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Assessment of Physical Dependence and Respiratory Toxicity in Rats Following Exposure to Mimosa pudica L. Smoke

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ABSTRACT

Mimosa pudica L., a member of the Fabaceae family, has been traditionally employed in Cuban culture through inhalation of smoke produced from burning its aerial parts or by smoking it in cigarette form, for both recreational purposes and therapeutic uses. This research sought to investigate the toxic effects and potential for physical dependence associated with M. pudica via acute inhalation toxicity testing and a spontaneous withdrawal experiment in rats subjected to the plant's smoke. Mimosine levels were quantified using a colorimetric assay. Acute inhalation toxicity was assessed in accordance with OECD Guideline 433. The potential for dependence from the burned dried aerial parts was examined using a non-precipitated withdrawal protocol in female Sprague-Dawley rats, with a dose of 1000 mg/kg applied in both experiments. Analysis revealed mimosine concentrations in the smoke of 0.62 ± 0.05 µg/mg dry weight. Toxicity evaluation indicated nasal cavity congestion, focal brain gliosis, and peribronchial pneumonitis. Exposure led to physical dependence indicators, including hyperactivity (manifested as excitability and aggression), piloerection, loose stools, reduced body weight, and elevated rectal temperature during withdrawal. Rats inhaling M. pudica smoke exhibited acute toxicity patterns resembling those seen with smoked substances like tobacco or cannabis, alongside physical dependence signs similar to those of chlordiazepoxide. These observations emphasize the health hazards potentially linked to the customary smoking of M. pudica.

Keywords: Mimosa pudica, Mimosine, Smoke inhalation injuries, Withdrawal symptoms

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Introduction

Humans have utilized plants for millennia, with consumption practices evolving over time based on their intended applications. A particularly influential method is inhaling the fumes from burning dried plant material or smoking it directly. This approach has served various purposes, from leisure activities (both permitted and prohibited) to ritualistic or spiritual practices [1], as well as therapeutic ones [2, 3]. Certain therapeutic plants possess compounds that exert pharmacological effects when inhaled [4, 5].

Mimosa pudica L., also referred to as "Moriviví," "sleeping grass," "touch-me-not," or "sensitive plant," is part of the Fabaceae family. Native to South and Central America, it is widely used globally for its varied pharmacological attributes. Chemical profiling has identified alkaloids, amino acids (including mimosine), phenolics, tannins, flavonoids (such as quercetin, gallic acid, apigenin, and luteolin), sterols, terpenoids, and fatty acids (e.g., linoleic, linolenic, palmitic, and stearic acids) [6, 7]. These compounds contribute to its reported preclinical benefits, encompassing anti-inflammatory, pain-relieving, anxiety-reducing, antioxidant, nerve-protective, mood-elevating, and calming effects [8-13].

Ethnobotanical surveys in eastern Cuba have recorded its application as a calming, sleep-inducing, and anxiety-alleviating remedy, administered by burning the leaves for smoke exposure or rolling them into cigarettes for

recreational smoking [14]. In certain Mexican areas, inhaling smoke from its leaves and flowers has been noted for managing sleeplessness [15]. Nevertheless, the plant contains the known toxin mimosine [16]. Given the lack of prior preclinical scrutiny of this inhalation route, evaluating mimosine transfer into smoke and related risks is crucial for informing safe clinical application [17].

Accordingly, this investigation focused on examining inhalation toxicity and physical dependence in rats exposed to smoke from burned M. pudica, mirroring traditional practices. To our knowledge, this represents the initial preclinical evaluation of acute inhalation toxicity and unprovoked withdrawal in rats from M. pudica smoke.

Materials and Methods

Ethical considerations

All animal procedures followed international Good Laboratory Practice standards and complied with the NIH Guidelines for the Care and Use of Laboratory Animals (USA). The protocol received approval from the Institutional Animal Research Ethics Committee and the Quality Assurance Department at the Center of Toxicology and Biomedicine in Santiago de Cuba (Protocol TOXIMED/UGC/010404).

Chemicals

Hydrochloric acid, formalin, hematoxylin, and eosin were obtained from Sigma Aldrich (USA). Ferric chloride was sourced from Merck KGaA (Germany). Microscopy immersion oil came from Leica Microsystems (Switzerland).

Plant material

Aerial parts (stems and leaves) of M. pudica were gathered from El Caney, Santiago de Cuba, Cuba (coordinates: 20.0569 latitude, -75.7719 longitude), during morning hours in March 2024. Botanical identification was confirmed by experts at the Eastern Center for Ecosystems and Biodiversity (BIOECO, Santiago de Cuba). A voucher specimen was deposited in the institution's herbarium under accession number 218237.

Determination of mimosine in smoke from aerial parts

Smoke from the aerial portions was captured in 0.1 mol/L hydrochloric acid (HCl) solution, adapted from the procedure outlined by Norani *et al*. [5]. Briefly, 1 g of dried aerial material was burned in a combustion apparatus, with generated smoke directed by a fan (0.5 L/min flow) into a chilled trapping tube containing the acid solution on ice. All smoke from combusting 1 g of dry material was absorbed into 10 mL of cooled 0.1 mol/L HCl.

Mimosine quantification employed a modified rapid colorimetric assay [18, 19]. In summary, 200 µL of trap solution was mixed with 400 µL of 0.1 mol/L HCl and 200 µL of 0.5% $FeCl_3$ (in 0.1 mol/L HCl), diluted to 5 mL with distilled water, and absorbance read at 535 nm using a PG Instruments UV/VIS spectrophotometer (T60U, UK). Concentrations were calculated from the standard curve equation $y = 0.0065x + 0.0018$ ($R^2 = 0.9986$), constructed with mimosine standards ranging 10–70 µg/mL (seven points).

Test animals and husbandry

Female Sprague-Dawley rats that were nulliparous and non-pregnant, aged between 6 and 8 weeks and with body weights ranging from 150 to 200 g, were acquired from the National Center for Laboratory Animal Production (CENPALAB, Havana, Cuba). The rats were maintained in standard housing conditions, with environmental temperature controlled at 22 ± 3 °C, relative humidity averaging 65%, and a 12-hour light/dark cycle. They had unrestricted access to drinking water and standardized pelleted rodent diet (EMO:1002 formula) supplied by ALYco (CENPALAB, Havana, Cuba).

Plant administration

Exposure of the study animals to smoke produced by burning the aerial parts of M. pudica was achieved using an established rodent smoke inhalation model [20]. The setup included an exposure chamber for the animals, a combustion unit containing the plant material, a circulation fan to distribute the smoke, and a regulated ignition system to manage burning **(Figure 1).** Inside the 7.0 L inhalation chamber, rats were exposed to the smoke generated from the burned plant material under controlled conditions of 23 ± 3 °C temperature and approximately 60% relative humidity. Smoke was circulated at a flow rate of 0.5 L/min provided by the fan.

Acute inhalation toxicity assessment

Evaluation of acute inhalation toxicity from the plant's combustion products followed OECD Guideline 433, adapted as a modified limit test suited to the inhalation route and the described single-exposure setup [21]. Rats were allocated into two groups of 5 females each (experimental and control), for a total of 10 animals. The treated group received a dose equivalent to 1000 mg/kg body weight, with exposure lasting one hour to avoid risk of suffocation [22, 23]. The control group was placed in the chamber for the identical period but breathed only filtered air.

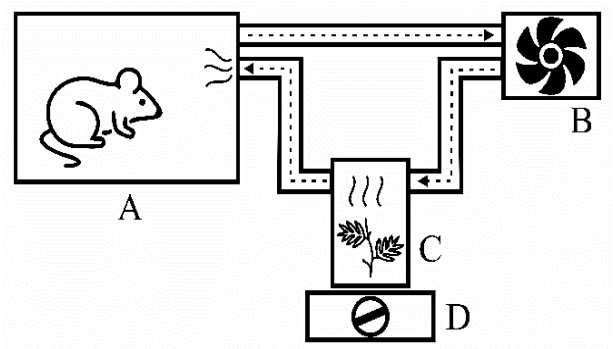


Figure 1. Smoke inhalation exposition system; A: inhalation chamber; B: circulating fan; C: incinerator; D: ignition source of the incinerator

Animals in both the treated and untreated groups underwent monitoring twice daily on the exposure day and at least once per day thereafter for a 14-day period. Special emphasis was placed on detecting signs such as shaking, seizures, drooling, loose stools, reduced activity, abnormal breathing patterns, drowsiness, or loss of consciousness.

At the end of the study period, animals were humanely euthanized through deep anesthesia induced by intraperitoneal injection of ketamine at 50 mg/kg body weight. Tissues from the brain, nasal passages, and lungs of subjects in both groups were examined microscopically. Specimens were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin before viewing under a light microscope (Leica DM 1000, Switzerland) equipped with a camera (Leica MC 170 HD, Heerbrugg, Switzerland).

Assessment of physical dependence

To investigate potential physical dependence associated with Mimosa pudica, a spontaneous abstinence protocol was implemented using Sprague Dawley rats [24]. Subjects were divided into three groups of five animals each. The treated group received daily inhalation of smoke from M. pudica aerial parts at 1000 mg/kg body weight, administered in two 10-minute sessions for 20 consecutive days, followed by an 8-day observation period without exposure. Body weight was tracked using a precision electronic scale (Sartorius, Germany), and core temperature was recorded with a probe thermometer (RG Medical Diagnostic, USA). Key signs monitored included leaping behaviors, shaking, increased activity, repetitive grooming, and loose stools. A positive comparator group received oral chlordiazepoxide at 64 mg/kg body weight, while the negative control group was given only the carrier solution, carboxymethylcellulose.

Statistical methods

Data are presented as mean values ± standard deviation. Variations in body weight and core temperature across days during the abstinence phase—for the untreated, chlordiazepoxide-treated, and M. pudica-treated groups—were evaluated using the Kruskal-Wallis one-way ANOVA. Withdrawal intensity was quantified by calculating a peak value for each subject (average of the highest reading and its two neighboring measurements), identifying the typical peak timing, and then deriving group severity scores by subtracting the corresponding averaged values from the vehicle control group across those same time points, thus adjusting for natural fluctuations over time [25]. Comparisons of severity indices for weight and temperature between the chlordiazepoxide and M. pudica groups were performed with the Mann-Whitney U test. Analyses utilized GraphPad Prism version 9 (Windows, 9.5.1, 2023), with statistical significance set at $p \leq 0.05$.

Results and Discussion

Quantification of mimosine in the smoke derived from combusted aerial parts of M. pudica yielded 0.62 ± 0.05 µg/mg of dry material. Prior reports on Indian specimens indicated mimosine levels of approximately 0.19% (via RP-HPTLC after methanol extraction) [26] or higher (1.938 mg/g via LC-MS-MS) [27]. The reduced levels in smoke likely result from thermal breakdown of compounds during burning [28]. Nonetheless, even at lower concentrations relative to direct plant ingestion [29], pulmonary delivery offers extensive absorptive surface, avoids hepatic first-pass effects, and enables quick transport of compounds to the brain [30].

Ethnopharmacological data suggest that customary therapeutic or informal use of combusted M. pudica (whether for healing or recreation, including rolled forms) typically involves exposures not exceeding one hour [14]. In the single-hour acute inhalation study, all exposed rats survived. Early signs in the treated subjects (within the first two hours) consisted of drowsiness, increased salivation, and erect hair. Over the following 14 days, behavior returned to normal.

Microscopic tissue analysis in the exposed group revealed distinct changes **(Table 1).** In the nasal passages, widespread blockage and bleeding beneath the lining were noted, along with damage to the surface layer; the blockage was the most frequent and intense lesion **(Figure 2a).** In the brain, marked reactive glial proliferation occurred in both gray and white regions with notable frequency **(Figure 2b),** accompanied by blood vessel blockage and swelling around vessels. The main lung abnormality was inflammation around the airways **(Figure 2c).**

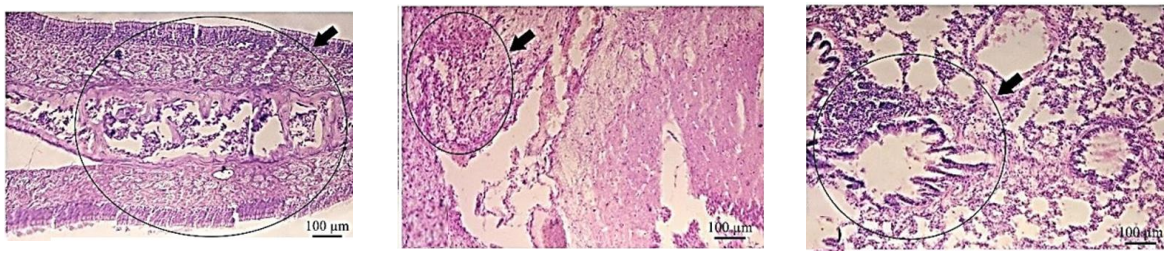
Table 1. Histopathological findings, incidence and severity score of damage in nasal cavity, brain and lungs after inhalation of *Mimosa pudica* smoke

Histopathological findings	Experimental Group	Control Group
Nasal cavity diffuse submucosal congestion	4/5 (2.75)	0/5
Nasal cavity hemorrhage	2/5 (2.00)	0/5
Nasal cavity degeneration of the mucosal epithelium	3/5 (1.67)	0/5
Brain focal gliosis	3/5 (5.00)	0/5
Brain vascular congestion	2/5 (1.50)	0/5
Brain perivascular edema	2/5 (2.00)	0/5
Lungs peribronchial pneumonitis	4/5 (2.67)	0/5

The values show the numbers of affected/evaluated (mean severity score of affected animals). Severity scores proposed by Hayes *et al*. [31], are out of a scale of 1 (minimal), 2 (slight), 3 (moderate), 4 (marked), 5 (severe)

The microscopic tissue changes observed aligned closely with those documented in research on inhalable materials like tobacco and cannabis. For instance, studies have described moderate vascular engorgement and influx of inflammatory cells in the nasal lining of Wistar albino rats subjected to cigarette smoke [32]. The lung tissue abnormalities, particularly inflammation around the airways, resembled patterns seen in rats inhaling cigarette smoke [33] or combustible cannabis products [34]. Inflammation in the lungs triggered by burning plant material has been explored in rat models of smoke exposure, where combustion generated gases such as carbon monoxide, hydrogen sulfide, and nitric oxide. In these models, smoke inhalation led to a significant elevation in interleukin-8 (IL-8), a key pro-inflammatory mediator involved in lung damage [35]. Additional studies on rats exposed to tobacco [36] or cannabis [37] have consistently reported reactive glial proliferation in the brain as a common pathological change [38].

Regarding the spontaneous withdrawal protocol, all animals survived the experiment. As detailed in **Table 2**, both the group receiving inhaled M. pudica smoke (at 1000 mg/kg) and the positive control group treated with chlordiazepoxide (64 mg/kg) showed behavioral and physiological patterns typical of central nervous system depressants. Notably, the animals exposed to the combustion products of the plant exhibited more prominent drowsiness (with greater frequency and intensity), diarrhea, excessive drooling, early rapid breathing in the first few days of exposure, and noticeable abdominal contractions suggestive of increased gut motility during sessions inside the inhalation chamber.



a) b) c)

Figure 2. Representative H&E-stained histopathological micrographs (100×) illustrating lesions in major target organs following acute inhalation toxicity induced by Mimosa pudica aerial parts, showing congestion in the nasal cavity (a), gliosis in the brain (b), and peribronchial pneumonitis in the lungs (c).

Table 2. Clinical signs observed during the 20-day administration period and after treatment cessation in control, reference (Chlordiazepoxide at 64 mg/kg b.w.), and experimental (*Mimosa pudica* at 1000 mg/kg b.w.) groups

Clinical signs	During administration			After administration		
	Control	Reference	Experimental	Control	Reference	Experimental
Piloerection	0/5	0/5	0/5	0/5	4/5 (1.75)	5/5 (1.60)
Somnolence	0/5	0/5	5/5 (5.00)	0/5	0/5	0/5
Salivation	0/5	0/5	4/5 (4.25)	0/5	0/5	0/5
Hyperactivity	0/5	5/5 (4.40)	5/5 (2.60)	0/5	5/5 (2.80)	5/5 (4.20)
Tremor	0/5	0/5	0/5	0/5	0/5	0/5
Tachypnea	0/5	0/5	2/5 (2.00)	0/5	0/5	0/5
Soft feces	0/5	3/5 (2.33)	3/5 (3.00)	0/5	0/5	1/5 (1.00)
Hypermobility	0/5	0/5	3/5 (3.00)	0/5	0/5	0/5

The values show the numbers of affected/evaluated (mean severity score of affected animals); severity scores proposed by Hayes *et al.* [31], are out of a scale of 1 (minimal), 2 (slight), 3 (moderate), 4 (marked), 5 (severe)

After returning to their individual housing areas, the animals showed increased levels of activity, including mild agitation and elevated thirst, leading to greater water intake. This rise in drinking behavior may have stemmed from dryness in the oral mucosa caused by the smoke exposure or from a strong salivation reaction. In contrast, the control group receiving chlordiazepoxide demonstrated pronounced restlessness and intense aggression right from the start of dosing, with these behavioral changes intensifying progressively. Changes in body weight for the animals exposed to the smoke and for the chlordiazepoxide control group during the last three days of the treatment phase and the following eight days are presented in **Figure 3.** The chlordiazepoxide control group followed a pattern consistent with that reported earlier [22]. A substantial drop in body weight was noted on day 22 of the study, which was the second day after cessation of administration. This weight loss gradually improved in the subsequent days. The group treated with M. pudica smoke exhibited a similar trend, although the decline in body weight was more severe on the third day post-administration, corresponding to day 23 of the experiment.

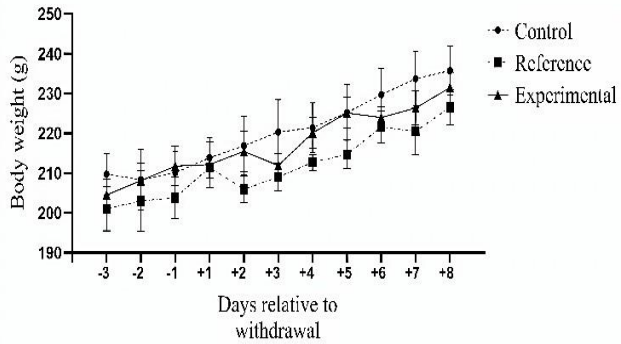


Figure 3. Variations in body weight among animals in the control group, the chlordiazepoxide reference group (64 mg/kg), and the Mimosa pudica experimental group (1000 mg/kg) across days following withdrawal; all data are presented as the mean ± standard deviation (n=5).

Additionally, the patterns of rectal temperature in the animals treated with smoke and in the chlordiazepoxide reference group during the last three days of the treatment phase and the following eight days are depicted in **Figure 4.** The group exposed to M. pudica smoke showed a statistically significant elevation in rectal temperature ($p<0.05$) on day 22 of the study, corresponding to the second day after cessation of exposure, relative to the control group. In the chlordiazepoxide reference group, rectal temperature rose on both days 22 and 23, with this elevation reaching statistical significance on day 23 ($p<0.05$).

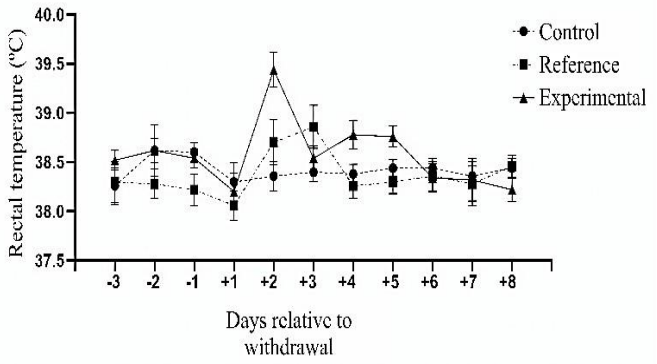


Figure 4. Changes in rectal temperature among animals in the control group, the chlordiazepoxide reference group (64 mg/kg), and the Mimosa pudica experimental group (1000 mg/kg) across days following withdrawal; all data are presented as the mean ± standard deviation (n=5).

Several approaches have been described for evaluating the intensity of withdrawal, either through individual analysis of each parameter or by integrating data from several parameters via validated equations [39, 40]. For instance, Cruz *et al*. suggested calculating withdrawal severity for various clinical signs by subtracting the measurements obtained in treated animals from those of the control group [41]. Applying a similar approach, the modification introduced by Metten *et al*. was utilized for scoring handling-induced convulsions [25]. However, as no seizures occurred in this investigation, the method was instead employed to score the parameters of body weight and rectal temperature. The calculated withdrawal severity scores for the M. pudica and chlordiazepoxide groups are displayed in **Table 3**. There were no marked differences in withdrawal severity related to body weight changes between the experimental and reference groups. Nevertheless, M. pudica showed a significantly higher withdrawal severity score for rectal temperature in comparison to chlordiazepoxide ($p<0.05$).

Particularly noteworthy in the M. pudica-treated animals was the sustained presence of sedative effects during the entire period of exposure. Yet, upon cessation of plant exposure, a clear shift in behavior toward marked hyperactivity—manifested as excitability and aggression—was observed. This pattern is reminiscent of the symptoms seen in withdrawal syndromes associated with alcohol and benzodiazepines [42, 43]. A methanol extract from the bark of M. pudica produced sedative effects that resembled the pharmacological profile of diazepam through binding to the benzodiazepine site on GABA-BDZ receptor complexes [44].

Table 3. Withdrawal severity scores following treatment with Mimosa pudica and chlordiazepoxide

Treatment group	Rectal temperature change	Body weight change
Chlordiazepoxide (64 mg/kg)	0.73 ± 0.15	7.56 ± 4.48
Mimosa pudica (1000 mg/kg)	1.30 ± 0.10*	3.11 ± 1.64

Values are presented as mean ± SD (n = 5); * denotes a statistically significant difference for the Mimosa pudica group compared with the chlordiazepoxide control, based on the Mann–Whitney U test ($p < 0.05$).

Although extensive investigations have clarified many of the classical pathways involved in addiction to commonly abused substances, the neurobiological bases underlying newer patterns of consumption with dependence potential remain incompletely understood and represent an ongoing scientific challenge. Mimosa pudica contains L-mimosine as its major metabolite, a compound that shares notable structural resemblance with L-tyrosine [45]. This similarity raises the possibility that mimosine may interfere with the biosynthesis or functional regulation of tyrosine-derived catecholamines, including dopamine (DA), norepinephrine, and epinephrine, thereby contributing to mechanisms relevant to substance dependence [46]. Supporting this

hypothesis, previous reports have described the inhibitory action of mimosine on dopamine β-hydroxylase, an enzyme crucial for the enzymatic conversion of dopamine into norepinephrine, an effect linked to its metal-chelating capacity [47]. Such inhibition may disrupt catecholamine balance, favoring dopamine accumulation as its downstream metabolism is suppressed **(Figure 5).**

It is well documented that drugs of abuse elevate dopamine levels within the nucleus accumbens through substance-specific molecular pathways, ultimately activating the mesolimbic reward system **(Figure 5).** This dopaminergic overstimulation plays a central role in reinforcing drug-seeking behavior and sustaining addiction in dependent individuals [48]. In line with this concept, exposure to mimosine in zebrafish has been associated with enhanced dopaminergic signaling [49]. Nevertheless, additional research is required to confirm whether this effect involves dopamine release within the nucleus accumbens, or whether it parallels the action of selective dopamine β-hydroxylase inhibitors, which preferentially increase dopamine availability in the medial prefrontal cortex [50].

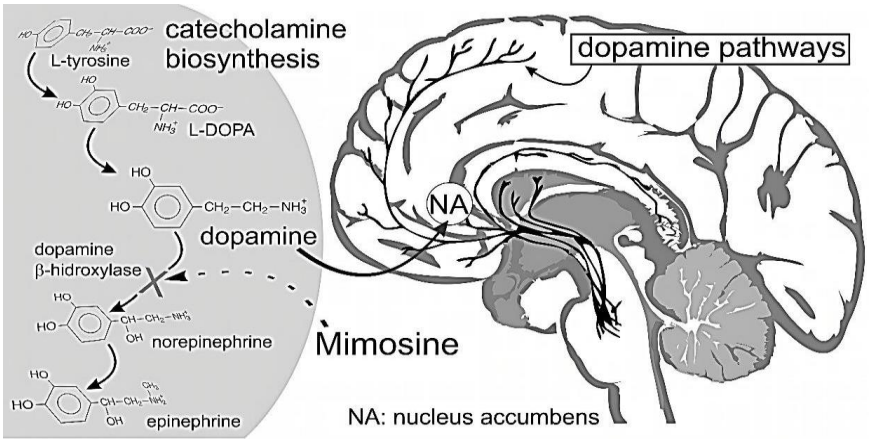


Figure 5. Schematic representation of the catecholamine synthesis cascade illustrating the proposed influence of L-mimosine on dopamine β-hydroxylase activity and the subsequent stimulation of dopaminergic reward pathways; illustration designed by the authors.

Conclusion

The findings of this work introduce an alternative interpretation of a customary practice associated with M. pudica, focusing on exposure via inhalation of its combustion byproducts. Experimental animals subjected to M. pudica smoke exhibited manifestations of acute respiratory toxicity comparable to those reported for commonly smoked materials, including tobacco and cannabis, and developed measurable physical dependence with a profile similar to that observed for chlordiazepoxide. In addition, the results give rise to a new conceptual framework suggesting that mimosine, the major metabolite of M. pudica, may interfere with central neurochemical regulators, a proposition that warrants targeted mechanistic studies in future research.

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Conflict of Interest: None

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Ethics Statement: None

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