UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOQUÍMICA

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Evaluation of the Acute Toxicity and Antioxidant Activity of *Justicia secunda* Methanolic Extract in a Murine Sepsis Model

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DEDICATÓRIA

Dedico este trabalho aos futuros pesquisadores que hão de levar em frente estudos da *Justicia secunda* Vahl, com intuito de comprovar o conhecimento empírico de uma joia Amazônica e contribuir no resgate da medicina alternativa.

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EPÍGRAFE

“For the Lord your God has blessed you in all the work of your hand. He knows your trudging through this great wilderness. [] the Lord your God has been with you; you have lacked nothing.” Deut. 2:7

RESUMO

A sepse é uma condição clínica que envolve uma resposta imune desregulada a uma infecção, levando a complicações como disfunção orgânica. A condição começa com hiperinflamação, seguida por um aumento no estresse oxidativo e esgotamento das defesas antioxidantes. As taxas de sepse e choque séptico aumentaram acentuadamente nas últimas duas décadas. Portanto, a busca por novas terapias para auxiliar no tratamento da sepse é importante. *Justicia secunda* Vahl é uma espécie amplamente utilizada na medicina tradicional. Possui propriedades anti-inflamatórias, antinociceptivas, antioxidantes, antianêmicas, antidiabéticas e antimicrobianas devido a metabólitos secundários, como flavonoides, polifenóis, alcaloides e terpenos. Avaliamos um extrato metanólico de *J. secunda* (JSLS) em um teste de toxicidade oral aguda e como pré-tratamento em um modelo de sepse de ligadura e perfuração cecal murina (CLP). No teste de toxicidade oral aguda, avaliamos os parâmetros clínicos por 14 dias após uma dose única de 2000 mg JSLS/kg de peso corporal, bem como o parâmetro de estresse oxidativo em amostras de órgãos. Posteriormente, avaliamos a sobrevida e os parâmetros antioxidantes de camundongos sépticos pré-tratados com 400 mg de JSLS/kg de peso corporal. No teste de toxicidade oral aguda, não houve sinais de toxicidade, sugerindo que o JSLS tem uma dose letal mediana superior a 2000 mg / kg de peso corporal. O pré-tratamento com JSLS melhorou a taxa de sobrevida, os parâmetros clínicos, os níveis de antioxidantes e o perfil hematológico de camundongos sépticos. Concluímos que o JSLS pode ser aplicado como agente coadjuvante no tratamento de doenças relacionadas ao estresse oxidativo. Estudos adicionais são necessários para melhor elucidar os mecanismos, bem como a dose efetiva dos compostos químicos presentes na JSLS.

Palavras-chave: *Justicia secunda*; toxicidade aguda; sepse

ABSTRACT

Sepsis is a clinical condition that involves a dysregulated immune response to an infection, leading to complications such as organ dysfunction. The condition begins with hyperinflammation, followed by an increase in oxidative stress and depletion of antioxidant defenses. The rates of sepsis and septic shock have increased markedly in the last couple of decades. Therefore, the search for new therapies to aid in sepsis treatment is important. *Justicia secunda* Vahl is a widely used specie in traditional medicine. It has anti-inflammatory, antinociceptive, antioxidant, antianemic, antidiabetic, and antimicrobial properties due to secondary metabolites such as flavonoids, polyphenols, alkaloids, and terpenes. We evaluated a *J. secunda* methanolic extract (JSLS) in an acute oral toxicity test and as a pretreatment in a murine cecal ligation and perforation (CLP) sepsis model. In the acute oral toxicity test, we evaluated clinical parameters for 14 days after a single dose of 2000 mg JSLS/kg body weight, as well as oxidative stress parameter in organ samples. Subsequently, we evaluated the survival and antioxidant parameters of septic mice pretreated with 400 mg JSLS/kg body weight. In the acute oral toxicity test, there were no signs of toxicity, suggesting that JSLS has a median lethal dose greater than 2000 mg/kg body weight. JSLS pretreatment improved the survival rate, clinical parameters, antioxidant levels, and hematological profile of septic mice. We conclude that JSLS could be applied as a coadjutant agent to manage oxidative stress-related diseases. Additional studies are needed to better elucidate the mechanisms, as well as the effective dose of the chemical compounds present in JSLS.

Keywords: *Justicia secunda*; acute toxicity; sepsis.

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LISTA DE SIGLAS E SÍMBOLOS

1. ANVISA: Agência Nacional de Vigilância Sanitária

2. IL-4: Interleucina-4

3. IL-10: Interleucina-10

4. DNA: Ácido desoxirribonucleico

5. ATP: Adenosina trifosfato

6. CAT: Enzima catalase

7. SOD: Superóxido dismutase

8. CAT: Catalase

9. GPx: Glutationa peroxidase

10. ROS: Espécies reativas de oxigênio

11. NO: Óxido nítrico

12. CLP: Ligadura e perfuração cecal

13. Js: Justicia secunda

14. JSLS: *J. secunda* syrup

15. WHO: World Health Organization

16. lL−1β: Interleukin-1beta

17. IL-6: Interleukin-6

18. TNFα: Tumor necrosis factor alpha

19. CEUA: Committee for Animal Ethics

20. UFPA: Federal University of Pará

21. °C: Degrees celcius

22. CONCEA: Animal Experimentation Control Council

23. NR: normative resolution

24. SISGEN: National Management System of Genetic Heritage and Associated Traditional Knowledge

25. OECD: Organization for Economic Co-operation and Development

26. m: minuet

27. h: hour

28. mg: milligram

29. kg: kilogram

30. PBS: phosphate-buffered saline

31. MDA: malondialdehyde

32. CEF: ceftriaxone

33. MSS: murine sepsis score

34. mM: millimolar

35. EDTA: ethylenediaminetetraacetic acid

36. µL: microliter

37. rpm: rotations per minuet

38. TBARS: Thiobarbituric Acid Reactive Substances

39. TEAC: Trolox Equivalent Antioxidant Capacity

40. ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

41. nm: Namometers

42. GSH: Glutathione

43. DTNB: 5,5′-dithiobis (2-nitrobenzoic acid)

44. mmol: Micromolar

45. ml: Milliliter

46. ±: Standard deviation

47. ANOVA: Analysis of variance

48. %: Percent

49. RBC: Red blood cell

50. MCV: Mean corpuscular volume

51. MCH: Mean corpuscular hemoglobin

52. MCHC: Mean corpuscular hemoglobin concentration

53. RDW: Red blood cell distribution width

54. WBC: White blood cell

55. ALT: Reduced alanine aminotransferase

56. AST: Aspartate aminotransferase

Sumário

1. REFERENCIAL TEÓRICO

1.1 Plantas medicinais

As plantas medicinais são utilizadas na prevenção e cura de doenças e representam um dos principais recursos terapêuticos de algumas comunidades, principalmente as comunidades de interiores ou ribeirinhas. Diante disso, Beltreschi (1) afirma que a sua utilização pela população funciona como um complemento para as medidas tomadas junto às orientações fornecidas pela assistência médica, que geralmente indica medicamentos industrializados e às vezes inacessíveis para algumas comunidades por questões econômicas. De acordo com a ANVISA (2), as plantas medicinais possuem a capacidade de amenizar ou curar determinadas enfermidades, além de caracterizar-se como uma tradição em populações que mantêm este hábito. A tradição familiar e a escolha por uma opção de tratamento mais saudável são os principais fatores que levam à transmissão de conhecimentos acerca das plantas medicinais, porém as pesquisas etnobotânicas são de extrema importância no resgate do conhecimento tradicional, seja pela confirmação da ação terapêutica ou pelo valor cultural que este conhecimento representa para sociedade. Brasileiro et al., (3), constatou que aqueles que possuem mais idade e pouca escolaridade, detinham mais informações sobre a prática da utilização de plantas medicinais para fins terapêuticos. Ademais, Macedo et al., (4), concluiu que esse conhecimento sobre plantas medicinais pode gerar o uso equivocado, podendo vir a provocar novas patologias por intoxicação do princípio ativo. Dessa maneira, o crescente uso de remédios e/ou medicamentos à base de plantas, traz a necessidade de maiores pesquisas, cujo objetivo se reflete na minimização dos efeitos adversos, além de assegurar o uso sustentável da biodiversidade, especialmente em países como o Brasil que apresenta uma diversidade de plantas como a Justicia secunda.

1.2 Aspectos botânicos de *Justicia secunda* Vahl

Acanthaceae é uma Família de ervas, subarbustos, arbustos, lianas e raramente árvores, tendo distribuição Pantropical e possuindo aproximadamente 85 gêneros e 2.000 espécies, quase metade sendo da flora Brasileira (5). *Justicia* é o maior gênero da Família Acanthaceae, com aproximadamente 600 espécies distribuídas em regiões tropicais onde 131 ocorrem em todos os biomas brasileiros. A

espécie *de Justicia secunda* que utilizamos neste estudo, é uma herbácea nativa de região tropical proveniente da América do Sul, mas atualmente é cultivada em outras regiões como, o continente Africano (6,7). A espécie cresce bem em solos úmidos próximo a margens de rios e riachos, possuindo folhas simples e agrupamento de flores caracterizadas por seu crescimento ramificado, como mostrado na **Figura 1** (8).

Figura 1. *Justicia secunda* em flor

Fonte: Autoria própria (2021)

1.3 Composição química

Plantas da Família Acanthaceae são ricas em lignanas, alcaloides, flavonoides, terpenos, taninos, monoaminas, quinonas, glicosídeos cardioativos, saponinas, carboidratos, proteínas e ácidos graxos (9,10). Já no gênero *Justicia*, foram isolados componentes como derivados da luteolina, pirrolidona, ácidos fenólicos e lignanas (11–13). O óleo essencial desta planta contém uma abundância de ácidos graxos poli-insaturados como o ácido octadecatrienoico (14). A composição mineral das folhas consiste na presença de sódio (Na), fósforo (P), zinco (Zn), potássio (K), magnésio (Mg), ferro (Fe), cobre (Cu) e manganês (Mn), sendo micronutrientes que desempenham papeis vitais na saúde e nutrição (8,15). Estudos mostram que alguns metabólitos secundários presentes no gênero são, alcalóides, flavonoides, taninos e terpenos (16). A literatura mostra que estudos da composição fitoquímica das folhas de *Justicia secunda*, revelam a presença de metabólitos secundários como, compostos fenólicos, flavonóides, taninos, alcaloides, antraquinonas, terpenos, saponinas, esteróides e derivados de cumarina (17–20). Esses metabólitos

secundários são responsáveis pelas propriedades biológicas das plantas, sendo potenciais candidatos para medicamentos e/ou antioxidantes (21).

1.4 Atividade biológica e farmacológica de Justicia secunda.

O gênero *Justicia* é amplamente empregado na medicina tradicional para o tratamento de doenças respiratórias, gastrointestinais, diabetes, inflamação e analgésico. A parte aérea das plantas são as mais utilizadas (22–24). A literatura demonstra que espécies deste gênero possuem uma gama de atividades biológicas como antioxidante (25), anti-inflamatório (26), antimicrobiano (27), antitumoral (28), antidiabético (29), anticâncer (30) e anti-HIV (31), além de possuírem efeitos hepatoprotetor (32,33) e antianêmico (34,35) (Tabela 1).

Tabela 1. Composição fitoquímica e atividade biológica do gênero *Justicia*

Componentes	**Atividade**	**Referência**
Flavonóides	Antioxidant; Antianemico	25;33
Compostos fenólicos	Anti-inflamatório; Antidiabético; Anti-HIV	26;29;31
Terpenos	Anti-inflamatório; Antimicrobiano; Antitumoral	27;28
Alcaloides	Anticâncer	30

A *Justicia secunda* é conhecida na medicina tradicional como “sanguinária”, “insulina” e “*blood root*”, devido a coloração vermelha da decocção preparada da parte aérea da espécie. A decocção é usada para diversos fins terapêuticos como anemia, cicatrização, antipirético, hipertensão e diabetes (13,34–36). Estudos demonstraram atividades biológicas da *Justicia secunda* como, anti-falciforme, antianêmico, hepatoprotetor, anti-inflamatório, antimicrobiano, antioxidante e sequestro de radicais superóxido (6,11,18–20,32,37–39).

Alguns metabólitos secundários importantes para as plantas são os taninos que têm a capacidade de desnaturar proteínas, e possuem propriedades antimutagênicas,

antivirais, antimicrobianas e redução de espécies reativas (17). Os terpenos são o maior grupo de metabólitos secundários, estando presentes nos óleos essenciais e possuindo atividades biológicas como, antituberculose, anticâncer, ansiolíticas e mutagênicas (21).

Os compostos fenólicos têm como principal atividade, suas propriedades antioxidantes, o principal mecanismos sendo por redox, inibindo ou retardando a oxidação de moléculas, tendo a capacidade de melhorar o sistema antioxidante endógena, prevenindo danos oxidativos e melhorando o equilíbrio redox, assistindo no tratamento de diversas patologias como: câncer, doenças hepáticas, doenças inflamatórias e infecções bacterianas (40). Contudo, quando os antioxidantes entram em desequilíbrio com espécies reativas, elevando suas concentrações, levam ao estresse oxidativo, sendo um gatilho que provoca danos ou morte celular e pode desenvolver diversas patologias. Os radicais livres têm papel essencial nos processos biológicos, como fagocitose de bactérias e sinalização redox (41–43).

Pertencente à família dos compostos fenólicos, podemos citar os flavonoides, que são abundantes em plantas, frutas e sementes, desempenhando funções como, regulação no crescimento celular, atração de polinizadores e proteção contra estresse biótico e abiótico. A síntese destes fitoquímicos, é induzida por estresse ultravioleta, toxicidade por metais pesados, ou baixa temperatura e condições escassas de nutrientes (44). A atividade biológica mais relevante dos flavonoides é sua capacidade antioxidante que envolve mecanismos de inibição, sequestro direto e quelação de radicais, como também ativação de defesas antioxidantes (45,46). Devido a estes mecanismos, os flavonoides têm a capacidade de regular a resposta inflamatória, possuem atividade antitumoral e o potencial de prevenir doenças neurodegenerativas pela redução do estresse oxidativo e aumento da função de células cerebrais (47,48).

Dessa maneira, o estresse oxidativo influencia diversas vias de sinalização celular, devido a produção exacerbada de citocinas pró-inflamatórias (interleucina -1β, IL-6 e fator de necrose tumoral alfa (TNFα) (49–52), essa produção implica forma subjacente os mecanismos fisiopatológicos de algumas doenças. Dentre essas, podemos citar: Câncer, dislipidemias, doenças renais crônicas e doenças hepáticas crônicas. Diante do exposto, o tratamento de doenças infecciosas envolve a morte direta do agente infeccioso ou através da modulação da resposta imune do hospedeiro para eliminar patógenos sem causar danos às células do hospedeiro (53). Com o intuito de investigar agentes terapêuticos inovadores, tem-se estudado o potencial dos

componentes bioativos de plantas que regulam as respostas imunes por mecanismos imunomoduladores, modulando a produção de citocinas e vias de sinalização imunológica (54).

1.5 Sepse

Sepse é uma resposta desregulada do hospedeiro frente a uma infecção mediado pelo aumento de citocinas pró-inflamatórias, estresse oxidativo e alterações na microcirculação, que pode levar à falência de múltiplos órgãos, sendo uma das principais causas de morte em unidades de terapia intensiva (UTI) (55,56). O diagnóstico precoce e início rápido da terapia, são fatores importantes que influenciam no prognóstico do paciente (57). Conforme o *World Health Organization* (58), em 2017, foram reportados 49 milhões de casos e 11 milhões de mortes relacionadas à sepse, sendo responsável por aproximadamente 20% das mortes, mundialmente. Neonatos e populações vulneráveis, como pacientes em UTI e imunossuprimidos são os mais atingidos. No Brasil, o ano de 2023 constatou o maior número de casos de infecção e choque séptico desde 2005, conforme o Instituto Latino-Americano de Sepse (59). Infecções bacterianas representam a maioria dos casos dessa patologia (60). Contudo, as infecções fúngicas são cada vez mais frequentes em pacientes imunossuprimidos e críticos, causando uma morbidade e mortalidade significativas (61). A sepse viral tem menor incidência e mesmo não tendo dados epidemiológicos, sua incidência tem se elevado em pacientes comprometidos (62). A terapia inicial, padrão em todos os casos de sepse, é o uso imediato de antibióticos de amplo espectro (63).

1.5.1 Patogênese da sepse

A patogênese da sepse é complexa, além do tipo de infecção e a resposta inicial do hospedeiro, envolve características heterogêneas de inflamação, ativação da coagulação, endotélio vascular, o sistema complemento, imunossupressão e alterações na microbiota (64). Na maioria dos casos, a sepse é causada por bactérias comumente gram-negativas. A lise bacteriana ou divisão celular, libera endotoxinas tais como os lipopolissacarídeos desencadeando uma cascata de eventos no organismo do hospedeiro. Esta resposta inflamatória exacerbada resulta em danos

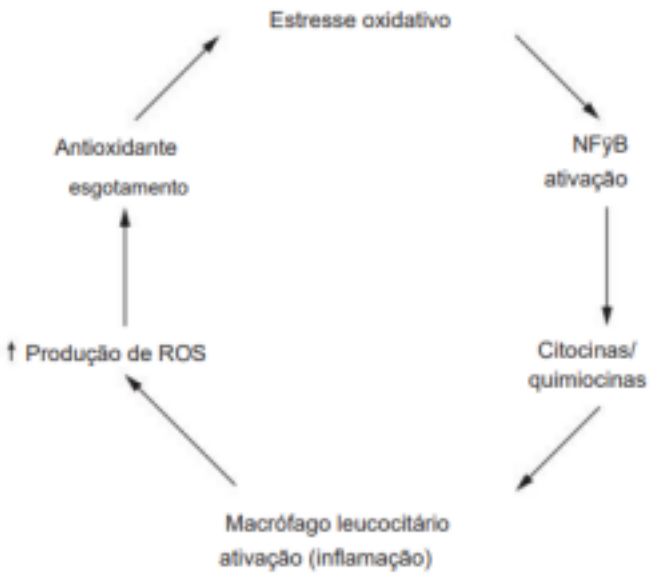
endoteliais e em órgãos, incluindo disfunções cardiovasculares, hepáticas, pulmonares e renais (57,60).

A disfunção orgânica correlacionada à imunossupressão pode evoluir para choque séptico, se caracterizando pela disfunção de múltiplos órgãos. Esse fenômeno é precipitado pelo aumento de citocinas anti-inflamatórias, como IL-4 e IL-10, resultando na perda da capacidade efetora das células do sistema imunológico, acompanhada de um aumento na apoptose e piroptose dessas células, bem como um incremento das células T reguladoras (Tregs) (65).

1.5.2 Estresse oxidativo na sepse

Quando há a instauração de um ambiente inflamatório, verifica-se um desequilíbrio na produção de espécies reativas de oxigênio e espécies reativas de nitrogênio, acarretando uma interrupção na sinalização redox e dano oxidativo tecidual, evidenciado nos componentes lipídicos, no DNA ou proteínas, sendo este fenômeno conceituado como quadro de estresse oxidativo, o qual culmina em uma piora do quadro clínico e na sobrevida dos pacientes (66). O cenário de estresse oxidativo também está intrinsecamente ligado à inflamação, tendo em vista que ele é responsável pela própria inflamação através da ativação direta do fator de transcrição nuclear kappa B (NF-kB), gerando uma espécie de circuito (Figura 2) (67,68)

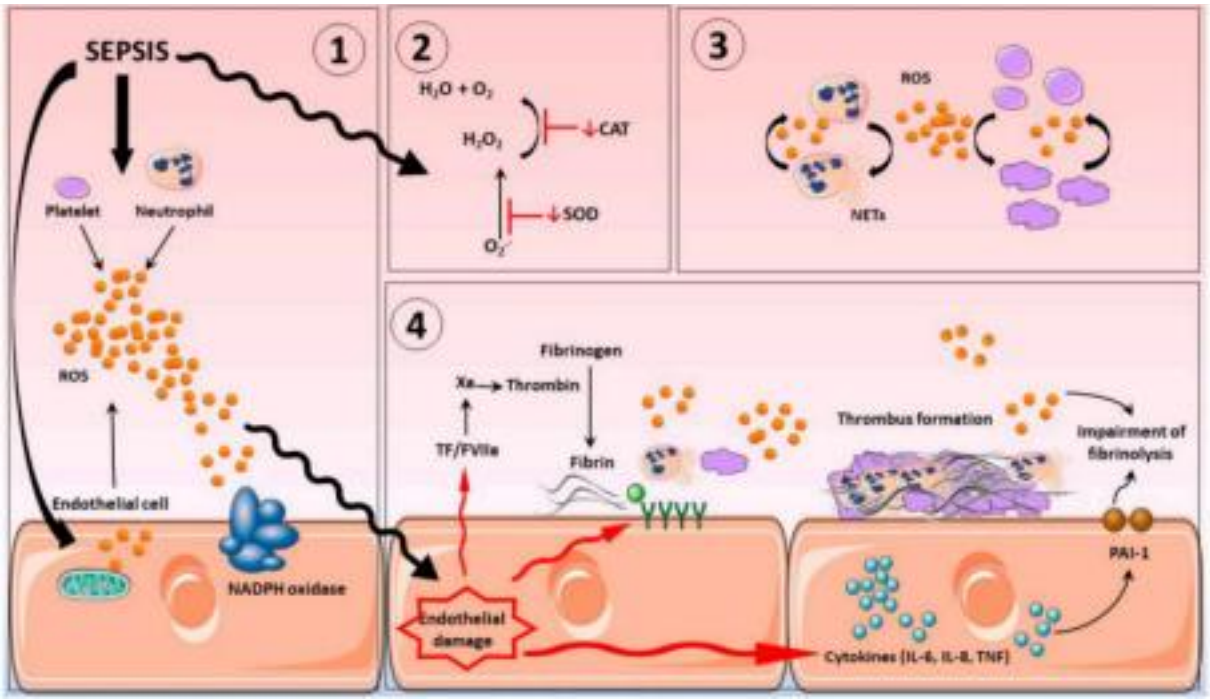
Figura 2. O ciclo envolve estresse oxidativo e inflamação. ROS: espécie reativa de oxigênio, NFkB: fator nuclear Kappa B.



Fonte: adaptado de Varizi e Rodriguez-Iturbe (2006) (68).

No contexto da resposta inflamatória da sepse, se observa um desequilíbrio entre componentes oxidantes e antioxidantes, levando a estresse oxidativo (69). Esse aumento de espécies reativas de oxigênio, consequentemente resulta em uma alta demanda de enzimas antioxidantes superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx) plasmáticas e teciduais, a fim de restaurar o equilíbrio oxidativo (70). Entretanto, com a resposta inflamatória intensa da síndrome, essas defesas não são suficientes para neutralizar a quantidade excessiva de espécies reativas de oxigênio (ROS), instaurando o quadro de estresse oxidativo, que causa danos oxidativo tecidual, com elevados níveis de substâncias reativas ao ácido tiobarbitúrico e diminuição da co-atividade antioxidante (Figura 3) (71).

Figura 3. Indução estresse oxidativo na sepse, através do aumento de Espécies Reativas de Oxigênio, sobrecarregando e esgotando o Sistema antioxidante endógeno (SOD, CAT e GPx), causando danos nos tecidos.



Fonte: Lopes-Pires, 2021 (71).

Na sepse, também ocorre um aumento na produção do vasodilatador, óxido nítrico (NO), provocando vasodilatação e permeabilidade do endotélio (72). Um aumento de NO leva a disfunção mitocondrial pela inibição da cadeia respiratória, sugerindo uma associação entre comprometimento mitocondrial e agravamento de sepse (73), isso ocorre devido ao desacoplamento parcial da fosforilação oxidativa mitocondrial, reduzindo os níveis de ATP e aumento de lactato nos vasos sanguíneos, sendo este um biomarcador de sepse.

Novas estratégias em relação aos sobreviventes da sepse incluem modulação do sistema imune e abordagem da disfunção mitocondrial (74). Algumas meta-análises mostraram que o uso de vitamina C intravenosa em pacientes sépticos apresentou redução na mortalidade a curto prazo, sendo tal vitamina um potente antioxidante e anti-inflamatório com efeito vasoconstrição (75). Enquanto Lamontagne et al (76), mostrou que o uso dessa vitamina concomitante com terapias vasopressoras em pacientes sépticos resultou em maior risco de morte.

Singer et al. (77), têm proposto modelos experimentais como de sepse devido à extrema variabilidade e às dificuldades em definir o estágio da sepse humana, estes modelos visam alcançar estudos reprodutíveis sobre a fisiopatologia e novos impactos terapêuticos. Os modelos experimentais devem replicar o ritmo e a gravidade desta patologia em humano na Unidade de Terapia Intensiva, estágios hemodinâmicos e imunológicos, características histológicas dos órgãos-alvo e mostrar variabilidade

entre os indivíduos. Três modelos principais são empregados para produzir sepse experimental, injeção de endotoxina, infusão de bactérias vivas intravasculares ou intraperitoneal, e indução de peritonite fecal.

O modelo mais utilizado em estudos pré-clínicos é a punção e ligadura cecal, devido sua capacidade de refletir a complexidade da sepse humana. Um dos principais focos infecciosos é de origem abdominal, sendo que as bactérias *Staphylococcus aureus* e *Escherichia coli* são comensais do trato gastrointestinal de camundongos e podem ser encontradas nas fezes desses animais. Este modelo satisfaz muitos critérios essenciais para um modelo experimental por apresentar lesão tecidual, ser uma fonte de tecido necrótico e uma fonte de infecção focal que eventualmente promove translocação bacteriana, ativação da resposta inflamatória e choque séptico (78).

Estudos utilizando o modelo de ligadura e perfuração cecal (CLP) demonstram que alguns extratos vegetais têm grande potencial para o tratamento da sepse, devido à diminuição da liberação de fatores inflamatórios e estresse oxidativo, sendo a principal base patológica da disfunção de órgãos causada pela sepse (79). Conforme (80), isoflavonas, da *Astragalus membranaceus*, que possuem funções anti-inflamatória, antioxidante e antitumoral, mitigaram danos patológicos nos pulmões de animais induzidos com sepse por CLP.

Na revisão sistemática proposto por Navegantes-Lima., et al (81), avaliou a integridade da barreira intestinal em animais que receberam compostos fenólicos, mostrou que estes agentes possuem efeitos benéficos sobre a inflamação intestinal, possivelmente devido à melhora da expressão de moléculas pró-inflamatórias, bem como *upregulation* da expressão de enzimas antioxidantes.

Além dos efeitos benéficos dos compostos fenólicos, podemos ressaltar o uso também de nutracêuticos no tratamento e/ou prevenção de algumas doenças crônicas e agudas prevalentes (82). Seus mecanismos de ação, aparentemente envolvem ativação e/ou supressão de células imunes e modulação do sistema redox, sendo benéficos no uso da prevenção patofisiológica (83).

Anteriormente nosso grupo mostrou que outros produtos naturais em modelo de sepse murino tem se mostrado eficaz como terapia alternativa. Segundo (81), nutracêuticos com efeitos imunomoduladores e antioxidantes, como *Agaricus brasiliensis*, protegeu camundongos contra a sepse, reduzindo os níveis sistêmicos de citocinas pró-inflamatórias, aumentando as enzimas antioxidantes e inibindo o

dano oxidativo.

Nesse contexto, considerando que a literatura demonstra atividade antioxidante, imunomoduladora e hepatoprotetor de *Justicia secunda*, é esperado que tenham resultados promissores que visam terapias de origem vegetal inovadoras. Além disso, no desenvolvimento de um nutracêutico, faz-se necessário avaliar sua toxicidade a fim de garantir a segurança da droga

Portanto, o presente estudo teve como objetivo avaliar a toxicidade oral aguda do extrato metanólico de *Justicia sp. in vivo* utilizando 2 g/kg de extrato vegetal, e o seu efeito em modelo de sepse induzida em animal. A metodologia de toxicidade aguda utilizada foi o teste limite presente no estudo proposto por de Onoja et al, (84).

Logo, é de grande importância avaliar diferentes atividades biológicas, tais como antimicrobiana, antioxidante e imunomoduladora, de produtos obtidos do extrato das folhas de Justicia secunda (Vahl), bem como avaliar em modelo de sepse em animal, visando identificar possíveis componentes com potente ação antimicrobiana e imunomoduladora in vitro e in vivo. Pois a sua caracterização e seus efeitos farmacológicos podem ser de grande significância tanto para a área da saúde como para a área industrial, mas principalmente para a economia. Além de comprovar a importância da etnobotânica.

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3. ARTIGO

Article

Evaluation of the Acute Toxicity and Antioxidant Activity of *Justicia secunda* Methanolic Extract in a Murine Sepsis Model

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Abstract: Sepsis is a clinical condition that involves a dysregulated immune response to an infection, leading to complications such as organ dysfunction. The condition begins with hyperinflammation, followed by an increase in oxidative stress and depletion of antioxidant defenses. Therefore, the search for new therapies to aid in sepsis treatment is important. *Justicia secunda* Vahl is a widely used herb in traditional medicine. It has anti-inflammatory, antinociceptive, antioxidant, antianemic, antidiabetic, and antimicrobial properties due to secondary metabolites such as flavonoids, polyphenols, alkaloids, and terpenes. We evaluated a *J. secunda* methanolic extract (JSLS) in an acute oral toxicity test and as a pretreatment in a murine cecal ligation and perforation (CLP) sepsis model. In the acute oral toxicity test, we evaluated clinical parameters for 14 days after a single dose of 2000 mg JSLS/kg body weight, as well as oxidative stress parameter in organ samples. Subsequently, we evaluated the survival and antioxidant parameters of septic mice pretreated with 400 mg JSLS/kg body weight. In the acute oral toxicity test, there were no signs of toxicity, suggesting that JSLS has a median lethal dose greater than 2000 mg/kg body weight. JSLS pretreatment slightly improved the survival rate, clinical parameters, antioxidant levels, and hematological profile of septic mice. We conclude that JSLS could be applied as a coadjutant agent to manage oxidative stress-related diseases. Additional studies are needed to better elucidate the mechanisms, as well as the effective dose of the chemical compounds present in JSLS.

Keywords: *Justicia secunda*; acute toxicity; sepsis.

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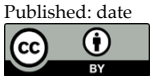

1. Introduction

Sepsis is defined as life-threatening organ dysfunction due to dysregulation of a host's response to an infection; it is mediated by an increase in multiple proinflammatory cytokines (1,2). According to the World Health Organization (WHO), each year approximately 20% of global deaths are related to sepsis, with the highest rates in lower-middle-income countries and vulnerable populations (3). Severe sepsis can develop rapidly due to a systemic inflammatory response, which is triggered by the activation of innate immune cells that lead to the

production of inflammatory cytokines. Thus, it is crucial to diagnose and start treating sepsis in its early stages (4,5).

In mice, cecal ligation and puncture (CLP) is often used to induce sepsis. Based on studies that use this model, some plant extracts have great potential for treating sepsis because they can attenuate the release of inflammatory factors and oxidative stress, the main pathological factors that lead to organ dysfunction in the context of sepsis (6,7). Studies using animal models have shown that the oral administration of phenolic compounds have beneficial effects on intestinal inflammation, possibly due to improved expression of pro-inflammatory molecules, as well as upregulation of antioxidant enzymes (8). According to Navegantes-Lima (9), nutraceuticals with immunomodulatory and antioxidant effects, such as *Agaricus brasiliensis*, protect mice against sepsis by reducing systemic pro-inflammatory cytokine levels, increasing antioxidant enzymes, and inhibiting oxidative damage. Phytochemicals, such as polyphenols, are potent immunomodulators with anti-inflammatory and cytoprotective activity; thus, they show protective and therapeutic effects in the context of sepsis (10).

Antioxidants can prevent and treat diverse pathologies due to the protection they provide to cells; an imbalance between these agents and reactive species results in oxidative stress (11). Oxidative stress influences several cellular signaling pathways that underlie chronic diseases, including inflammation, disruption of iron homeostasis, cancer, dyslipidemia, and chronic kidney and liver diseases. These conditions may begin with a low-grade inflammatory response, followed by excessive production of pro-inflammatory cytokines, such as interleukin (IL)−1β, IL-6, and tumor necrosis factor alpha (TNFα) (12–17).

Justicia is the largest genus of the Acanthaceae family, with nearly 600 species, many of which are widely used in traditional medicine. We focused on *Justicia secunda* Vahl, a natural herb from Central and South America that is widely used in traditional medicine (18). Studies on *J. secunda* leaf extracts have demonstrated its antioxidant, anti-inflammatory, antinociceptive, and antimicrobial properties, and efficacy in treating sickle-cell disease (19–24). Animal studies have shown that *J. secunda* leaves have hepatoprotective and antioxidant activity as well as lipid peroxidation inhibition, possibly through bioactive compounds such as phenols, flavonoids, tannins, saponins, and alkaloids (25,26). Thus, we aimed to evaluate the antioxidant effect of a *J. secunda* leaf methanolic extract in a murine sepsis model.

2. Materials and Methods

2.1 Ethics Statement

This study was carried out in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the Brazilian National Council of Animal Experimentation and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The institutional Committee for Animal Ethics of the Federal University of Pará/UFPA (CEUA) approved the procedures carried out in this study (protocol no. 6932251121.

2.2 Mice

Male and female Swiss mice (*Mus musculus*) weighing 25–30 g and 7–8 weeks old, obtained from the Animal Facility of the Federal University of Pará, were used in this study. The mice were kept in cages at 25 ± 1 °C, a 12-h photoperiod, and with food and water available *ad libitum*. They were acclimatized before use. All experimental animals were euthanized by high doses of anesthetics, followed by exsanguination, in accordance with the Animal Experimentation Control Council (CONCEA) normative resolution (NR) n° 37, 15 of February 2018. All euthanized animals were frozen until proper disposal in accordance with international standards (27).

2.3 Plant Collection, Extract Preparation, and Antioxidant Assay

Research authorization was obtained from the National Management System of Genetic Heritage and Associated Traditional Knowledge (SISGEN), in the form of provisional measure N°A90EE8D, for the purpose of collecting *J. secunda* leaves. Samples were collected from the municipality of Dom Eliseu in the State of Pará (4°24'15"S, 47°41'16"W). The leaves were collected in October, the dry season in Pará, and then identified and authenticated at the *João Murça Pires* herbarium at the *Museu Paraense Emílio Goeldi* with voucher specimen number MG 236144. The leaves were oven-dried at 40 °C for 48 h and then pulverized using a mechanical grinder. The extraction was carried out using a published method (28). In brief, the dry sample was incubated in methanol for 10 days, with intermittent shaking. After filtration on cotton wool and Whatman filter paper, the filtrate was concentrated using a rotary evaporator and dried in a hot air oven at 35 °C to obtain a methanolic brute extract of *J. secunda*. The brute extract was submitted to antioxidant, cytotoxic, and antimicrobial assays (data not shown).

2.4 Preparation of Justicia sp. Syrup (JSLS)

For the *in vivo* tests, a simple syrup was used as a vehicle to prepare the herbal formulation, according to a previous study (29) in which the authors used syrup formulations to orally administer dried aqueous root extract. The simple syrup (85% sucrose) was purchased from a compounding pharmacy and prepared in accordance with the *Formulário Nacional da Farmacopeia Brasileira* (30). A methanolic extract of *J. secunda* was used to prepare JSLS that was used for oral administration in the following assays.

2.5 Design of In Vivo Experiments

2.5.1 Oral Acute Toxicity Test

The oral acute toxicity of JSLS was evaluated *in vivo* with an oral acute toxicity test, according to the Organization for Economic Co-operation and Development (OECD) guideline n° 425 (up and down procedure) (31). For this test, 10 female Swiss mice, 8–12 weeks old and weighing 20–30 g, were used. Five mice were submitted to the limit test—they received 2000 mg JSLS/kg body weight—and five were dosed with the vehicle. In accordance with the guidelines, in the first step, the aforementioned dose was administered to a single female mouse, which was observed at 0 min, 30 min, 4 h, and 24 h after administration. After this treated mouse had survived for 24 h, four additional mice were

administered the same dose under the same conditions. Following this period, the mice were observed daily for 14 days to evaluate clinical parameters and to record body weight, feed consumption, and death. After day 14, all mice were euthanized by the recommended method with a high dose of anesthetic (subcutaneous administrations of > 100 mg ketamine/kg body weight and > 10 mg xylazine/kg body weight), followed by exsanguination, in accordance with CONCEA NR n° 37, 15 of February 2018 (27), and NR n° 6, 10 of July 2012 (32). The same procedure was followed for the vehicle-treated control group comprising five mice; the vehicle was a simple syrup administered at the same volume as that of the treated group. The signs of toxicity that mice can display include changes in skin, fur, eyes, and mucus membranes; changes in the respiratory, circulatory, autonomic, and central nervous systems; altered somatomotor activity and behavioral patterns; convulsions; the lateral position; tremor; salivation; diarrhea; lethargy; sleep; coma; gasping; and vocalization. These clinical signs were used as humane endpoints for experimental animals as described previously (33).

The liver, kidney, and heart were collected for macroscopic evaluation. They were washed in 1× phosphate-buffered saline (PBS), homogenized (Ultra Turrax T25 Basic) in 1× PBS, and centrifuged at 3,000 rpm for 10 min. The supernatant was collected and stored in microtubes at –80 °C and submitted to the malondialdehyde (MDA) assay.

2.5.2 Survival and Pre-Treatment Assessment

A murine model of moderate sepsis induced by CLP was used. The mice received oral treatment 24 h prior to and immediately before undergoing the CLP surgery. The mice were divided into the following groups: (i) sham (n = 5), no JSLS pretreatment and underwent sham surgery; (ii) CLP (n = 5), the mice received vehicle 24 h prior to and immediately before undergoing the CLP surgery; (iii) JSLS+CLP (n = 5), the mice received 400 mg JSLS/kg body weight 24 h prior to and immediately before undergoing the CLP surgery (n = 5); and (iv) CEF+CLP (n = 5), the mice received 20 mg ceftriaxone/kg body weight intraperitoneally 24 h prior to and immediately before undergoing the CLP surgery. The JSLS test dose was chosen based on studies with *J. secunda* methanolic extracts carried out in rodent models (34,35). The survival rate, food consumption, and body weight were evaluated daily. In addition, the murine sepsis score (MSS) was used to assess the severity of sepsis in mice based on observation of their behavioral pattern after CLP. After 12 days, all surviving mice were euthanized as described previously. The survival rate was determined based on a survival assessment score (36) with adaptations (37).

2.5.3 CLP Model

The CLP model was adapted to mice according to a published study (38). The mice were anesthetized with an intraperitoneal injection of 75 mg ketamine/kg body weight and 15 mg xylazine/kg body weight. The abdominal region was shaved, and then a small incision was made to externalize, bind, and puncture the cecum with a 22G needle 1 cm from the ileocecal valve. The mice were sutured using a 3-0 silk suturing kit, the area was cleaned with 70% alcohol, and the mice were administered

sterile saline solution subcutaneously to prevent dehydration. The mice were allowed to recover in a cage under a heating lamp for 1 h.

2.5.4 Obtaining Peritoneal Lavage, Blood, and Organs, and Hematological Analysis

Twenty-four hours following CLP induction, the mice were sedated with an intraperitoneal injection of 75 mg ketamine/kg body weight and 15 mg xylazine/kg body weight. The peritoneum was washed with 3 mL of 1× PBS containing 1 mM ethylenediaminetetraacetic acid (EDTA). Blood samples (500 µL) were collected intracardially 24 h after CLP induction. The blood samples were stored in tubes containing EDTA for a hemogram that measured red blood cells, hemoglobin, hematocrit, the mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCHC) in a Hematoclin Vet 2.8 hematological system. The heart, liver, and lungs were collected 24 h after CLP. Each collected organ was washed with and stored in 1× PBS. The organs were macerated in a tissue homogenizer (Ultra Turrax T25 Basic) in 1× PBS and centrifuged at 3000 rpm for 10 min. Then, the supernatant was collected and stored at –80 °C for further analysis.

2.5.5 Evaluation of Oxidative Stress Parameters

2.5.5.1 Thiobarbituric Acid Reactive Substances (TBARS) Assessment

Lipid peroxidation was evaluated in organ and peritoneal liquid samples based on the reaction of MDA and other substances with thiobarbituric acid (TBA; Sigma-Aldrich T5550) at a low pH and high temperature. This reaction forms a pinkish MDA-TBA complex, the absorbance of which is measured at 535 nm. The procedure was performed as described previously (39)(40).

2.5.5.2 Trolox Equivalent Antioxidant Capacity (TEAC) Assay

The TEAC was determined according to the antioxidant capacity equivalent to 6-hydroxy-2,5,7,8-tetramethylcromono-2-carboxylic acid (Trolox, Sigma-Aldrich 23881-3), which describes the dynamic balance between pro-oxidant and antioxidant compounds. The diammonium salt, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Sigma-Aldrich) was incubated with potassium persulfate (Sigma-Aldrich) to produce ABTS·+ radical, a green-blue chromophore. The inhibition of ABTS·+ formation by antioxidants in the samples was expressed as Trolox equivalents, determined based on absorbance at 740 nm using a calibration curve plotted with different amounts of Trolox (Sigma-Aldrich). The method was performed as described previously (41), with modifications (42).

2.5.5.3 Determination of the Glutathione (GSH) Levels

The GSH levels in the organ supernatants (after deproteinization) and peritoneal liquid samples. This assay is based on the production of a yellow color when 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) is added to compounds containing sulfhydryl groups. The GSH level was determined using a standard curve constructed with different concentrations of GSH in the reduced form. The absorbance was recorded at 412 nm, and the results are expressed in µmol/mL (43).

2.6 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8 software (GraphPad Software Inc.). The data are presented as mean ± standard deviation (SD). Kaplan–Meier analysis followed by the log-rank test was used to assess survival. Other data were analyzed using analysis of variance (ANOVA) followed by the Tukey test for multiple comparisons. A p-value < 0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. Acute Oral Toxicity Test

3.1.1. Survival, Behavior, Body Weight, and Feed Consumption

In the acute oral toxicity test conducted with a dose of 2000 mg JSLS/kg body weight, none of the mice died (Figure 1A). Throughout the 14-day observation period, the body weight of the test and control groups increased slightly, with no difference between the groups (Figure 1B). Feed consumption remained constant in the control group throughout the entire study period, while the test group showed decreased feed consumption until day 11, after which time it increased until day 14 (Figure 1C). The first 4 h following administration are crucial in determining the toxicity of a chemical. During this time, there were alterations in behavioral patterns in the control group. The test group showed slight alterations in the fur, eyes, and level of consciousness, as well as decreased activity. These clinical signs normalized after the first 4 h and throughout the 14-day observation. The test group did not present respiratory impairment. There were no clinical signs of acute toxicity (convulsions, the lateral position, tremor, gasping, and vocalization) throughout the 14-day observation (Figure 1D).

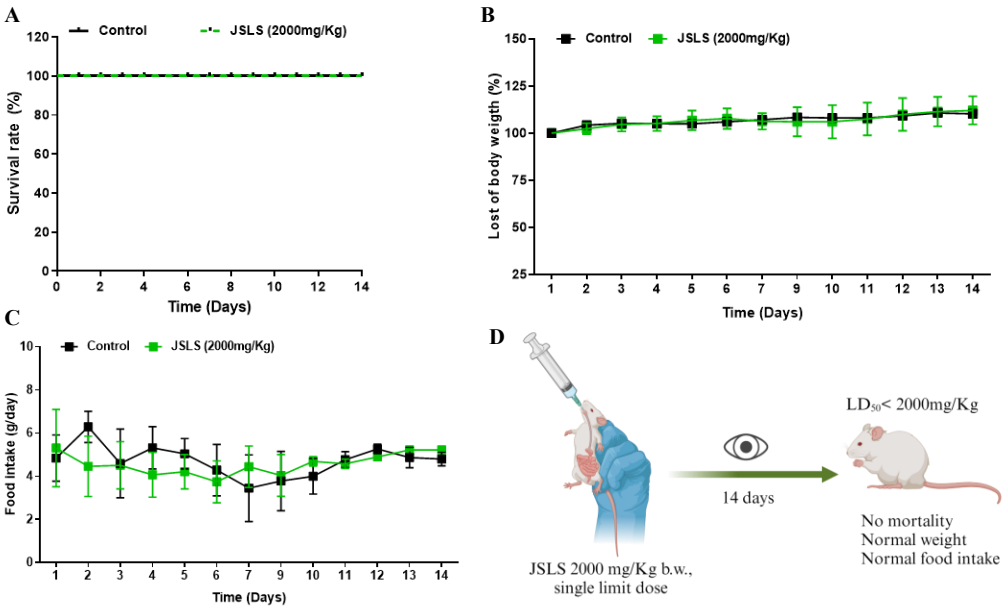

Figure 1. The (A) survival rate, (B) body weight, and (C) feed consumption of the control (vehicle) and test (2,000 mg JSLS/kg body weight) groups during the acute oral toxicity test. (D) An outline of the acute oral toxicity test.

3.1.2 Relative Organ Weights and Oxidative Stress in Organs

We calculated the liver, kidney, and heart weights relative to the body weight by using the following formula: (organ weight × 100)/body weight. The relative liver weight was 4.97 ± 0.45 mg/kg for the test group and 4.72 ± 0.48 mg/kg for the control group. The relative kidney weight was 1.15 ± 0.13 mg/kg for the test group and 0.98 ± 0.02 mg/kg for the control group. The relative heart weight was 0.39 ± 0.02 mg/kg for the test group and 0.43 ± 0.01 mg/kg for the control group (Figure 2A). There were no significant differences between the groups. Regarding oxidative stress, the MDA levels did not differ significantly between the test and control groups in the liver, kidney, or heart (Figure 2B).

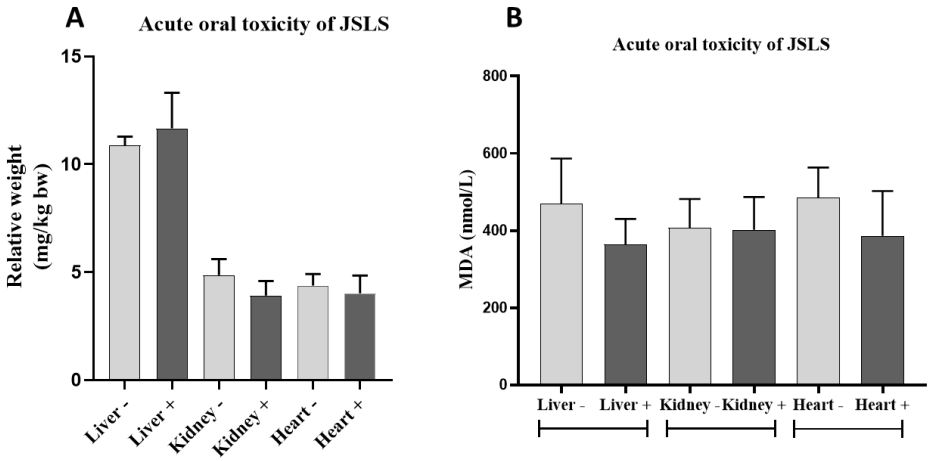

Figure 2. (A) The relative organ liver, kidney, and heart weights in the control (–) and test (+) groups. (B) The MDA levels in the liver, kidney, and heart in the control (–) and test (+) groups.

3.2 Survival Rate, MSS, Feed Consumption, and Relative Weight After JSLS Pretreatment Followed by CLP

We observed all mice for 12 days after sham surgery or CLP induction. None of the mice died in the sham group (Figure 3A). In the CLP group, 20% of the mice died within 24 h following CLP, and by 96 h, 50% of the mice had died. In the JSLS group, 40% of the mice died within 24 h (Figure 3A). The 12-day survival rate was 100% for the sham group, 60% for the JSLS group, 50% for the CLP group, and 80% for the CEF+CLP group (Figure 3A).

Compared with the sham group, the three groups submitted to CLP showed an increase in the MSS up to 72 h following the procedure, confirming that sepsis induces alterations in appearance, level of consciousness, activity, stimulus response, eyes, and respiratory rate and quality (Figure 3B). All groups submitted to CLP showed a significant difference in the MSS compared with the sham group up to 120 h following CLP, after which the MSS returned to 0, indicating normal clinical behaviors (Figure 3B). The MSS remained slightly elevated in the surviving mice in the CLP until 168 h after CLP induction; after that time, it remained stable until the end of the study (Figure 3B). Beginning at 144 h following CLP induction, the JSLS group showed significant improvements: The mice were active and had a normal appearance and normal breathing until the end of the study (Figure 3B).

Figure 3C shows data on food intake. All groups submitted to CLP showed reduced feed intake up to 24 h after CLP. At 48 h after CLP, the

CEF+CLP group presented feed intake very close to the sham group. For the JSLS group, feed intake returned to the sham group level by 120 h. Subsequently, the JSLS group showed slightly increased feed intake at 216 and 240 h compared with the other groups.

All groups submitted to CLP showed weight loss up to 96 h following the procedure. The relative weight was reduced for the CLP group compared with the sham group up to 120 h. Over time, the CLP group was able to recover from this weight loss. The JSLS group showed variations in relative weight throughout the observation period. The relative weight of this group was reduced by 12% compared with the sham group, and it remained at this reduced level until the end of the study.

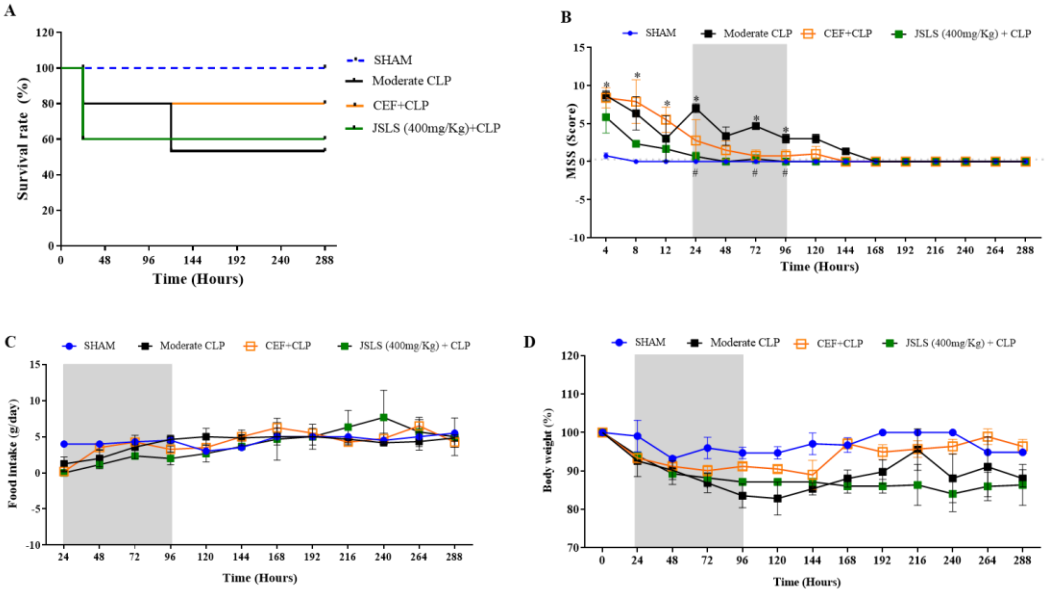

Figure 3. The (A) survival rate, (B) MSS, (C) feed consumption, and (D) relative body weight for the four groups of mice. Moderate CLP, vehicle + CLP; JSLS+CLP, 400 mg JSLS/kg body weight + CLP; CEF+CLP, 20 mg ceftriaxone/kg body weight + CLP. * $p < 0.05$ compared with the sham group; # $p \leq 0.05$ compared with the CLP group.

3.3 Hematological Profile

Table 1 shows the hematological profile for each group. The JSLS group showed slight alterations compared with the other groups. Specifically, it presented a significant increase in granulocytes compared with the sham and CLP groups. There was a significant decrease in lymphocytes in the JSLS and CLP groups compared with the sham group. In sepsis models, elevated granulocytes are expected. Thus, the increased granulocytes in the JSLS group suggest that the tested JSLS dose stimulated granulocyte production. This alteration might explain the increased mortality in this group 24 h after CLP compared with the CLP group.

Table 1. The hematological parameters of the four groups of mice.

Parameters	Reference value[+]	Sham	Moderate CLP	JSLS+CLP	CEF+CLP
RBC (x10⁶/uL)	7.42 ± 0.85	6.85 ± 0.63	6.51 ± 0.80	7.16 ± 0.71	6.09 ± 0.83

Hemoglobin (g/dL)	12.92 ± 0.34	10.73 ± 2.05	11.44 ± 1.58	12.32 ± 1.2	10.55 ± 2.05
Hematocrit (%)	39.81 ± 1.64	38.11 ± 5.34	33.26 ± 4.76	36.06 ± 3.07	31.5 ± 6.22
MCV (fL)	57.81 ± 7.79	54.45 ± 3.04	51.08 ± 1.83	50.48 ± 1.19	51.55 ± 3.18
MCH (pg)	18.58 ± 2.03	15.46 ± 1.75	17.5 ± 0.48	17.14 ± 0.33	17.2 ± 0.98
MCHC (g/dL)	31.78 ± 0.97	28.6 ± 3.20	34.38 ± 0.72	34.08 ± 0.65	34.08 ± 0.65
RDW (%)	15.64 ± 1.47	14.15 ± 2.01	12.78 ± 0.64	13.4 ± 0.68	13.7 ± 1.41
WBC (× 10^3/uL)	4.98 ± 1.26	4.35 ± 1.17	2.91 ± 0.64	4.55 ± 0.75	3.65 ± 2.89
Lymphocytes (× 10^3/uL)	–	4.24 ± 2.04	1.35 ± 0.42*	1.3 ± 0.2*	1.8 ± 1.27
Monocytes (× 10^3/uL)	–	0.2 ± 0.1	0.17 ± 0.07	0.3 ± 0.1	0.25 ± 0.35
Granulocytes (× 10^3/uL)	–	1.3 ± 0.53	1.34 ± 0.43	2.95 ± 0.45*#	1.6 ± 1.27

Abbreviations: RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; WBC, white blood cell. ⁺Reference parameters for male Swiss mice (44). Moderate CLP, vehicle + CLP; JSLS+CLP, 400 mg JSLS/kg body weight + CLP; CEF+CLP, 20 mg ceftriaxone/kg body weight + CLP. * $p < 0.05$ compared with the SHAM group; # $p \leq 0.05$ compared with CLP group.

3.4 MDA

Compared with the CLP group, the JSLS group showed a significant decrease in MDA in the heart, lung, liver, and peritoneal lavage samples compared with the CLP group, as shown in figure 4. The JSLS group showed slightly higher MDA levels in the lung and liver and slightly lower MDA levels in the heart and peritoneal lavage samples compared with the sham group, but the differences were not significant. The CLP group showed elevated MDA levels in all tested organs, changes that are expected due to sepsis induction. Elevated MDA levels indicate oxidative stress; thus, a decrease in the MDA levels the JSLS group may be due to the antioxidant activity of JSLS.

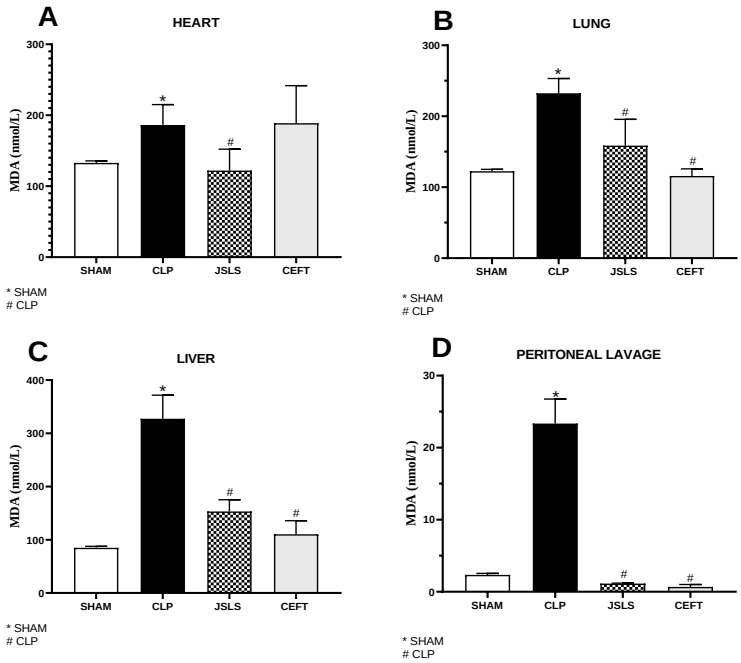

Figure 4. The malondialdehyde levels in the (A) heart, (B) lung, (C) liver, and (D) peritoneal lavage samples from the four groups of mice. CLP, vehicle + CLP; JSLS, 400 mg JSLS/kg body weight + CLP; CEFT, 20 mg ceftriaxone/kg body weight + CLP. * $p < 0.05$ compared with the sham group; # $p \leq 0.05$ compared with the CLP group.

3.5 TEAC

The TEAC was not significantly different between the JSLS and CLP groups, except for the liver, as shown in figure 5. The JSLS group showed a significant decrease compared with the CLP group. For the CEF+CLP group, the TEAC was significantly decreased in the heart compared with the other groups. A decrease in the TEAC in the liver samples from the JSLS group may be from the consumption of antioxidant compounds due to sepsis and/or the tested dose or dosing schedule.

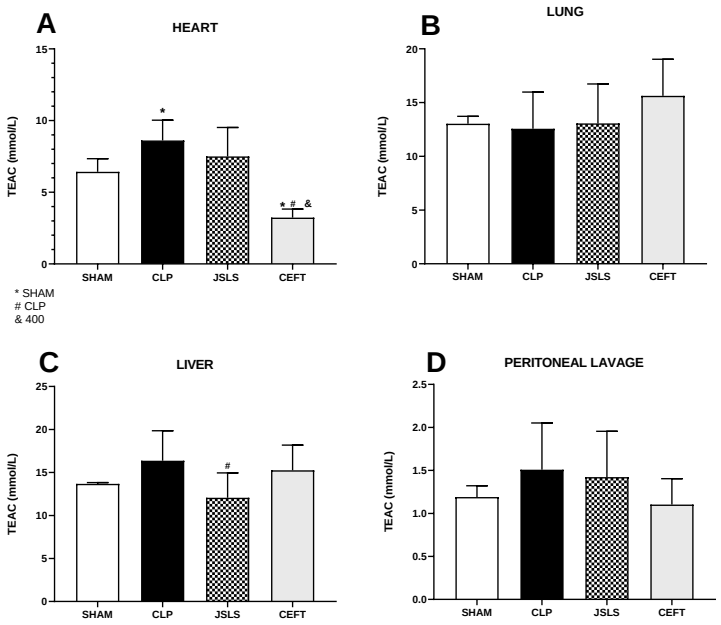

Figure 5. The TEAC levels in the (A) heart, (B) lung, (C) liver, and (D) peritoneal lavage samples from the four groups of mice. CLP, vehicle + CLP; JSLS, 400 mg JSLS/kg body weight + CLP; CEFT, 20 mg ceftriaxone/kg body weight + CLP. * $p < 0.05$ compared with the sham group; # $p \leq 0.05$ compared with the CLP group.

3.6. GSH

The CLP group showed a significant decrease in GSH in the heart and lung compared with the sham group, as shown in figure 6. However, the JSLS showed a significant increase in GSH in these organs compared with the CLP group. In the liver, GSH was significantly higher in the CLP, JSLS, and Ceft+CLP groups compared with the sham group. The JSLS group also showed an increase in GSH relative to the sham group. These findings indicate that JSLS may exert antioxidant activity in the context of sepsis.

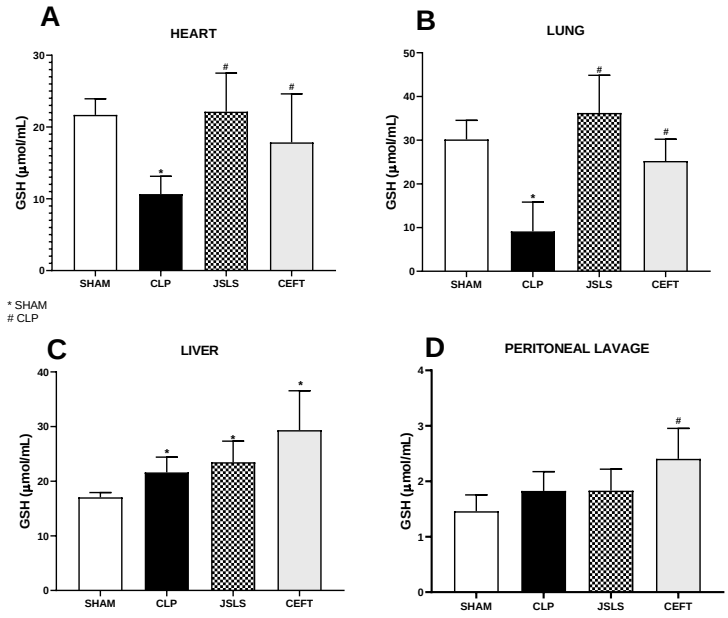

Figure 6. The GSH levels in the (A) heart, (B) lung, (C) liver, and (D) peritoneal lavage from the four groups of mice. CLP, vehicle + CLP; JSLS, 400 mg JSLS/kg body weight + CLP; CEFT, 20 mg ceftriaxone/kg body weight + CLP. * $p < 0.05$ compared with the sham group; # $p \leq 0.05$ compared with the CLP group.

4. Discussion

Research has shown that plant extracts can be used to manage sepsis in mouse models of this condition due to their bioactive secondary metabolites that have the capacity to modulate immune responses and redox imbalance. Thus, plant extracts could be used as potential therapeutic agents in the management of sepsis.

Phytochemicals, also known as secondary metabolites, naturally present in plants, are considered to be responsible for the traditional medicinal effects of plants and their extracts that have been used to prevent and treat various diseases (45,46). The phytochemical screening of *J. secunda* leaves and stems has revealed the presence of bioactive compounds such as anthocyanins and other flavonoids, tannins, saponins, steroids, alkaloids, essential oil rich in polyenoic fatty acid methyl esters, as well as volatile constituents such as terpenes and their derivatives (19,23,47–50). According to previous studies, secondary metabolites (e.g., phenolic compounds) in *J. secunda* leaves are responsible for the antioxidant activity in the extracts (51). The imbalance between antioxidant and pro-oxidant compounds leads to oxidative stress, a principal factor in the development and progression of diverse pathologies. Thus, plant-based therapeutics could prove to be promising candidates to treat conditions with an inflammatory component due to their antioxidant effects.

In the acute oral toxicity test, the daily cage-side notes showed no alterations in behavioral pattern following JSLS administration. These data suggest that the median lethal dose (LD50) of JSLS is > 2000 mg/kg body weight. The globally harmonized classification system divides chemicals based on their LD50; the low toxicity class is for compounds with an LD50 > 2000 mg/kg. Thus, JSLS could be classified in the low toxicity group. According to (34), a *J. secunda* leaf methanolic extract did

not induce signs of toxicity in the acute oral toxicity test at the limit dose of 2000 mg/kg body weight. Similarly, in a sub-acute toxicity test, 500 mg/kg body weight of *J. secunda* did not cause death nor alterations in the fur, eyes, skin, general behavioral patterns, and respiratory system in rats (52). An acute oral toxicity test is necessary to determine the safe dose range to manage the potential negative effects of a drug.

We found slightly decreased MDA levels in the liver and heart in the test group compared with the control group. The kidney showed no significant difference between the control and test groups, suggesting that JSLS did not provoke nephrotoxicity. Consistently, Aimofumeh (53) demonstrated that *J. secunda* extracts inhibited lipid peroxidation, reduced alanine aminotransferase (ALT), aspartate aminotransferase (AST), and increased GSH levels in plasma. Hence, the bioactive compounds in JSLS could reverse free radical production and damage, serving to ameliorate oxidative stress and exerting hepatoprotective activity. In another study, plasma urea and creatinine levels were reduced in animals treated with *J. secunda* extract (25), suggesting that the plant extract contains bioactive compounds with nephroprotective properties.

There are limited data demonstrating the activity of *J. secunda* extracts in sepsis models. However, some plant extracts have been shown to ameliorate oxidative stress and inflammatory responses in CLP-induced sepsis models (54–56). According to Wasshausen (57), sepsis-induced mice administered a plant extract with a high total flavonoid and phenolic content had a higher survival rate (80%) compared with the untreated group (33%). In a CLP model, green propolis elevated the survival rate to 70%, while the saline-treated animals had a 40% survival rate; this benefit was possibly due to the extract's anti-inflammatory, antioxidant, and immunomodulatory properties (58). In contrast, Soleimanipour (59) demonstrated that a plant extract with antimicrobial activity diminished the survival rate in animals submitted to CLP, possibly due to an increased inflammatory response and mortality rate in rats with polymicrobial sepsis. Researchers have also shown that pretreatment with a nutraceutical exerts beneficial effects in sepsis animal models (9,60). In patients with sepsis, excessive oxidative stress accompanied by a decreased antioxidant capacity characterizes redox imbalance, which disrupts cellular homeostasis and leads to cell death (61). Plant extracts with the capacity to regulate oxidative stress and inflammatory responses have protective effects on sepsis-induced mice (62). Ceftriaxone is a broad-spectrum antibiotic that is empirically used to reduce the risk of mortality in patients with sepsis, when the causative pathogen is sensitive to this antibiotic. In our model of moderate CLP, the clinical parameters were highest at 24 h following CLP-induction in the CLP group, while the mice pretreated with JSLS showed similar clinical parameters to those of the ceftriaxone group at this time. According to Okoyomoh (63), rats dosed with a *J. secunda* ethanolic extract at 100, 200, and 400 mg/kg body weight did not show altered albumin, globulin, alkaline phosphatase (ALP), ALT, and AST levels compared with the normal group that did not receive treatment. Evaluating different doses of *J. secunda* extract on hematological and oxidative parameters, Oyewale (52) reported that lower doses (10, 100, and 1000 mg/kg body weight) increased the red blood cell and hemoglobin count, while higher doses

(1600, 2900, and 5000 mg/kg body weight) reduced the hematological parameters. Animals dosed at 10–2900 mg/kg body weight had increased MDA and superoxide dismutase levels, while the dose of 5000 mg/kg body weight decreased these parameters. However, catalase activity was similar for all tested doses. According to Irinmwinwa (64), extracts from *J. secunda* have hemopoietic effects in anemic mice, elevating hemoglobin as well as the red blood cell, white blood cell, and platelet counts at varying doses under 64 mg/kg body weight.

MDA is commonly used as a biomarker to assess oxidative stress. When oxidative stress is prolonged, there is impaired redox imbalance, which is linked to the progression of inflammatory diseases, aging processes, and carcinogenesis (65,66). Lipid peroxidation provides a constant supply of free radicals, which in turn cause further peroxidation, accumulation of free radicals, and increased levels of MDA, resulting in oxidative stress. The TBARS method is used most frequently to determine MDA levels (67). Studies suggest that elevated MDA is common in patients with cancer: It is linked to an increase in the production of reactive oxygen species and a deficient antioxidant defense (68). In our study, 400 mg JSLS/kg body weight administered 24 h and immediately before CLP diminished reactive oxygen species in the heart, lungs, liver, and peritoneal lavage samples of CLP mice compared with the untreated group. Although oxidative stress is necessary when the body is faced with a systemic infection, excessive oxidative stress leads to numerous negative effects. Therefore, the ability to revert redox imbalance is important in order for the body to properly react and heal.

The TEAC is employed to measure the overall ability that the body has to counteract oxidative stress. Enzymatic and non-enzymatic antioxidants work together to prevent the formation of free radicals and to protect against cellular and tissue damage (69). We observed a slightly increase in TEAC in the groups submitted to CLP, possibly due to consumption and saturation of the antioxidant system that is responsible for scavenging and neutralizing reactive oxygen species. The JSLS group had a slight decrease in the TEAC tested organs compared with the CLP group. The decrease in antioxidant parameters in our CLP model suggests that sepsis depletes antioxidants through pro-oxidant stimulation.

GSH is an endogenous antioxidant whose levels are altered during infection or disease (46). It is used to determine an imbalance in redox homeostasis. The JSLS group presented reduced GSH in the heart, lung, and liver, while the CLP group showed a greater decrease in GSH levels, especially in the heart and lung. GSH was higher in the heart and lung of the JSLS group compared with the ceftriaxone group. The GSH decrease observed in the CLP group may be due to excess reactive metabolites and/or the production of reactive oxygen species in the body, indicating consumption of antioxidant agents during infection, which can lead to antioxidant depletion. On the other hand, the increase in GSH in the JSLS group suggests that JSLS could reverse the redox imbalance induced by sepsis. According to Aimofumeh (53), *J. secunda* extract (100, 300, and 500 mg/kg body weight) elevated GSH in acetaminophen-induced rats, indicating that this extract could protect against acetaminophen-induced hepatic damage. According to Mobisson (70), male rats treated with *J. secunda* extract presented elevated GSH.

Sepsis is a major cause of mortality in critically ill patients and poses a huge economic burden on the healthcare system. The invading microbes trigger a hyperinflammatory response, leading to an imbalance in cell homeostasis and multi-organ failure. Antibiotic resistance and delayed diagnosis are causes for concern regarding sepsis treatment; thus, it is necessary the search for alternative therapeutic strategies (71). During sepsis and septic shock, the heart, lung, and liver are among the major organs adversely affected; the severity depends on organ dysfunction and hemodynamic disorders. Lipopolysaccharides from invading pathogens and uncontrolled inflammatory mediators as a host response initiate endothelial damage, resulting in microvascular disorders; coagulopathy; and cardiovascular, liver, pulmonary, and renal dysfunction. Cardiovascular dysfunction is caused by intrinsic and extrinsic factors, as well as blood vessel dilation and permeability. Hepatic dysfunction is caused by cytotoxic cytokines released from Kupfer cells. Endothelial damage, as well as impaired blood flow and permeability cause alveolar edema and alter gas exchange (72). Herbal extracts have been shown to ameliorate sepsis-induced damage in rodent models (73–75). Oxidative stress has been shown to be associated with patient mortality: It increases substantially during sepsis and decreases after therapy (76). Thus, phytochemicals could be used to ameliorate damaging effects of sepsis through balancing specific biochemicals and mediators.

In summary, our acute toxicity text following the OECD guidelines showed that JSLS is not toxic to Swiss mice at the dose of 2000 mg/kg body weight. The MDA assay revealed a slight decrease in the liver and heart of the test group compared with the control group, while the MDA level in the kidney was similar in both groups. Regarding JSLS as a potential sepsis therapy, the JSLS group showed a slight improvement in the survival rate, clinical parameters, the hematological profile and redox homeostasis compared with the untreated CLP group. However, it is necessary to determine a more efficacious dose and dosing schedule. We recommend that JSLS be further explored as a chemoprotective drug candidate in the management of oxidative stress–related disorders.

5. Conclusions

Our data suggest that JSLS is non-toxic at the tested dose (2000 mg/kg body weight). We observed that JSLS influences the survival and clinical parameters of septic mice compared with untreated septic mice. Previous studies on *J. secunda* have shown the presence of diverse secondary metabolites, such as flavonoids, phenolic compounds, saponins, tannins, terpenoids, and alkaloids. The synergistic activity of these bioactive compounds is responsible for the biological properties of *J. secunda*. Thus, *J. secunda* represents a natural product that can modulate redox imbalance through decreasing oxidative parameters and increase antioxidant agents. It could be a promising therapeutic agent in the adjuvant treatment of oxidative stress–related disorders, including sepsis. We strongly recommend further investigation of JSLS to elucidate the safest and most efficacious dose and to evaluate its use as an adjuvant and/or preventive therapy for sepsis and other oxidative stress–related diseases.

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Authors: Kelly Davis, Ana Paula Holanda Santana, Alexandre Guilherme da Silva Dias, Rana Karen Mesquita da Silva, Rayane Caroline dos Santos Pereira, Lucas da Silva Gato, Antônio Rafael Quadros Gomes, Sávio Monteiro dos Santos, Bruno Alexandre Quadros Gomes, Kely Campos Navegantes-Lima, Marta Chagas Monteiro *
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