



Repeated dose oral toxicity of *Mimosa pudica* L. (Fabaceae) aerial parts in mice

[Toxicidad oral a dosis repetidas de partes aéreas de *Mimosa pudica* L. (Fabaceae) en ratones]

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Abstract

Context: *Mimosa pudica* L., belonging to the Fabaceae family, has been widely used in natural and traditional medicine because of its proven pharmacological properties. The present study examines through a preclinical toxicity assay, the safety of leaves and stems decoction of this plant used in popular medicine.

Aims: To evaluate the oral toxicity at repeated doses of the *M. pudica* aerial parts.

Methods: The toxicity assay was carried out using the limit test method by the administration of *M. pudica* decoction at repeated doses (1000 mg/kg of weight) for 28 days, to NMRI mice of both sexes. The body weight and clinical signs were assessed throughout the experimental period; and hematological and biochemical parameters, as well as, histopathological analysis of the main organs were evaluated at the end of the study.

Results: The body weight of the animals treated with the plant decoction showed a significant decrease with respect to controls ($p < 0.05$). The hematological parameters did not show alterations, while the biochemical markers exhibited a decrease in plasma proteins and an increase in glucose and the alanine aminotransferase enzyme activity. The histopathological study showed slight alterations in the brain, liver, lung, heart, thymus, and kidneys of experimental mice.

Conclusions: The biochemical and histopathological results suggest that the decoction of *M. pudica*, administered orally at repeated doses, caused slight alterations that warn about the risks of its traditional use.

Keywords: *Mimosa pudica* L; NMRI mice; repeated dose toxicity.

Resumen

Contexto: La planta *Mimosa pudica* L. perteneciente a la familia de las Fabaceae, ha sido ampliamente empleada en la medicina natural y tradicional por sus propiedades farmacológicas demostradas. El presente estudio evalúa a través de un ensayo preclínico de toxicidad la seguridad de la decocción de hojas y tallos de esta planta usada en la medicina popular.

Objetivos: Evaluar la toxicidad oral a dosis repetidas de las partes aéreas de la planta *M. pudica*.

Métodos: El ensayo de toxicidad se realizó empleando el método de test límite por administración a dosis repetidas durante 28 días, de la decocción de la planta *M. pudica* a dosis de 1000 mg/kg de peso en ratones NMRI de ambos sexos. Se evaluó el comportamiento del peso corporal durante todo el proceso experimental y al final del estudio se determinaron parámetros hematológicos y bioquímicos, y se realizó el estudio histopatológico de los principales órganos.

Resultados: El comportamiento del peso corporal de los animales tratados con la decocción de la planta mostró una disminución significativa con respecto a los controles. Los parámetros hematológicos no mostraron alteraciones, mientras que en los bioquímicos se pudo apreciar una disminución de las proteínas plasmáticas y un incremento de la glucosa y la enzima alanina aminotransferasa. El estudio histopatológico mostró alteraciones ligeras en encéfalo, hígado, pulmón, corazón, timo y riñones.

Conclusiones: Los hallazgos bioquímicos e histopatológicos encontrados muestran que la decocción de la planta *M. pudica*, administrada por vía oral a dosis repetidas, provoca ligeras alteraciones que alertan sobre las precauciones en el uso tradicional de esta.

Palabras Clave: *Mimosa pudica* L; ratones NMRI; toxicidad a dosis repetidas.

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INTRODUCTION

Natural and traditional medicine has been used by various cultures around the world to prevent and treat diseases since ancient times. Also known as "green medicine", it is still widely used in many countries and plays an important role in the healthcare of millions of people, despite the progress of modern medicine. Although this millenary practice has been popularly spread, it has often been the subject of controversy, especially with respect to its safety and efficacy (Salmerón-Manzano et al., 2020; Upadhyay, 2023). In this sense, preclinical toxicological studies of medicinal plants are fundamental to ensure their safety in traditional and complementary medicine. These studies allow the evaluation of the possible toxic effects of compounds present in plants, the identification of the safe dose, and the determination of any adverse effects that may occur. Preclinical toxicological studies of medicinal plants are important to protect the health of people who use them and to ensure their therapeutic efficacy. Furthermore, these studies are essential for developing new drugs and therapies derived from medicinal plants (Ntabaza et al., 2023; Shauli et al., 2023).

Mimosa pudica L. (*Fabaceae*) is a plant native of Central and South America, also known as "moriviví", "sensitive plant" or "dormilona" due to its characteristic of closing its leaves upon physical contact. In addition to its responsiveness to stimuli, this plant also has various medicinal properties used for centuries by traditional cultures in the region (Keshamma and Srusti, 2022; Saraswat et al., 2020). *M. pudica* is rich in alkaloids, fatty acids, non-protein amino acids (mimosine), flavonoids, steroids, terpenoids, and tannins, compounds that possess antioxidant, anti-inflammatory, analgesic, and antimicrobial activities. These molecules are responsible for a wide range of health benefits, including the ability to treat skin conditions, pain, inflammation, and infections (Hassan et al., 2019; Kumar, 2021; Majeed et al., 2021). Despite its traditional use, little is known about its safety and toxicity, mainly in long-term exposures, which limits its clinical use as an alternative treatment for various diseases.

In Cuba, the main ethnobotanical uses of this plant are based on its sedative and antidepressant properties, being consumed by the population through decoctions of the aerial parts of the plant, alone or in combination with other plant species. There are also reports from Cuban authors that refer to the use of this plant as an abuse substance due to its effects on the Central Nervous System (Cutié-Bressler et al., 2015). Taking into account the extensive use of the

aerial parts of *M. pudica* by the Cuban population as a sedative and antidepressant and the lack of evidence that supports its safety, this study aimed to evaluate the toxicity at repeated doses of leaves and stems decoction of *M. pudica*, simulating the way of consumption in popular medicine.

MATERIAL AND METHODS

Plant material and decoction preparation

Stems and leaves of *M. pudica* were collected from El Caney town in Santiago de Cuba, Cuba (Latitude: 20.0569 and Longitude: -75.7719). Taxonomy identification was performed by specialists at the Eastern Center for Ecosystems and Biodiversity (BIOECO, Santiago de Cuba). A vegetal sample was stored at the botanic herbarium of this institution with code 218237.

M. pudica decoction was prepared according to the British Pharmacopoeia, the USP XXI Pharmacopoeia of the United States, and the Practical Pharmacy of Remington. Briefly, 10 g of shadow-dried leaves and stems were added to 100 mL of water, boiled for 5 min, filtered, and then stored at 4°C until use. The concentration of the decoction was expressed on the basis of its total dried weight.

Experimental animals

Male and nulliparous female NMRI mice between 6 and 8 weeks and weighing 20-25 g (National Center for Laboratory Animal Production, CENPALAB, Havana) were used. They were kept under conventional conditions with a temperature of $22 \pm 3^{\circ}\text{C}$, relative humidity of 65% on average, and a photoperiod of 12 hours light and 12 hours dark. They were fed with pelleted feed (Formula EMO:1002) (ALYco, CENPALAB, Havana, Cuba) and water *ad libitum*.

Ethical considerations

The study was conducted in compliance with the Good Laboratory Practice conditions of the Food and Drug Administration (FDA) and the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH, USA). The experimental design was reviewed and approved by the Ethics Committee for Research at TOXIMED and the Quality Assurance Unit, according to Protocol TOXIMED/UGC/010404.

Repeated dose toxicity assay

The repeated dose toxicity study of the subacute effects of *M. pudica* decoction was conducted using the limit test method, based on the Regulatory Guide-

line No. 407 of the Organization for Economic Cooperation and Development (OECD, 2008).

The animals were divided into three groups: experimental, control, and satellite groups, with 10 individuals per group (5 males and 5 females), for a total of 30 mice in the study. The decoction was administered orally at a dose of 1000 mg/kg of body weight through a gastric tube to the experimental and satellite groups for a period of 28 days, while the control group received the decoction vehicle (sterile water). The animals were weighed weekly to detect possible body weight variations and adjust the sample volume to be administered to maintain the dose used.

Clinical observations

The control and experimental groups were observed for 28 days after administration, while the satellite group was monitored for 42 days to detect delayed appearance, and improvement or recovery of toxicity symptoms. Clinical observations were performed twice daily after administration, looking for changes in skin, hair, eyes, mucous membranes, the occurrence of secretions/excretion, autonomic activity, posture, locomotion, and response to manipulation, as well as the presence of clonic or stimulant movements, stereotypies or bizarre behavior.

Clinical laboratory assays

At the end of the experimental stage, euthanasia was performed by narcosis with injectable ketamine (50 mg/kg of body weight) intraperitoneally. Blood samples were obtained to determine hematological variables, such as hematocrit and total and differential leukocyte counts. Moreover, biochemical parameters were determined in plasma: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activities, and metabolites such as glucose, total cholesterol, total proteins, albumin, and globulins. Colorimetric methods were performed, and measurements were made using a UV/VIS spectrophotometer (PG Instrument T60U, Leicestershire, UK).

Anatomopathological studies

Anatomopathological studies included macroscopic studies through the examination of the external surface of the body, all orifices and cavities (cranial, thoracic, and abdominal), as well as organs such as the brain, liver, kidneys, thymus, spleen, lungs, and heart of all animals. The organs were placed on Whatman filter papers for 5 min (Akhigbemen et al., 2018) and weighed on an analytical balance (OHAUS, USA). The relative organ weight was calculated by

the relation between the weight of each organ and the body weight of the animal before euthanasia (Wahyuni et al., 2023).

For histopathological studies, samples were taken from the following organs: brain, cerebellum, pons, spinal cord (cervical, thoracic, and lumbar regions), thyroid gland, parathyroid gland, thymus, esophagus, salivary glands, stomach, small and large intestine (including Peyer's patches), liver, pancreas, kidneys, adrenal glands, spleen, heart, trachea and lungs, aorta, gonads, accessory sex organs (mammary glands, prostate), uterus, urinary bladder, lymph nodes (one near the administration site and one distant from it), peripheral nerve (sciatic or tibial) preferably closer to the muscle, and a section of bone marrow of the animals from all groups. Samples were fixed in 10% neutral formalin, processed by the paraffin method, sectioned at 4 μ m and stained with hematoxylin-eosin for subsequent observation under a transmitted light microscope (Leica DM 1000, Switzerland) coupled to a digital camera (Leica MC 170 HD, Heerbrugg, Switzerland).

Statistical analysis

Statistical analysis was performed using the statistical software GraphPad Prism 9 (GraphPad Software Inc., La Jolla, CA, USA). The experimental results were expressed as the arithmetic means $\pm$ standard deviations. The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test to compare body weight, hematological and biochemical parameters, and relative organ weight. Differences at $p \leq 0.05$ were accepted as significant.

RESULTS

Repeated dose toxicity evaluation of *M. pudica* aerial parts

Effect on the general behavior of NMRI mice

The assay concluded with a 100% survival rate. In treated animals, signs of generalized piloerection and semi-soft feces were observed during the first seven days of administration. The remaining 21 days maintained normal behavior, with typical postural reflexes, grooming habits, and habitual response to nociceptive stimuli.

Effect on mice body weight

All groups increased their weight during the experimental period; however, animals of both sexes in experimental and satellite groups had a significantly lower increase than control animals ($p < 0.05$) (Fig. 1).

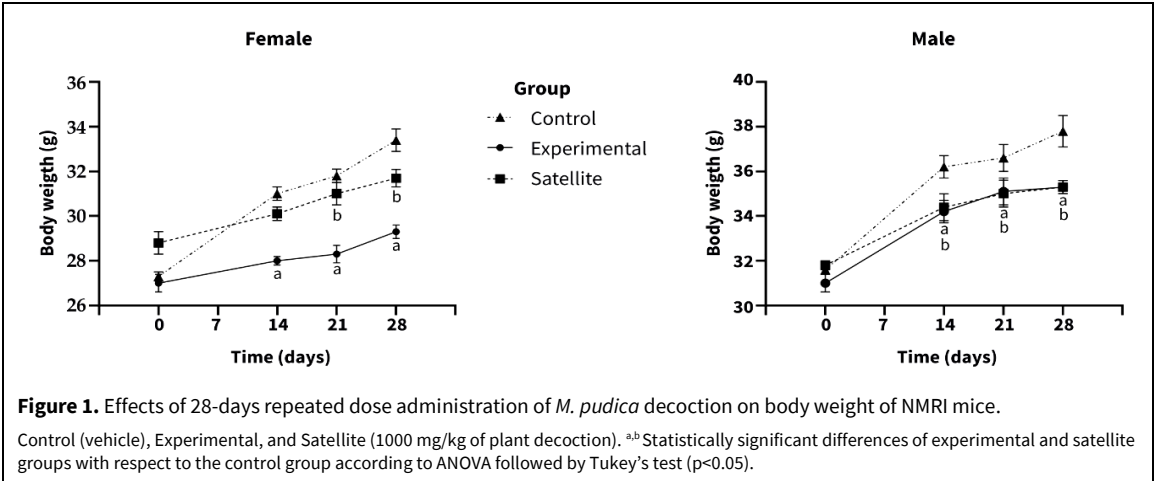


Table 1. Effects of 28 days repeated dose administration of *M. pudica* decoction on hematological and biochemical parameters.

Parameters	Female			Male		
	Control	Experimental	Satellite	Control	Experimental	Satellite
Hematocrit (mm)	0.53 ± 0.03	0.54 ± 0.048	0.54 ± 0.02	0.53 ± 0.05	0.52 ± 0.07	0.51 ± 0.03
Leucocytes (× 10 ⁹ /L)	6.92 ± 1.39	5.88 ± 0.55	5.98 ± 0.71	6.60 ± 0.72	6.12 ± 1.05	5.66 ± 0.85
Lymphocytes (× 10 ⁹ /L)	5.74 ± 1.21	4.48 ± 0.77	4.70 ± 1.39	5.08 ± 0.87	4.66 ± 1.36	4.20 ± 0.12
Neutrophils (10 ⁹ /L)	1.16 ± 0.39	1.36 ± 0.48	1.26 ± 1.13	1.50 ± 0.94	1.42 ± 1.03	1.46 ± 0.79
Eosinophils (× 10 ⁹ /L)	0.02 ± 0.04	0.04 ± 0.055	0.02 ± 0.045	0.02 ± 0.04	0.04 ± 0.09	0.00 ± 0.00
Glucose (mmol/L)	3.68 ± 1.19	3.16 ± 1.02	8.36 ± 0.84 ^b	3.82 ± 0.37	3.62 ± 0.82	8.24 ± 1.70 ^b
Creatinine (mmol/L)	42.86 ± 24.98	42.57 ± 17.32	42.89 ± 14.54	40.59 ± 17.37	34.45 ± 14.61	29.39 ± 13.19
ALT (IU/L)	53.60 ± 13.94	117.84 ± 16.95 ^a	63.86 ± 30.29	77.42 ± 36.67	58.94 ± 13.83	64.86 ± 11.47
AST (IU/L)	135.20 ± 20.36	130.80 ± 38.24	116.00 ± 54.18	156.00 ± 36.89	127.00 ± 19.68	105.60 ± 24.54 ^b
Total cholesterol (mmol/L)	3.48 ± 0.47	2.74 ± 0.55	2.80 ± 0.44	3.92 ± 0.28	3.79 ± 0.49	2.55 ± 0.38 ^b
Total proteins (g/L)	63.84 ± 1.65	62.68 ± 3.35	58.66 ± 1.15 ^b	66.58 ± 3.55	63.48 ± 2.59	57.34 ± 2.07 ^b
Albumin (g/L)	38.56 ± 1.78	39.70 ± 1.70	34.90 ± 1.24 ^b	37.32 ± 2.87	36.48 ± 1.59	32.52 ± 1.51 ^b
Globulins (g/L)	25.20 ± 0.83	22.80 ± 2.39	23.60 ± 0.55 ^b	29.40 ± 1.67	27.00 ± 3.08	24.80 ± 0.84 ^b

All values are expressed as the arithmetic mean ± SD (n = 5). ^{a,b}Statistically significant differences of experimental and satellite groups with respect to the control group according to ANOVA followed by Tukey's test ($p<0.05$).

Effect on hematological and biochemical blood parameters

Table 1 shows the behavior of hematological and biochemical parameters evaluated. No significant differences were observed between groups in relation to measured hematological parameters, which were in all cases within the normal range reported for NMRI mice. The most relevant findings with respect to the biochemical parameters were the decrease in total proteins and a marked increase in ALT enzyme in females of the experimental group. Moreover, there were significant differences ($p<0.05$) in glucose levels in both sexes of the satellite group compared to the control and experimental groups (Table 1).

Effect on the organ weights and histopathological results

No macroscopic lesions were found in the anatomicopathological study of the animals. The relative weights of certain organs, such as the brain, liver, lung, spleen, and kidneys, decreased significantly in some of the groups treated with the plant decoction (Table 2). In the case of the heart, a significant increase ($p<0.05$) was observed in females of the satellite group. The thymus showed no differences in relative weight in any of the evaluated groups.

Table 3 shows the main histopathological findings in the affected organs. According to the incidence of the histopathological evidence calculated by the relation between the number of affected animals and the total of animals in each group, and the severity score proposed by Hayes et al. (2020), the means of severity

were in the scales of 1.00-2.50, corresponding to minimal and slight damages (Hayes et al., 2020). A slight degeneration and cerebral congestion were observed in animals of the experimental group (Fig. 2A). Additionally, hepatic vacuolar degeneration, congestion, focal necrosis, and mononuclear (MN) infiltrates were observed in the liver of animals in the experimental group (Fig. 2B).

On the other hand, peribronchial and perivascular pneumonitis with mononuclear and lymphocyte infiltrates, vessel congestion, and peribronchial and mucosal epithelial desquamation were observed in the lungs of female animals in the experimental group. In

contrast, no histopathological alterations were observed in males (Fig. 2C).

In the histopathological study of the heart, congestion of myocardial vessels, focal subepicardial mild myocarditis, and lymphomononuclear infiltrates and diffuse Zenker degeneration in myocardium were detected in males of the experimental group (Fig. 2D). A slight cortex hyperplasia was observed in the thymus of animals in the experimental group (Fig. 2E), as well as, a slight renal cortical-medullary congestion in the kidneys (Fig. 2F). No apparent histopathological alterations were found in the remaining organs.

Table 2. Effects of 28 days repeated dose treatment of *M. pudica* decoction on relative organ weight.

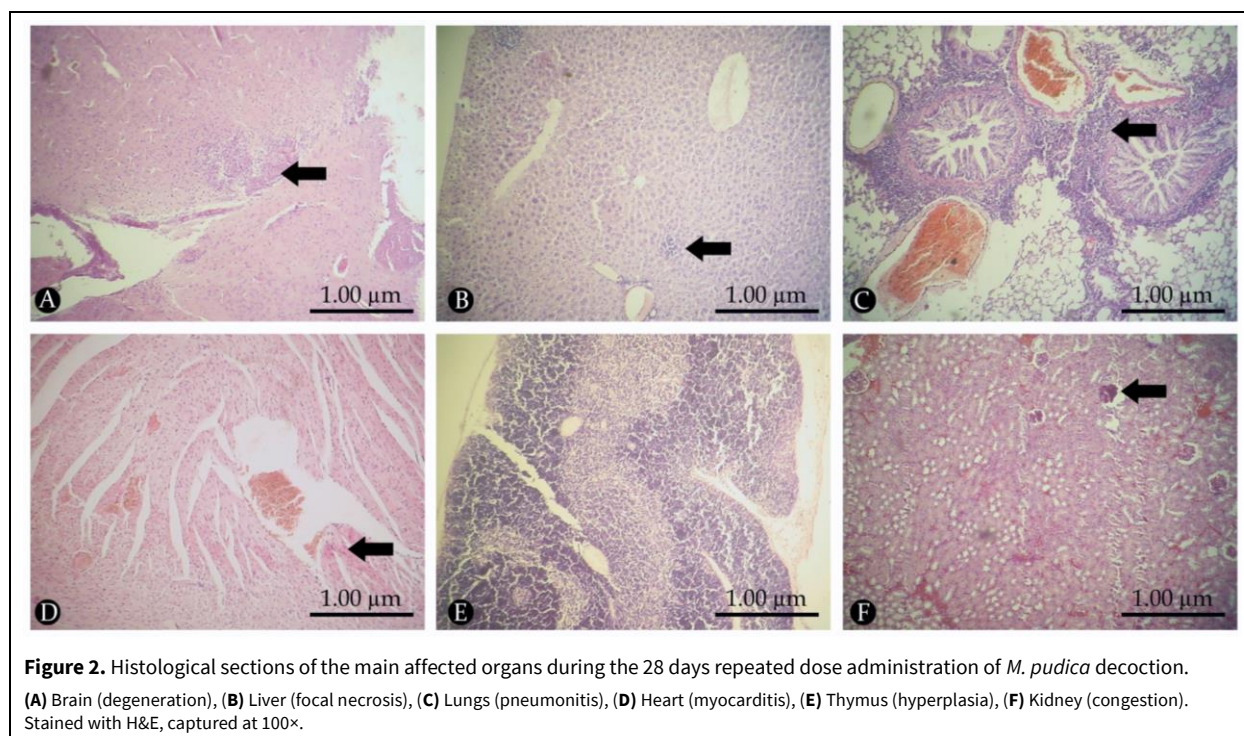
Relative organ weight (g)	Female			Male		
	Control	Experimental	Satellite	Control	Experimental	Satellite
Brain	1.74 ± 0.19	1.86 ± 0.10	1.33 ± 0.08 ^b	1.45 ± 0.08	1.46 ± 0.12	1.34 ± 0.09
Liver	5.72 ± 1.21	4.55 ± 0.28	4.72 ± 0.29	5.24 ± 0.33	4.72 ± 0.21 ^a	4.77 ± 0.24 ^b
Lungs	0.97 ± 0.16	0.93 ± 0.07	0.79 ± 0.04 ^b	0.82 ± 0.18	0.96 ± 0.09	0.72 ± 0.06
Heart	0.54 ± 0.08	0.56 ± 0.07	0.71 ± 0.07 ^b	0.66 ± 0.12	0.63 ± 0.13	0.57 ± 0.11
Thymus	0.40 ± 0.11	0.34 ± 0.12	0.25 ± 0.07	0.26 ± 0.06	0.29 ± 0.06	0.25 ± 0.06
Spleen	0.68 ± 0.09	0.51 ± 0.07 ^a	0.63 ± 0.07	0.55 ± 0.07	0.50 ± 0.09 ^a	0.56 ± 0.10
Right kidney	0.70 ± 0.10	0.70 ± 0.05	0.74 ± 0.08	0.81 ± 0.05	0.77 ± 0.07	0.65 ± 0.12 ^b
Left kidney	0.62 ± 0.06	0.62 ± 0.05	0.57 ± 0.12	0.79 ± 0.03	0.77 ± 0.07	0.62 ± 0.02 ^b

All values are expressed as the arithmetic mean ± SD (n = 5). ^{a,b} Statistically significant differences of experimental and satellite groups with respect to the control group according to ANOVA followed by Tukey's test (p<0.05).

Table 3. Main histopathological findings, incidence and severity score of damage in a 28-days repeated dose oral toxicity study of *M. pudica* decoction in NMRI mice.

Histopathological finding	Female			Male		
	Control	Experimental	Satellite	Control	Experimental	Satellite
Brain degeneration	0/5	1/5 (2.00)	0/5	0/5	2/5 (2.00)	0/5
Brain congestion	0/5	2/5 (2.00)	0/5	0/5	2/5 (2.00)	0/5
Liver degeneration	0/5	1/5 (2.00)	0/5	0/5	0/5	0/5
Liver congestion	0/5	1/5 (2.00)	0/5	0/5	1/5 (2.00)	0/5
Liver necrosis	0/5	3/5 (2.33)	0/5	0/5	2/5 (2.50)	0/5
Liver MN infiltrates	0/5	2/5 (2.00)	0/5	0/5	1/5 (2.00)	0/5
Lung pneumonitis	0/5	2/5 (2.00)	0/5	0/5	0/5	0/5
Lung congestions	0/5	2/5 (1.50)	0/5	0/5	0/5	0/5
Lung epithelial desquamation	0/5	1/5 (1.00)	0/5	0/5	0/5	0/5
Heart vessels congestion	0/5	2/5 (2.00)	0/5	0/5	1/5 (2.00)	0/5
Heart subepicardial myocarditis	0/5	2/5 (2.50)	0/5	0/5	1/5 (2.00)	0/5
Heart myocardium Zenker degeneration	0/5	1/5 (2.00)	0/5	0/5	0/5	0/5
Thymus cortex hyperplasia	0/5	2/5 (2.00)	0/5	0/5	1/5 (2.00)	0/5
Kidney congestions	0/5	1/5 (1.00)	0/5	0/5	2/5 (1.50)	0/5

The values show the numbers of affected/evaluated (mean severity score of affected animals). Severity scores are out of a scale of 1 (minimal), 2 (slight), 3 (moderate), 4 (marked), 5 (severe).



DISCUSSION

Preclinical toxicological studies of medicinal plants are essential to evaluate their safety and efficacy in humans. Conducting these studies ensures that the safety and quality standards required by health regulations are met. Therefore, it is guaranteed that medicinal plants are safe and effective for use, and the risk of unwanted side effects is avoided (Thakur et al., 2020).

M. pudica, which is used by the population as a sedative and antidepressant, is utilized with caution due to its marked effects on the Central Nervous System. On the other hand, phytochemical studies of the plant reported the presence of the non-protein amino acid mimosine (Madal et al., 2022), to which toxic properties have been attributed when studied in other plant species (Honda and Borthakur, 2019).

M. pudica was administered in the form of a decoction for 28 days, and it did not cause deaths in experimental animals at a dose of 1000 mg/kg body weight. These survival results are in agreement with other studies conducted on the plant but in acute toxicity assays. A 100% survival rate and no signs of toxicity were found in Sprague Dawley rats receiving a methanolic extract of the plant at doses of up to 2000 mg/kg body weight in an oral acute toxicity test as part of the study of the antidiabetic and anti-hyperlipidemic effects of *M. pudica* (Parasuraman et al., 2019). Moreover, the safety of an alcoholic extract of *M. pudica* leaves was reported in an oral acute toxicity assay at a dose of 1000 mg/kg body weight

when evaluating its hepatoprotective effect in rats (Paras et al., 2022).

However, the effects of a substance may not be evident in a single acute exposure and could manifest over time. Then, repeated dose toxicity studies conducted over a prolonged period are necessary for the detection of cumulative, chronic, and delayed effects (Alkahtani et al., 2022).

Certain variations in the normal parameters of NRMI mice related to the administration of the plant were observed after 28 days of testing (Falkenberg et al., 2019). The performance of body weight may be related to the decrease in certain biochemical parameters such as proteins and lipids. Studies on plant species that contain mimosine as the main secondary metabolite, such as *Leucaena leucocephala*, have shown a decrease in the weight of different experimental biomodels when included in the diet, which has been attributed to the toxic effects of mimosine. This non-protein amino acid has been reported as a metabolite capable of decreasing protein biosynthesis due to its structural similarity with L-tyrosine, acting as an analog or antagonist of this amino acid (Gotardo et al., 2021). Similarly, due to its chelating properties of iron (Fe^{3+}), it can affect the formation of ferroproteins, and because of its ability to covalently bind to pyridoxal phosphate, an important cofactor of aminotransferase enzymes, amino acid metabolism could be affected (Honda and Borthakur, 2019). On the other hand, the ability to form conjugates between mimosine and bile acids can cause atypical bile salts that decrease the

absorption of lipids and fat-soluble vitamins in the gastrointestinal tract, affecting the incorporation of these nutrients into the body (Sethi and Kulkarni, 1995).

The long-term hyperglycemic effect found in this study has been described in albino Swiss mice treated with an ethanol extract of *M. pudica* leaves; however, these reported effects appear between 1-2 hours post-administration (Amalraj and Ignacimuthu, 2002). On the other hand, studies of repeated administration of extracts of the aerial parts of the plant in murine bio-models of diabetes showed that *M. pudica* is capable of restoring blood glucose levels after 21 days of treatment (Parasuraman et al., 2019; Thirukkalukundram-Singarapriyavardhanan et al., 2022). Glucose determinations in both cases were always made within 24 hours after the last administration of the plant preparation, similar to the experimental group in our study, in which glucose levels were within the normal range. However, hyperglycemic effects were found 14 days after treatment when evaluating the satellite group. These long-term effects, which should be further studied, corroborate the importance of including this satellite group in toxicity studies to evaluate the persistence of toxic effects or the delayed onset of such effects and their possible improvement or recovery (OECD, 2008).

In some cases, slight alterations in the brain, liver, lungs, heart, thymus, and kidneys correspond to the decrease found in the relative weight of these organs. In most cases, the effects were not pronounced enough to alter the levels of blood biomarkers, excepting the ALT enzyme, whose values are correlated with liver damage. *In vitro* studies conducted with mimosine in liver cells showed that this metabolite is capable of causing slight alterations in the intracellular enzymes of hepatocytes (Brahamchari et al., 2005). However, the administration of different doses of mimosine for 28 days in male Wistar rats did not lead to alterations in liver enzymes at the evaluated doses (Gotardo et al., 2021). On the other hand, *M. pudica* possesses metabolites with antioxidant properties, such as phenols and flavonoids (Pramanik and Sur, 2021), and the hepatoprotective activity of a hydroalcoholic extract of the plant was reported after its administration for 21 days to Wistar rats with liver damage induced by carbon tetrachloride (Paras et al., 2022). In our study, 14 days after completing treatment, the satellite group showed a recovery of the liver and hepatic enzymes.

CONCLUSION

The present study provides valuable information for the safety evaluation of *M. pudica*, which grows in Cuba. The biochemical and histopathological results

suggest that the decoction of *M. pudica*, administered orally at repeated doses, caused slight alterations that warn about the risks of its traditional use and highlight the importance of further research on the biological effects of this plant on different models.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

- Akhigbemen AM, Ozolua RI, Bafor EE, Okwuofu EO (2018) Subacute toxicological profile of *Caladium bicolor* Aiton (Araceae) methanolic leaf extract in rat. *J Pharm Pharmacogn Res* 6: 503-516. https://doi.org/10.56499/jppres18.411_6.6.503
- Alkahtani S, Hasnain MS, Algamdy H, Aljarba NH, AlKahtane A (2022) Acute and sub-acute oral toxicity *Lagerstroemia speciosa* in Sprague-Dawley rats. *Saudi J Biol Sci* 29(3): 1585-1591. <https://doi.org/10.1016/j.sjbs.2021.11.005>
- Amalraj T, Ignacimuthu S (2002) Hyperglycemic effect of leaves of *Mimosa pudica* Linn. *Fitoterapia* 73(4): 351-352. [https://doi.org/10.1016/s0367-326x\(02\)00079-5](https://doi.org/10.1016/s0367-326x(02)00079-5)
- Brahamchari AK, Asha M, Meena K (2005) *In vitro* effect of mimosine on certain enzymes in goat liver slice system. *Indian J Vet Res* 14(2): 35-39.
- Cutié-Bressler A, Rosales-Rodríguez RC, Gámez-Pérez RN (2015) *Mimosa pudica*: A local modality of abuse substance MEDISAN 19: 1556-1560.
- Falkenberg MK, Teilman AC, Henriksen T, Hau J, Poulsen HE, Abelson KS (2019) Clinical, physiologic, and behavioral evaluation of permanently characterized NMRI mice. *J Am Assoc Lab Anim Sci* 58(3): 380-389. <https://doi.org/10.30802/AALAS-JAALAS-18-000060>
- Gotardo AT, Dipe VV, de Almeida ERM, Hueza IM, Pfister JA, Górniak SL (2021) Potencial toxic effects produced by L-mimosine in the thyroid and reproductive systems. Evaluation in male rats. *Toxicol* 203: 121-128. <https://doi.org/10.1016/j.toxicol.2021.10.003>
- Hassan NA, Karunakaran R, Abdulmumin S (2019) A review on the pharmacological and traditional properties of *Mimosa pudica*. *Int J Pharm Pharm Sci* 11: 12-16. <http://dx.doi.org/10.22159/ijpps.2019v11i3.30452>
- Hayes AW, Hardisty JF, Harris SB, Okazaki Y, Weber K (2020) Oral repeated-dose toxicity studies of BIA 10-2474 in Wistar rat. *Regul Toxicol Pharmacol* 111: 104540. <https://doi.org/10.1016/j.yrtph.2019.104540>
- Honda MD, Borthakur D (2019) Mimosine concentration in *Leucaena leucocephala* under various environmental conditions.

- Trop Grassl-Forrajes Trop 7: 164-172. [https://doi.org/10.17138/tgft\(7\)164-172](https://doi.org/10.17138/tgft(7)164-172)
- Keshamma E, Srusti SNR (2022) Ethnopharmacological perspectives on *Mimosa pudica* - A review. Int J Eng Technol Manag Sci 6: 114-119. <https://doi.org/10.46647/ijetms.2022.v06i06.018>
- Kumar V (2021) Phytochemical, pharmacological activities and ayurvedic significances of magical plant *Mimosa pudica* Linn. Mini Rev Org Chem 18: 296-312. <http://dx.doi.org/10.2174/1570178617999200629155204>
- Madal AK, Pandey A, Sah RK, Baral A, Sah P (2022) *In vitro* antioxidant and antimicrobial potency of *Mimosa pudica* of Napalese Terai Region: Insight into L-mimosine as an antibacterial agent. Evid Based Complement Alternat Med 2022: 6790314. <https://doi.org/10.1155/2022/6790314>
- Majeed I, Rizwan K, Ashar A, Rasheed T, Amarowicz R, Kausar H, et al (2021) A comprehensive review of the ethnotraditional uses and biological and pharmacological potential of the genus *Mimosa*. Int J Mol Sci 22: 7463. <https://doi.org/10.3390/ijms22147463>
- Ntabaza VN, Amuri B, Ompey JMV, Simbi L, Byanga K (2023) Acute and sub-acute oral toxicities of aqueous leaf extract of *Vitex madiensis* Oliv. in guinea pig model. S Afr J Bot 153: 157-162. <https://doi.org/10.1016/j.sajb.2022.12.028>
- OEDC (2008) Organization for Economic Cooperation and Development. Guideline for the testing of chemicals. Revised draft guideline 407. Document on Repeated Dose 28-Day Oral Toxicity Study in Rodents: 1-13. <https://doi.org/10.1787/9789264070684-en>
- Paras H, Tahir M, Mehwish R, Akhtar S, Maqbool T, Chand Y, Qureshi JA, Hadi F, Atif M, Mehdi S (2022) Hepatoprotective effect of *Mimosa pudica* leaves ethanoic extract in CCl4-induced hepatotoxicity. J Xi'an Shiyu Univ Nat Sci 18(11): 1120-1131.
- Parasuraman S, Ching TH, Leong CH, Banik U (2019) Antidiabetic and antihyperlipidemic effects of a methanolic extract of *Mimosa pudica* (Fabaceae) in diabetic rats. Egyptian J Basic Appl Sci 6: 137-148. <https://doi.org/10.1080/2314808X.2019.1681660>
- Pramanik T, Sur TK (2021) Presence of antioxidants and nitric-oxide precursors in *Mimosa pudica* extract. GSC Adv Res Rev 07: 104-108. <https://doi.org/10.30574/gscarr.2021.7.3.0124>
- Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F (2020) Worldwide research trends on medicinal plants. Int J Environ Res Public Health 17: 3376. <https://doi.org/10.3390/ijerph17103376>
- Saraswat N, Wal P, Pal RS, Wal A, Pal Y, Pandey A (2020) Pharmacognostic evaluation and standardization of the leaves of *Mimosa pudica*, leaves of *Murraya koenigii* and root of *Asparagus racemosus*. Res J Pharm Tech. 13: 5743-5748. <https://doi.org/10.5958/0974-360X.2020.01000.8>
- Sethi P, Kulkarni PR (1995) *Leucaena leucocephala*: A nutrition profile. Food Nutr Bull 16(3): 1-16. <https://doi.org/10.1177/156482659501600307>
- Shauli M, Sewani-Rusike C, Abura S, Kolosa I, Gondwe MM, Gari M, Gqaza B, Rajesh N, Wickens N, Smith N (2023) Acute toxicity and 28-day oral administration of *euclea natalensis* extract in Swiss albino mice. J Med Lab Sci Technol S Afr 5(1): 1-10. <https://doi.org/10.36303/JMLSTSA.115>
- Thakur M, Singh K, Khedkar R (2020) Phytochemicals: Extraction process, safety assessment, toxicological evaluations, and regulatory issues. In: Prakash B (Ed), Functional and preservative properties of phytochemicals. Academic Press, pp. 341-361. <https://doi.org/10.1016/B978-0-12-818593-3.00011-7>
- Thirukkalukundram-Singarapriyavardhanan S, Timiri-Shanmugam PS, Koppala-Narayana SK, Ammari AA, Amran RA, Alhimaidi AR (2022) *Mimosa pudica* alleviates streptozotocin-induced diabetes, glycemic stress and glutathione depletion in Wistar Albino Rats. J King Saud Univ-Sci 34(4): 102037. <https://doi.org/10.1016/j.jksus.2022.102037>
- Upadhyay HC (2023) Exploring nature's treasure for drug discovery. Lett Drug Des Discov 20: 373-374. <https://doi.org/10.2174/157018082004230113144404>
- Wahyuni TS, Ilmi H, Kristiaji AS, Dewi MC, Nisa HK, Hafid AF, Widayawaruyanti A (2023) Acute and repeated dose 28-day oral toxicity of *Ruta angustifolia* Pers. leaves ethanolic extract in Wistar rats. J Pharm Pharmacogn Res 11(3): 437-447. https://doi.org/10.56499/jppres23.1609_11.3.437

AUTHOR CONTRIBUTION:

Contribution	Fong-Lores O	Berenguer-Rivas CA	de la Vega-Acosta JE	Mora-Tassé Y	Morris-Quevedo HJ
Concepts or ideas	x	x			x
Design	x	x			x
Definition of intellectual content	x	x			x
Literature search	x	x	x		x
Experimental studies	x	x	x	x	
Data acquisition	x	x	x	x	
Data analysis	x	x	x		
Statistical analysis	x	x	x		
Manuscript preparation	x	x	x		
Manuscript editing	x	x			x
Manuscript review	x	x	x	x	x

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