjana erecta extract - EESe

2.1 Plant material, extraction, isolation and identification of compounds

S. erecta was collected in Aquidauana (MS - Brazil) and a voucher specimen (deposit number: HMS: 8355) was deposited at the Herbarium of Brazilian Agricultural Research Corporation (EMBRAPA) in Mato Grosso do Sul State, Brazil. The code registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) is A055721.

The leaves (600 g) of *S. erecta* were extracted successively with hexane, ethyl acetate and ethanol solvents at room temperature. Besides, in this study we used only the ethanol extract of leaves. The extract was fractionated by XAD-2 resin column chromatography eluted with of water, methanol and finally acetone solvents. Furthermore, methanolic fraction was fractionated by Sephadex LH-20 column chromatography and eluted with methanol solvent. The fractions were combined by thin-layer chromatography (silica gel plates, ethyl acetate/*n*-propanol/water, 120:8:70 by volume, upper phase). The compounds obtained were further purified by repeated column chromatography on polyvinylpolypyrrolidone eluted with methanol. The isolated compounds were analyzed by NMR (Nuclear Magnetic Resonance) spectra were recorded on a Bruker DPX 300 spectrometer, IR (Infrared) spectrum were performed in a FT-IR-Nicolet Impact IMACT-400 and UV (Ultraviolet) spectrum were performed in a Hitachi 110 spectrophotometer.

2.2 Animals

The animals were provided by the central animal house of Federal University of Mato Grosso do Sul (UFMS) in 60 mice (*Mus musculus*) of the Swiss strain of both sexes at reproductive age: 40 females with an average weight of 30 g and 20 males with an average weight of 35 g. All animals went through a 7-day adaptation period and were housed in mini-isolators (Alesco® ventilated rack) with Pinus sp. wooden chips. They were kept under temperature control (22 ± 2°C), light by photoperiod (12h light/12h dark), relative humidity of 55 ± 10% and access to feed and water *ad libitum*. The research was conducted according to the protocols of the Universal Declaration of Animal Rights and with approval from the Ethics Committee on Animal Use (CEUA) of the UFMS, under registered number of 965/2018.

2.3 Experimental design

The animals were mated during the night in the ratio of 1 male: 2 females. The detection of vaginal plugs in females determined the occurrence of pregnancy and this day was considered day zero of gestation (Oliveira et al., 2009; Oliveira et al., 2015). Pregnant females were randomLy divided into 4 experimental groups (n = 10): Control Group - animals received 0.1 mL/10 g body weight (b.w.) of vehicle (Tween 80 at 1%) by oral gavage (p.o.) throughout gestation (gestational day 1 to 18); EESe Groups - animals received EESe by gavage at doses of 500 mg/kg (b.w, p.o.) (Group EESe 500), 1000 mg/kg (p.o.) (Group EESe 1000) and 2000 mg/kg (p.o.) (Group EESe 2000) during gestation. The dose of 500 mg/kg was chosen from the effective dose described by Arruda et al. (2009) in a model of gastric ulcer induced by absolute ethanol. From this dose, the doses 2x and 4x higher (1000 and 2000 mg/kg) than the one with the desired activity were defined, following the recommendation of the guidelines of the area (ANVISA, 2013; OECD 421, 2016; OECD 443, 2018). Furthermore, we opted for the higher dose (2000 mg/kg) since Arruda et al. (2009) classified the chloroform extract of *S. erecta* as practically non-toxic since the dose of 5000 mg/kg had not caused signs of acute toxicity.

2.4 Biological Tests

Peripheral blood was collected by intravenous puncture on the 16^{th}, 17^{th} and 18^{th} gestational days (g.d.), when the females were euthanized by deep anesthesia, followed by laparotomy, omphalectomy and hysterectomy for collection, weighing and proper storage of organs, fetuses and placentas. The fetuses underwent systematic analysis for external malformations and sexing and were then randomLy assigned to two subgroups. The first subgroup was destined for visceral analysis and were fixed in Bodians' solution composed of

distilled water (142 mL), acetic acid (50 mL), formaldehyde (50 mL) and 95% alcohol (758 mL) for at least seven days and submitted to incision/microdissection with strategic cuts proposed by Barrow and Taylor (1969) for the study of the thorax and abdomen by Wilson, 1965, then Oliveira et al. (2009) modified for the study of the head. The classification of visceral changes was based on the works of Taylor (1986), Manson and Kang (1994), Wise et al. (1997), Damasceno et al. (2008), and Oliveira et al. (2009). The second subset, intended for skeletal analysis, were fixed in absolute acetone for at least seven days and after removal of the viscera, they were immersed in potassium hydroxide (KOH, 0.8%) and stained with Alizarin Red during the diaphanization process as proposed by Straples and Schenell (1964) with modifications by Oliveira et al. (2015). After the fetuses were stained, the KOH solution was replaced with the bleaching solution (1 liter glycerin: 1 liter ethyl alcohol: 0.5 liter benzyl alcohol) and changed every 24 h for five days. The analyses of fetal viscera and skeletons were performed under a stereomicroscopic magnifying glass (Nikon® - SMZ 745T) at 1.6x magnification.

2.5 Determination of organ weight

The organs weights were calculated from the records of initial weight of animals (females weighed at day zero), final weight (females weighed at 18^{th} g.d.), weight gain (final weight - initial weight), uterus weight, net weight gain (weight gain - uterus weight), absolute and relative weights of heart, lung, spleen, kidneys and liver.

2.6 Reproductive performance and embryofetal development

The number of implantation sites (no. of live fetuses + no. of dead fetuses + no. of resorptions) and resorption were recorded, in addition to the number of live and dead fetuses and the fetal and placental weight. Based on these data, we obtained: fetal viability (number of live fetuses × 100 / number of implantations), post-implantation loss rate [(number of implantations - number of resorptions) × 100 / number of implantations], resorption rate (number of resorptions × 100 / number of implantations), placental index (placenta weight / fetal weight), placental efficiency (fetal weight / placental weight) and sex ratio (number of male fetuses / number of female fetuses).

The classification of fetal weight according to gestational age (CFWGA) was done as proposed by Soares et al. (2018) which classifies fetal weights individually. Thus, fetuses were considered adequate for gestational age (AGA) when they did not diverge from the range of the mean ± 1.7 × the standard deviation of the mean (SD) of the control fetuses. They were considered small for gestational age (SGA) when their body weight was less than the mean -

1.7 × SD in relation to the mean of the control group fetuses and were considered large for gestational age (LGA) when their body weight was greater than the mean + 1.7 × SD in relation to the mean of the control group.

2.7 Micronucleus in peripheral blood

The micronucleus test was performed according to Carvalho et al. (2015). Peripheral blood, collected by puncture of the tail vein (about 20 μL), was deposited on a slide prepared with Acridine Orange (1mg/mL). The blood was covered with a coverslip and the material was stored in a freezer (-20ºC) for a minimum of seven days. A total of 2,000 reticulocytes were examined per animal using an EVOS M7000 microscope (Zeiss®) at 40x magnification with an excitation filter 470 nm ± 22 and an emission filter 525 nm ± 50.

2.8 Statistical Analysis

For data with normal distribution, the one-way ANOVA test with Tukey post-test was used. For the other data, the Kruskal-Wallis test with Dunn's post-test were used. For frequency comparisons, the Chi-square test was used. The analyses were performed using the GraphPad Instat® program. Data were presented as mean ± standard error of the mean or mean ± standard deviation and the established significance level was $p < 0.05$.

3. Results

3.1 Phytochemical analysis

The ethanolic extract of leaves showed the presence of kaempferol, kaempferol-3,7-di-O-a-L-rhamnopyranoside, (-)-epicatechin, apigenin-6-C-b-D-glucopyranoside (isovitexin) and apigenin-8-C-b-D-glucopyranoside (Cardoso et al., 2013). The ethanolic extract of leaves continued to be fractionated resulting in the isolation and identification of the kaempferol-3-O-a-l-rhamnopyranoside.

Spectral data: 1 H NMR [(300 MHz, CD3OD, J (Hz)]: d 6.19 (s, H-6), 6.39 (s, H-8), 7.76 (d, J= 8.4 Hz, H-2', H- 6'), 6.91 (d, J = 8.4 Hz, H-3', 5'), 5.36 (d, J = 1.8 Hz, H-1''), 0.90 (d, J=5.4 Hz, H-6''), 3.2-4.3 (m, sugar). RMN 13C (70 MHz, DMSO-d6,): d 157.8 (C-2), 134.0 (C-3), 177.6 (C-4), 161.9 (C-5), 98.5 (C-6), 164.1 (C-7), 93.5 (C-8), 156.8 (C-9), 103.9 (C-10), 121.8 (C-1'), 130.3 (C-2', C-6'), 115.3 (C-3', C-5'), 161.3 (C-4'), Rha'': 101.9 (C-1''), 70.4 (C-2''), 70,6 (C-3''), 70.8 (C-4''), 69,8 (C-5''), 16,6 (C-6''). UV lmax: 264 nm e 345 nm. IV umax: 3242, 1655, 1607, 1505 cm-1.

3.1 Maternal Toxicity and Biometric Parameters

The dose of 2000 mg/kg induced maternal toxicity and the females died within 6 days of treatment. The signs of toxicity observed were hair raising, hypothermia, hypoactivity, altered ambulation, reduced water and feed intake, and diarrhea. Thus, the results that presented correspond to the doses of 500 and 1000 mg/kg.

When comparing to the control, EESe (500 and 1000 mg/kg) groups, it was observed that the initial weight, final weight, weight gain, uterine weight, net weight gain, absolute and relative weight of the spleen, kidneys and liver did not present statistically significant differences ($p>0.05$). The relative and absolute weight of the heart of the EESe 500 mg/kg increased ($p<0.05$) compared to the other groups. The absolute weight of the lungs did not differ between groups ($p>0.05$). However, the relative lung weight differed between the EESe 500 and 1000 mg/kg groups ($p<0.05$). But, both did not differ from the control group (**Table 1**).

Table 1. Absolute weight and relative weight of organs of females treated with the ethanolic extract of *Serjania erecta*:

Organ weight (g)					
Experimental Groups (mg/kg)	Initial weight[2]	Final weight[1]	Weight Gain[1]	Uterine Weight[1]	Net Weight Gain[2]
Control	29.17±0.23[a]	48.02±1.34[a]	18.86±1.29[a]	15.38±1.09[a]	3.48±0.74[a]
EESe 500	29.01±0.40[a]	48.16±1.49[a]	19.15±1.40[a]	15.91±1.68[a]	3.24±1.61[a]
EESe 1000	29.41±0.57[a]	46.38±1.41[a]	16.97±1.08[a]	14.87±0.89[a]	2.10±0.40[a]
Absolute Organ Weight (g)					
	Heart[1]	Lungs[2]	Spleen[2]	Kidney[1]	Liver[1]
Control	0.16±0.00[a]	0.23±0.01[a]	0.14±0.01[a]	0.37±0.01[a]	2.26±0.09[a]
EESe 500	0.21±0.02[b]	0.21±0.01[a]	0.16±0.02[a]	0.36±0.01[a]	2.31±0.04[a]
EESe 1000	0.15±0.01[a]	0.25±0.01[a]	0.12±0.01[a]	0.37±0.02[a]	2.19±0.09[a]
Relative Weigth of Organs (g)					
	Heart[1]	Lungs[1]	Spleen[2]	Kidney[1]	Liver[2]
Control	0.0033±0.0001[a]	0.0048±0.0001[ab]	0.0029±0.0002[a]	0.0077±0.0004[a]	0.0471±0.0014[a]
EESe 500	0.0042±0.0003[b]	0.0043±0.0002[a]	0.0033±0.0005[a]	0.0074±0.0003[a]	0.0483±0.0016[a]
EESe 1000	0.0033±0.0001[a]	0.0054±0.0002[b]	0.0026±0.0001[a]	0.0080±0.0002[a]	0.0473±0.0015[a]

EESe - ethanolic extract of *Serjania erecta*; g - grams. Different letters (a,b) indicate statistically significant differences. Mean ± Standard error of the mean. [1]Anova/Tukey's test; [2]Kruskal-Wallis/Dunn; $p>0.05$.

3.2 Reproductive performance

EESe treatment caused no changes ($p>0.05$) in the number of implants, live fetuses, dead fetuses, average number of fetuses per litter, fetal viability, post-implantation loss rate, and sex ratio. However, the number of resorptions and resorption rate increased ($p<0.05$) in the EESe 1000 mg/kg. An increasing trend in the number of resorptions and resorption rate was also observed for the EESe 500 mg/kg ($p>0.05$) **(Table 2).**

Table 2. Reproductive parameters of females treated with the ethanolic extract of *Serjania erecta*:

Reproductive Performance			
	Experimental Groups		
Parameters	Control	EESe 500 mg/kg	EESe 1000 mg/kg
Implants	11.55±0.78[a]	12.00±0.62[a]	12.62±0.97[a]
Live Fetuses	10.78±0.92[a]	11.11±0.99[a]	10.50±0.80[a]
Dead Fetuses	00.11±0.11[a]	00.00±0.00[a]	00.00±0.00[a]
Average number of fetuses	10.89±0.92[a]	11.11±0.99[a]	10.50±0.80[a]
Fetal Viability	92.41±2.75[a]	90.87±5.46[a]	83.88±3.45[a]
Post-implantation loss rate	07.58±2.75[a]	09.13±5.46[a]	16.11±3.45[a]
Reabsorption	00.44±0.24[a]	00.89±0.45[ab]	2.12±0.44[b]
Reabsorption Rate	04.55±2.53[a]	09.12±5.46[ab]	16.11±3.45[b]
Sexual Reason	81.72±15.38[a]	108.77±17.48[a]	203.02±72.28[a]

EESe - ethanolic extract of *Serjania erecta*; Different letters (a,b) indicate statistically significant differences. Mean ± Standard error of the mean. ANOVA/Tukey's test; $p>0.05$.

3.3 Embryofetal Development and Placental Efficiency

Treatment with EESe caused no changes in placental index, placental efficiency and fetal weight. However, the placental weight of the EESe 500 mg/kg decreased ($p<0.05$) compared to the control group and the EESe 1000 mg/kg group. The percentage of fetuses classified as GIP and GIG was significantly higher ($p<0.05$) only in the dams treated with EESe 500, differing from the other groups **(Table 3).**

Table 3. Embryofetal development parameters and placental efficiency of females treated with ethanolic extract of *Serjania erecta*:

Embryo Development and Placental Efficiency			
	Experimental Groups		
Parameters	Control	EESe 500 mg/kg	EESe 1000 mg/kg
Placental Weight (g)[1]	0.0918±0.00[a]	0.0860±0.00[b]	0.1148±0.01[a]
Placental Index[1]	0.08±0.00[a]	0.10±0.00[a]	0.10±0.01[a]
Placental Efficiency[1]	13.08±0.35[a]	13.05±0.56[a]	12.66±0.39[a]
Fetal Weight (g)[1]	1.17±0.01[a]	1.03±0.03[a]	1.15±0.01[a]
% SGA[2]	6.18	28*	8.33
% AGA[2]	92.78	65	91.67
% LGA[2]	1.03	7*	0

EESe - ethanolic extract of *Serjania erecta*; g - grams; SGA - small for gestational age, AGA - adequate for gestational age, LGA - large for gestational age. Statistical test: [1]ANOVA/Tukey - Mean ± Standard

error of the mean. Different letters indicate statistically significant differences; [2]Chi-square test. *Indicates statistically significant difference in relation to the control group; $p<0.05$.

Systematic analysis of external malformations indicated the occurrence of curved tail, unilateral posterior paw hyperflexion, posterior paw hyperextension (uni and bilateral), paw rotation, gastroschisis, open eye and scoliosis. There were no variations ($p>0.05$) in the frequencies of these malformations when comparing the different groups, except for paw rotation. The frequency of this malformation was higher ($p<0.05$) in the EESe 500 mg/kg compared to the control group and the EESe 1000 mg/kg. When the sum of the different external malformations was compared, no differences were observed between the different groups ($p>0.05$) (**Table 4**).

Table 4. External malformations found in the offspring of females treated with ethanolic extract of *Serjania erecta*:

External Malformation			
	Experimental Groups		
Parameters	Control	EESe 500 mg/kg	EESe 1000 mg/kg
Fetuses Analysed	97	99	66
Normal Fetuses	89	84	58
Curly Tail	1	0	5
Unilateral posterior paw hyperflexion (HFPU)	0	4	0
Unilateral posterior paw hyperextension (HFPU)	1	0	5
Hyperextension of the posterior paw (HEPB)	0	0	1
Gastroschisis	1	1	0
Open eye	1	0	0
Scoliosis	4	3	0
Paw Rotation	0	8*	0
Frequency of Malformation	8	16	11
%M.F.	8.25	16.16	16.67

EESe - ethanolic extract of *Serjania erecta*; HFPU - unilateral posterior paw hyperflexion; HEPU - unilateral posterior paw hyperextension; Hyperextension of the posterior paw (HEPB); %F.P. - percentage of fetuses with malformation. Statistical test: Chi-square. *Indicates statistically significant difference in relation to the Control group; $p<0.05$.

In relation to visceral malformations, hydrocephalus (mild, moderate and severe grades), hydronephrosis (mild and moderate grades) and absence of suprarenal were identified. An increase ($p<0.05$) in the frequency of mild hydrocephalus and hydronephrosis was observed in the EESe 1000 mg/kg when compared to the control group. In the analysis of the percentage of fetuses with malformations, the EESe 500 mg/kg showed a significant increase ($p<0.05$) in

relation to the control group both in the brain and in the urogenital region, while the EESe 1000 mg/kg showed the same difference only for the urogenital region (**Table 5**).

Table 5. Visceral malformations found in the offspring of females treated with ethanolic extract of *Serjania erecta*:

Visceral Malformation			
	Experimental Groups		
Parameters	Control	EESe 500 mg/kg	EESe 1000 mg/kg
Analyzed fetuses	49	48	27
Normal Fetuses	36	26	11
Brain - Hydrocephalus			
Mild Hydrocephalus	8	16	9*
Moderate Hydrocephalus	3	4	0
Severe Hydrocephalus	1	0	0
Frequency of Malformations	12	20	9
% F.M	24.49	41.67*	33.33
Urogenital Region - Hydronephrosis			
Hydronephrosis Mild	1	7	5*
Moderate Hydronephrosis	0	2	0
Absence of Suprarenal	1	0	0
Frequency of Malformations	2	9	5
% F.M	4.08	18.75*	18.52*

EESe - ethanolic extract of *Serjania erecta*; % F.M. - percentage of fetuses with malformation. Statistical test: Chi-square. *Statistically significant difference; $p<0.05$.

The skeletal malformations found were fusion of the metacarpal (MTC) and metatarsal (MTT), absence and reduced ossification (OR) of the ischium and pubis, OR of the sternum and absence of the last sternum, OR of the nasal, OR or absence of the parietal, OR or absence of the interparietal, absence of the vomer, absence of the palate, OR of the presphenoid, OR or absence of the basiphoid, OR of the basoccipitoid, absence of the orbitosphenoid, OR or absence of the Timpanic and various rib malformations.

Significant differences ($p<0.05$) were found in the EESe 500 group compared to the control group for absence of anterior and posterior phalanges, MTC/MTT, sternum, supraoccipitoid, presphenoid and hamulus and for OR of vomer and vertebrae.

Regarding the frequency of malformations, a significant difference ($p<0.05$) was found in the SESe 500 group compared to the control group for absence of anterior and posterior phalanges, absence/fusion of the MTC/MTT, and absence/OR of the ischium and pubis.

Differences were found ($p<0.05$) in the absence of anterior and posterior phalanges and in the absence/OR of the ischium and pubis.

Regarding the percentage of fetuses with malformation, the EESe 500 mg/kg was significantly higher ($p<0.05$) than the control group in all malformations related to limbs, sternum, head bone, vertebrae and ribs. For the EESe 1000 mg/kg, the difference found in relation to the same percentage was in the absence of anterior and posterior phalanges, absence/OR of the ischium and pubis, sternum and head bones (**Table 6**).

Table 6. Skeletal malformations found in the offspring of females treated with ethanolic extract of *Serjania erecta*:

Skeletal Malformation				
Experimental Groups				
Parameters		Control	EESe 500 mg/kg	EESe 1000 mg/kg
Analyzed fetuses		48	51	39
Normal Fetuses		30	30	30
Members				
Anterior phalanges	Absence	0	12*	8
Posterior phalanges	Absence	0	12*	4
Frequency of Malformations		0	24*	12*
%M.F.		0	41.18*	30.77*
MTC and MTT	Absence	1	20*	0
	Merger	0	1	0
Frequency of Malformations		1	21*	0
%M.F.		2.08	41.18*	0
Ischium and Pubis	Absence	0	6	0
	O.R.	0	6	4
Frequency of Malformations		1	12*	4*
% M.F.		2.08	23.53*	10.26*
Sternum				
Sternum Bones	O.R.	1	4	6
	Absence of the Last Sternal Centre	3	7	3
Absence		0	10*	0
Frequency of Malformations		4	21	9
%M.F.		8.33	41.18*	23.08*
Head				
Nasal	O.R.	0	1	0
Parietal	O.R.	0	5	0
	Absence	0	4	0

Interparietal	O.R.	0	5	0
	Absence	0	5	0
Supra occipital	Absence	0	7*	0
Vomer	O.R.	0	7*	4
	Absence	0	1	0
Paw	Absence	0	4	0
Presphenoid	O.R.	0	1	1
	Absence	0	8*	0
Basiphoenoid	O.R.	0	2	0
	Absence	0	1	0
Hamulus	Absence	0	9*	1
Basocciptal	O.R.	0	1	0
Orbitosphenoid	Absence	0	2	0
Tympanic	O.R.	0	2	0
	Absence	0	1	0
Frequency of Malformations		0	35	6
% F.M		0	41.18*	15.38*
Vertebrae and ribs				
Vertebrae	O.R.	0	7*	0
Ribs	Malformations	5	7	3
Frequency of Malformations		5	14	3
% F.M		10.42	34.15*	7.69

EESe - ethanolic extract of *Serjania erecta*; R.O. - Reduced Ossification; % F.M.-percentage of fetuses with malformation. Statistical test: Chi-square. *Statistically significant difference; $p<0.05$.

3.4 Genetic toxic assessment: micronucleus test

The frequency of chromosomal damage did not vary between the different experimental groups indicating that EESe is not genotoxic ($p>0.05$). There were also no significant variations between the different times of analysis indicating that EESe does not have a cumulative effect ($p>0.05$), as showed in Figure 1.

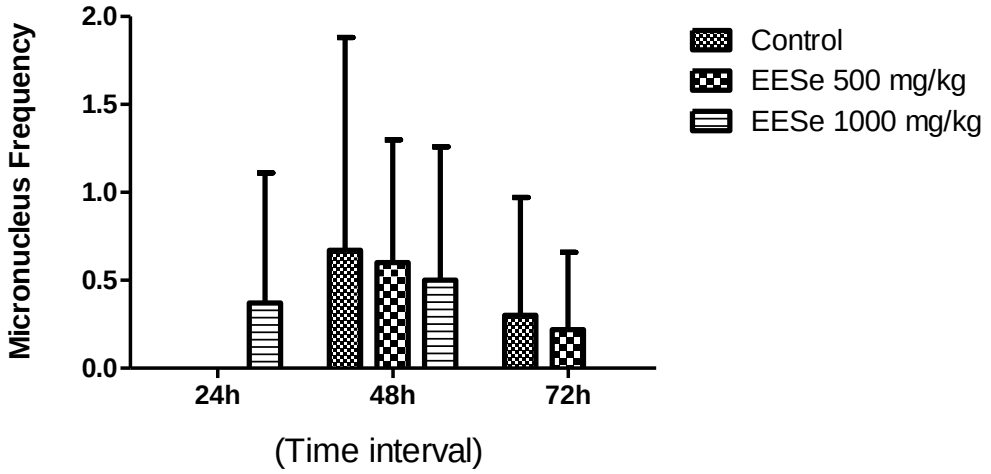


Fig. 1. Frequency of micronucleus in female animals treated with ethanolic extract *S. erecta* (EESe). Statistical test: ANOVA/Tukey ($p>0.05$).

4. Discussion

According to the literature consulted there are no data available on the maternity-toxicity, teratogenic and genotoxic effects of *S. erecta*, which demonstrates the importance, uniqueness and pioneering of this study since its leaves are popularly used for stomach pain and gastric ulcers and its root for hypertension (Guarim-Neto, Santana and Silva, 2000), symptoms that may arise during pregnancy (Berstein et al., 2020). In addition, our findings may help in its ethnopharmacological indication.

The treatment of diseases based on cultural traditions with the use of natural products, based on ethnopharmacology, is gaining increasing popularity worldwide due to the increasing difficulty of access to medicines especially in traditional and/or isolated populations. Moreover, since the beginning of human history, herbal formulas or compounds have been used to treat diseases and symptoms during pregnancy and childbirth based on previous family experiences. However, the lack of scientific studies on the effects of medicinal plants offers risk to the health of the fetus and pregnant woman (Araújo et al., 2016; Berstein et al., 2020; Theshome et al., 2021).

According to Arruda et al. (2009), the chloroform extract of *S. erecta* did not show acute toxicity at a dose of 5,000 mg/kg. The same author demonstrated that the dose of 500 mg/kg showed gastroprotective effect. The literature further mentions that the hydroalcoholic extract of *Serjania marginata* (Pericó et al., 2015) and the hydroethanolic extract of *S. caracasana* (Silva et. al., 2017) also showed no acute toxicity up to the dose of 5000 mg/kg. However, our

results indicated that the dose of 2000 mg/kg (4x the effective dose) induced maternal-toxicity and females died by day 6 of treatment. It should be clarified that this dose was used in multiple doses since the females were treated during the entire gestational period.

For the analysis of the 500 and 1000 mg/kg doses, only small alterations in the biometrical parameters were observed, suggesting the absence of toxicity and safety in the use of EESe at these doses. A significant increase in the absolute and relative weight of the heart was observed in the EESe 500 mg/kg when compared to the control and EESe 1000 mg/kg. However, this isolated data does not have biological relevance, because in general, only an increase in the weight of the heart is not considered a sign of toxicity. It is expected that xenobiotics that cause damage to the body determine, in particular, enlargement of the liver and kidneys that are organs directly involved in metabolism and excretion (Vani et al., 2018). Therefore, our results suggest no maternal toxicity at doses of 500 and 1000 mg/kg.

These safety results are reinforced by the absence of genotoxic effects evaluated by the micronucleus test. Micronuclei are fragments of DNA that are not able to incorporate into the main nucleus forming in the anaphase of mitosis. This inability may occur due to chromosomal breaks or mitotic spindle errors and that, therefore, are not amenable to repair (Valente et al., 2017). The micronucleus test, conducted to assess chromosomal damage, which is accepted by international agencies to predict the risk of consumption of a product. Therefore, it is considered an important biomarker of toxicity (Oliveira et al., 2018). In our study, we also used the collection over 72 h to verify if EESe had a cumulative effect. Still there was absence of chromosomal damage in all the analyzed times and, therefore, no cumulative effects. This reinforces the idea that EESe does not cause maternal toxicity at doses of 500 and 1000 mg/kg.

A study conducted by Moreira et al. (2019), evaluated toxicological effects after single and repeated exposure in rats to *S. marginata*, which belongs to the same family as the plant used in our study. One of the parameters evaluated were biometric parameters, of males and females, by analysing the weight of organs similar to those analysed here, and they concluded, as in our study, that there was no change in these parameters, i.e., they suggested absence of toxicity.

During pregnancy exposure to harmful agents, which easily cross the placental barrier, can lead to different effects ranging from embryo non-implantation, functional or morphological changes, delayed development, malformation and even lethality (Moraes-Souza et al., 2017).

Regarding the reproductive performance, the treatment with EESe showed a significant increase in the number of resorptions and in the resorption rate in the EESe 1000 mg/kg and a tendency to increase the number of the same parameters for the EESe 500 mg/kg. These facts suggest attention to the use of EESe in the gestational period.

Fetal resorption is an alteration resulting from the death of the fetus, with its incomplete resorption occurring after the formation of the placenta. This fetal loss can be consequent to environmental and toxicological factors (Bolon, 2014; Paula et al., 2020), a fact that deserves to be highlighted since the use of this plant in high doses has been shown to be embryofetotoxic with consequent fetal death.

Embryotoxicity refers to the disturbance in the embryonic or foetal development, at the expense of dosages that do not necessarily affect the maternal organism, which should be considered as a general toxic effect of the plant that has as a consequence the interruption of pregnancy with the death of the embryo or foetus together with its annexes (Rodrigues et al., 2011).

The placenta is the interface responsible for the transport of nutrients, gases and energy substrates that allow the proper development of the offspring, and understanding its normal development is necessary in order to recognize possible abnormalities (Fullanet et al., 2016). Placental efficiency is a parameter used to evaluate placental function in relation to maternal-fetal exchange (Fowden et al., 2009). In the present study, no differences were found in placental index or even placental efficiency. However, there was a significant decrease in the placental weight of the EESe 500 mg/kg. This fact was considered as an isolated event since the other parameters did not differ from the control group.

In relation to fetal weight no differences were observed between the groups. However, calculation of the percentage of fetuses classified as SGA, AGA and LGA in each group showed differences both for fetuses considered small, the majority, and large for gestational age in the SESe 500 group. It was also identified that for the same group the placental weight was lower ($p<0.05$) in relation to the other groups although there were no differences in the placental index, placental efficiency and mean fetal weight. It is inferred that these data are a dose induced result, but without direct correlation between them since the other parameters showed no differences. Guimarães, Meirelles and Fernandes (2015) describe that lighter placentas may be even more efficient than heavier ones, and that they adapt to maintain fetal growth by increasing

their efficiency, and may eventually limit intrauterine growth and decrease efficiency shortly before birth due to increased demand for nutrients for the fetus.

Regarding external malformations, only the rotation of the paw was found as a statistically significant alteration ($p<0.05$) in the EESe 500 mg/kg in relation to the control group, without any other statistically relevant alteration. This alteration does not compromise fetal viability, but requires attention.

As regards visceral malformations, a significant increase in hydrocephalus and hydronephrosis was observed. Most of these malformations were mild. It is known that this type of alteration can be reverted at or after birth. Thus, they may be considered as variants of normality since they are also present in the control group even if at a lower frequency. These malformations may also occur because the pregnancy was interrupted early (Taylor, 1986; Gonçalves et al., 2013; David et al., 2014).

Regarding skeletal malformations, we observed a significant increase in the frequency of absence of bones that are considered small such as phalanges in the forelimbs and hind limbs, absence of the MTT and MTC in the EESe 500 mg/kg. These alterations may be due to the premature removal of the fetuses, although the procedure is protocol and indicated by the specialized literature of the area (Taylor, 1986; Gonçalves et al., 2013). However, this fact requires attention.

According to Chahoud and Paumgartten (2009), the apparent decrease in the amount of mineralized bone, compared to what is expected for the age of development, can be classified as delayed ossification. However, due to the apparent absence of damage to cartilage and long bones of the foetuses analysed, it is likely that mineralisation occurred later. Therefore, these situations would be transitory and, therefore, would have less impact on the survival or health of the individual. Delayed ossifications are therefore included in the variations of skeletal normality (Sartori et al., 2020).

Absence of the sternum, OR of the vomer, absence of the supraocciput, presphenoid and hamulus and OR of the vertebrae were also observed in the EESe 500 group in comparison with the control group. This may be justified by the presence of fetuses considered small for gestational age, present in the control, EESe 500 and 1000 mg/kg groups (6.18%, 28% and 8.33% respectively). Even if in a global analysis of % F.M. the EESe 500 mg/kg showed an increase in frequency, we suggest that these alterations are caused in particular by the premature collection of fetuses and not only by the treatment.

The phytochemical analysis of the ethanol extract of *S. erecta* resulted in the identification and characterization as kaempferol-3-O-a-l-rhamnopyranoside compound by column chromatography and NMR spectral analysis. *S. erecta* is a rich source of phytochemicals and their previous phytochemical studies have been reported many secondary metabolites such as polyphenols, flavonols, flavones, tannins, triterpenes, diterpenes and saponins with their biological activities including antioxidant, anti-inflammatory, antimicrobial (Cardoso et al., 2013; Broggini et al., 2010), hypertension, gastroprotective activity (Broggini et al., 2010). Therefore, the ethanolic extract of *S. erecta* was evaluated its maternotoxicity nor genotoxicity effect using animal model to confirm its safety. Our preliminary study results demonstrated that higher dose caused embriolethal effect in the gestational period.

5. Conclusion

The ethanolic extract of *S. erecta* was maternotoxic at a dose of 2000 mg/kg. However, lower doses caused neither maternotoxicity nor genotoxicity. The extract increased the number and rate of resorptions which is indicative of embryolethality. Furthermore, it caused a slight increase in malformations suggesting a low teratogenic effect. In view of the above, we advise against the use of this extract in the gestational period due, in particular, to the embriolethal effect.

Conflict of interest

The authors declare no conflict of interest.

CRediT author statement

Hudman Cunha Ortiz: Biometric and analysis of biological materials

Silvia Cordeira das Neves: Biometric and analysis of biological materials

Cândida Aparecida Leite Kassuya: scientific review and statistical analysis

Henrique Rodrigues Scherer Coelho: scientific review and statistical analysis

Marcelo Luiz Brandão Vilela: scientific review and statistical analysis

Valter Aragão do Nascimento: scientific review and statistical analysis

Arunachalam Karuppusamy: scientific revision and translation of the article

Roberto da Silva Gomes: scientific review and statistical analysis:

Cláudia Andrea Lima Cardoso: scientific review and statistical analysis

Rodrigo Juliano Oliveira: study guidance, data analysis and writing of the manuscript.

Acknowledgements

The authors acknowledge to the CNPq, FUNDECT, CAPES and UFMS for providing various research grants.

References

ANVISA - Agência Nacional de Vigilância Sanitária. Guia para a condução de estudos não clínicos de segurança necessários ao desenvolvimento de medicamentos. Versão 2, Brasília, 2013.

Araújo, C. R. F. D., Santiago, F. G., Peixoto, M. I., Oliveira, J. O. D. D., & Coutinho, M. D. S. 2016. Use of medicinal plants with teratogenic and abortive effects by PregnantWomen in a City in northeastern Brazil. Rev. Bras. Ginecol. Obstet. 38, 127-31, 2016.

Arruda, A. P. C. C. B. N., Coelho, R. G., Honda, N. K., Ferrazoli, C., Pott, A., & Hiruma-Lima, C. A. 2009. Gastroprotective effect of Serjania erecta Radlk (Sapindaceae): involvement of sensory neurons, endogenous nonprotein sulfhydryls, and nitric oxide. J. Med. Food. 12(6), 1411-1415.

Barrow M. V., Taylor W. I. 1969. A rapid method for detecting malformation in rat fetuses. J. Morphol. 127, 291-306

Bernstein, N., Akram, M., Yaniv-Bachrach, Z., & Daniyal, M. 2021. Is it safe to consume traditional medicinal plants during pregnancy?. Phytother. Res. 35(4), 1908-1924.

Bolon, B. 2014. Pathology Analysis of the Placenta. In: Croy, A., Yamada, A. T., DeMayo, F. J., Adamson, S. L. The Guide to Investigation of Mouse Pregnancy. London: Elsevier. p. 175-88, 2014.

Broggini, L. S. C., Fernandes, R. S., Nogueira, T., Suzano, F. R., Caetano, A. L., Buck, H. S., Couto, L. B., França, S. C., Pereira, P. S. 2010. Behavioral and enzymatic bioassays with *Serjania erecta* Radlk., Sapindaceae, correlated with cognitive dysfunctions. Rev. Bras. Farmacogn. 20, 519-28

Cardoso, C. A. L., Coelho, R. G., Honda, N. K., Pott, A., Pavan, F. R., Leite, C. Q. F. 2013. Phenolic compounds and antioxidant, antimicrobial and antimycobacterial activities of *Serjania erecta* Radlk. (Sapindaceae). Braz. J. Pharm. Sci. 49, 775-82.

Carvalho, P.C., Santos, E. S., Schneider, B. U. C., Matuo, R., Pesarini, J. R., Cunha-Laura, A. L., Monreal, A. C. D., Lima, D. P., Antoniolli, A. C. M. B., Oliveira, R. J. 2015. Diaryl sulfide analogs of combretastatin A-4: oxicogenetic, immunomodulatory and apoptotic valuations and prospects for use as a new chemotherapeutic drug. Environ. Toxicol. Pharmacol. 40, 715–21, 2015.

Chahoud, I., Buschmann, J., Clark, R., Droga, A., Falke, H., Faqi, U., Hansen, E., Heinrich-Hirsch, B., Hellwig, J., Linck, W., Parkinson, M., Paumgartten, F. J., Pfeil, R., Platzek, T., Scialli, A. R., Semente, J., Stahlmann, R., Ulbrich, B., Wu, X., Yasuda, M., Younes, M., Solecki, R. 1999. Classification terms in developmental toxicology: need for harmonisation. Report of the second workshop on the terminology in developmental toxicology Berlin, 27-28 august 1998. Reprod. Toxicol.13, 77-82, 1999.

Cordova, S. M., Benfatti, C. S., Magina, M. D. A., Guedes, A., Cordova, C. M. M. 2010. Análise da atividade antimicrobiana de extratos isolados de plantas nativas da flora brasileira frente a *Mycoplasma arginini*, M. hominis e *Ureaplasma urealyticum*. RBAC, 42, 241-44, 2010.

Damasceno, D. C., Kempinas, W. G., Volpato, G. T., Consoni, M. 2008. Anomalias Congênitas: Estudos Experimentais. Coopmed Editora Médica, Belo Horizonte. 2008.

David, N., Maurício, M. O., Gonçalves, C. A. G., Pesarini, J. R., Strapasson, R. L. B., Kassuya, C. A. L., Stefanello, M. E. A., Cunha-Laura, A. L., Monreal, A. C. D., Oliveira, R. J. 2014. Gochnatia polymorpha ssp. floccosa: Bioprospecting of an anti-inflammatory phytotherapy for use during pregnancy. J Ethnopharmacol. 154, 370-9, 2014.

Fernandes, R. S., Costa, T. R., Marcussi, S., Bernardes, C. P., Menaldo, D. L., Rodriguéz, G. Ii, Pereira, P. S., Soares, A. M. 2011. Neutralization of pharmacological and toxic activities of Bothrops jararacussu snake venom and isolated myotoxins by *Serjania erecta* methanolic extract and its fractions. J. Venom. Anim. Toxins Incl. Trop. Dis. 17, 85-93.

Fowden, A. L., Sferruzzi-Perri, A. N., Coan, P. M., Constancia, M., Burton; G. J. 2009. Placental efficiency and adaptation: endocrine regulation. J. Physiol, 587, 3459-72.

Fulanetti, F. B., Camargo G. G. R., Ferro M. C., Randazzo-Moura P. 2016. Toxic effects of the administration of *Mikania glomerata* Sprengel during the gestational period of hypertensive rats. Open Vet. J. 6, 23-9, 2016.

Gonçalves, C. A., Silva, N. L., Mauro, M. O., David N., Cunha-Laura, A. L., Auharek, S. A., Monreal A. C. D., Vieira, M. C., Silva, D. B., Santos, F. J. L., Siqueira J. M., Oliveira R. J. 2013. Evaluation of mutagenic, teratogenic, and immunomodulatory effects of Annona nutans hydromethanolic fraction on pregnant mice. Genet. Mol. Res. 13, 4392-4405.

Gomig, F., Pietrovski, E. F., Guedes, A., Dalmarco, E. D., Calderari, M. T., Guimarães, C. L., Pinheiro R. M., Cabrini, D. A., Otuki, M. F. 2008. Topical anti-inflammatory activity of *Serjania erecta* Radlk (Sapindaceae) extracts. J. Ethnopharmacol. 118, 220–24.

Guarim-Neto, G., Santana, S. R., Silva, J. V. B. 2000. Notas etnobotânicas de espécies de sapindaceae jussieu. Acta botânica brasileira, 14, 327-34.

Guimarães, C. C., Oliveira, D. D., Valdevitea, M., Saltorattoa, A. L. F., Pereira, S. I. V., França, S. C., Pereira, A. M. S., Pereira, P. S. 2015. The glycosylated flavonoids vitexin, isovitexin, and quercetrin isolated from *Serjania erecta* Radlk (Sapindaceae) leaves protect PC12 cells against amyloid-β25-35 peptide-induced toxicity. Food Chem. Toxicol. 86, 88–94.

Guimarães, C. F., Meirelles, M. G., Fernandes, C. B. 2015. Eficiência placentária na espécie equina: quais fatores podem estar relacionados? Braz. J. Vet. Res. Anim. Sci. 52, 98-105.

Hayashi, M., Kodama, Y., Sofuni, T., Ishidate Jr., M. 1990. The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. Mut. Res. 245, 245-49.

Loomis, T. A., Hayes, A. W. 1996. Essentials of Toxicology. 4th ed. Academic Press, London, 33-46.

Manson, J. M., Kang, Y. J.1994. Test Methods for Assessing Female Reproductive and Developmental Toxicology. In: Principles and Methods of Toxicology (Hayves AW, ed.). Raven Press, New York.

Martins, J. L. R., Rodrigues, O. R. L., Sousa, F. B., Fajemiroye, J. O., Galdino, P. M., Florentino, I. F., Costa, E. A. 2015. Medicinal species with gastroprotective activity found in the Brazilian Cerrado. Fundam. Clin. Pharmacol 29, 238–51.

Moraes-Souza, R. Q., Soares, T. S., Carmo, N. O. L., Damasceno, D. C., Campos, K. E., Volpato G. T., 2017. Adverse effects of *Croton urucurana* B. exposure during rat pregnancy. J. Ethnopharmacol. 199, 328-33.

Moreira, S. S., Tamashiro, L. K., Jorge, B. C., Balin, P. S., Heredia-Vieira, S. C., Almeida, G. L., Cardoso, C. A. L., Kassuya, C. A. L., Cristina, A. A., 2019. Toxicological safety evaluation in acute and 28-day studies of aqueous extract from Serjania marginata Casar. (Sapindaceae) leaves in rats. J. Ethnopharmacol. 231, 197-204.

OECD - Test No. 421: Reproduction/Developmental Toxicity Screening Test, 2016.

OECD – Test No. 443: Extended One-Generation Reproductive Toxicity Study, 2018.

Oliveira, R.J., Kanno, T.Y.N., Salles, M.J.S., Lourenã, O.A.C.S., Ribeiro, L.R., Freira, G.A., Matiazi, H.J.O., Mantovani, M.S., Silva, A.F., 2009. Effects of the polysaccharide beta-glucan on clastogenicity and teratogenicity caused by acute exposure to cyclophosphamide in mice. Regul. Toxicol. Pharmacol. 53, 164-73.

Oliveira, R. J., Mantovani, M. S., Pesarini, J. R., Mauro, M.O., Silva, A. F., Souza, T. R., Ribeiro, L. R., 2015. 6-Dimethylaminopurine and cyclohexamide are mutagenic and alter reproductive performance and intrauterine development in vivo. Genet. Mol. Res. 14, 834-49.

Oliveira, R. J., Santos, N. D. C. L., Pesarini, J. R., Oliveira, B. C. D., Berno, C. R., Araújo, F. H. S. D., Silveira, I. O. M. F., Nascimento, R. O., Antoniolli-Silva, A. C. M. B., Monreal, A. C. D., Beatriz, A., Lima, D. P. D., Gomes, R. D. S., 2018. Assessment of genetic integrity, splenic phagocytosis and cell death potential of (Z)-4-((1,5-dimethyl-3-oxo-2-phenyl-2,3dihydro-1H-pyrazol-4-yl)amino)-4-oxobut-2-enoic acid and its effect when combined with commercial chemotherapeutics. Genet. Mol. Biol. 41, 154-66.

Paula, V. G., Cruza, L. L., Sene, L. B., Gratão, T. B., Soares, T. S., Moraes-Souza, R. Q., Damasceno, D. C., Volpato, G. T., 2020. Maternal-fetal repercussions of Phyllanthus niruri L. treatment during rat pregnancy. J. Ethnopharmacol. 254, 1-8.

Périco, L. L., Heredia-Vieira, S. C., Beserra, F. P., Santos, R. C., Weiss, M. B., Resende, F. A., Ramos, M. A. S., Bonifácio, B. V., Bauab, T. M., Varanda, E. A., Gobbi, J. I. F., Rocha, L. R. M., Vilegas, W., Hiruma-Lima, C. A., 2015. Does the gastroprotective action of a medicinal plant ensure healing effects? An integrative study of the biological effects of *Serjania marginata* Casar. (Sapindaceae) in rats. J. Ethnopharmacol. 172, 312-24.

Pott, A and Pott, V., 1994. Plantas do Pantanal. Corumbá: Embrapa – SPI,

Potrich, F. B., Baggio, C. H., Freitas, C. S., Mayer, B., Santos, A. C., Twardowschy, A., Guedes, A., Marques, M. C. A., 2014. Ação de extratos de plantas medicinais sobre a motilidade do trato gastrointestinal. Rev. Bras. Pl. Med., Campinas, 16, 750-54, 2014.

Rodrigues, H. G., Meireles, C. G., Lima, J. T. S., Toledo, G. P., Cardoso, J. L., Gomes, S. L., 2011. Efeito embriotóxico, teratogênico e abortivo de plantas medicinais. Rev. Bras. Pl. Med., Botucatu, 13, 359-66, 2011.

Roman, S. S., Dambros, A., Rosset, A., Pistore, M., Schimid, A. R., Cansian, R. L., 2014. Toxicidade materna e reprodutiva de extrato hidroalcoólico de Sonchus oleraceus l. em ratos wistar. Perspectiva, Erechim, 38, 69-77.

Sharma, N., and Vijayvergia, R., 2011. Study of primary metabolites and antimicrobial activity of Gomphrena celosioides mart. Int. J. Pharma. Bio. Sci. 2, 581-6.

Silva, F. L., Silva, J. L. V., Silva, J. M., Marcolin, L. S. A., Nouailhetas, V. L. A., Yoshida, M., Vendramini, P. H., Eberling, M. N., Barbosa-Filho, J. M., Moreno, P. R.H., 2017. Antispasmodic activity from *Serjania caracasana* fractions and their safety. Rev. Bras. Farmacogn. 27, 346-52.

Sartori, A. S., Hollenbach, C. B., Jardim, L. H., Silva, P., Mello, F. B., Mello, J. R. B., 2020. Avaliação da toxicidade pré-natal: estudo de teratogenicidade do inseticida piriproxifeno em ratos Wistar. Arq. Bras. Med. Vet. Zootec. 72, 719-728.

Staples, R. E., Schnell, V. L., 1964. Refinements in rapid clearing technic in the koh-alizarin red s method for fetal bone. Stain Technol, 39, 61-63.

Taylor, P., 1986. Pratical Teratology. Academic Press, New York.

Teshome, D., Tiruneh, C., Berhanu, L., Berihun, G., Belete, Z. W., 2021. Developmental Toxicity of Ethanolic Extracts of Leaves of Achyranthes aspera, Amaranthaceae in Rat Embryos and Fetuses. J. Exper. Pharmacol. 13, 555-63.

Valente, D., Costa-Amaral, I. C., Carvalho, L. V. B., Santos, M. V. C., Castro, V. S., Rodrigues, D. R. F., Falco, A., Silva, C. B., Nogueira, S. M., Gonçalves, E. S., Moreira, J. C., André, L. C., Teixeira, L. R., Sarcinelli, P. N., Sisenando, H. A., Oliveira, M. S., Perini, J. A., Mattos, R. C. O. C., Larentis, A. L., 2017. Utilização de biomarcadores de genotoxicidade e expressão gênica na avaliação de trabalhadores de postos de combustíveis expostos a vapores de gasolina. Ver. Bras. Saude Ocup. 42.

Vani, J. M., Monreal, M. T. F. D., Auharek, S. A., Cunha-Laura, A. L., Arruda, E. J., Lima, A. R., Silva, C. M., Antoniolli-Silva, A. C. M. B., Lima, D. P., Beatriz, A., Oliveira, R. J., 2018. A mistura do líquido da casca da castanha de caju e óleo de mamona resulta em um larvicida eficiente contra o Aedes aegypti que não altera o desenvolvimento embriofetal, o desempenho reprodutivo ou a integridade do DNA. PLoS One, 13, 1-21.

Vasconcelos, P. C. P., Spessotto, D. R., Marinho, J. V., Salvador, M. J., Junior, A. G., Kassuya, C. A. L., 2017. Mechanisms underlying the diuretic effect of Gomphrena celosioides Mart. (Amaranthaceae). J. Ethnopharmacol. 202, 85-91.

Wilson J. G., 1965. Methods for administering agents and detecting malformation in experimental animal. In: Wilson J. C., Warkany J. Teratology principles and techniques. Chicago: Univ of Chicago Press; p. 262-77.

Wise, L. D., Beck, S. L., Beltrame, D., Beyer, B. K., Chahoud, I., Clark, R. L., Clark, R., Druga, A. M., Feuston, M. H., Guittin, P., Henwood, S.M., Kimmel, C.A., Lindstrom, P., Palmer, A.K., Petrere, J.A., Solomon, H. M., Yasuda, M., York, R. G., 1997. Terminology of developmental abnormalities in common laboratory mammals (version 1). Teratology, 55, 249-92.