Evaluation of the antispasmodic and toxicity potential of methanol extract of *Chromolaena odorata* on Ileum muscle in animal models

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ABSTRACT

Background: *Chromolaena odorata* plant is widely used in traditional medicine with a great potential for treating various ailments, including diabetes, malaria, wounds, gastrointestinal disorders and inflammation, and its bioactive compounds could lead to new therapeutic applications.

Objectives This study was designed to determine the *in vitro* effect (contraction or relaxation) of orally administered methanol extract of *C. odorata* on ileum muscle as well as to evaluate its in vivo effect on the histological architecture integrity of the ileum tissue in rat models.

Methods: Thirty adults mice weighing between 20 g to 30 g were randomly selected and divided into six groups. They were used to determine acute toxicity using the modified Lorke's method. Also, twenty matured Wistar rats 180 g - 200 g were divided into four groups of five animals each were utilized in the *in vitro* studies. Standard experimental procedures were observed. The Organ bath was filled with Tyrode's solution and mounted with a strip of ileum muscle tissues. Histological analysis was also performed on the harvested ileum using Mayer's haematoxylin and eosin staining techniques.

Results: The LD_{50} was 2738.61 mg/kg which is indicative of a relatively low acute toxicity. The extract exhibited dose-dependent antispasmodic effects on ileum smooth muscle, mediated at least in part through muscarinic receptors, as demonstrated by atropine's antagonistic effects. *C. odorata* extract (2×10^{-2} mg/ml) induced the maximum relaxation response (-7.0 ± 0.58 mm, 100 % of max response), though less potent than atropine, but statistically not significant at $P < 0.05$. Histological findings suggest that low doses are safe, while higher doses induced moderate mucoal damage.

Conclusion: The findings suggest that the extract exhibits dose-dependent antispasmodic effects on ileum smooth muscle, mediated partly through muscarinic receptors. Low doses are safe, while higher doses induce moderate mucosal damage, indicating a need for careful dose optimization. These findings validate the traditional use of *C. odorata* in treating gastrointestinal disorders and suggest its potential as a natural antispasmodic agent.

Keywords: *Chromolaena odorata;* antispasmodic; toxicity; ileum; methanol

Évaluation du potentiel antispasmodique et toxique de l'extrait méthanolique de *Chromolaena odorata* sur le muscle de l'iléon chez des modèles animaux

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RESUME

Contexte: La plante *Chromolaena odorata* est largement utilisée en médecine traditionnelle, et elle présente un grand potentiel pour le traitement de diverses affections, notamment le diabète, le paludisme, les plaies, les troubles gastro-intestinaux et l'inflammation. Ses composés bioactifs pourraient déboucher sur de nouvelles applications thérapeutiques.

Objectifs: Cette étude a été conçue pour déterminer l'effet in vitro (contraction ou relaxation) de l'extrait de méthanol de *C. odorata* administré par voie orale sur le muscle de l'iléon ainsi que pour évaluer son effet in vivo sur l'intégrité de l'architecture histologique du tissu de l'iléon chez des modèles de rat.

Méthodes: Trente souris adultes pesant entre 20 et 30 g ont été sélectionnées aléatoirement et réparties en six groupes. Elles ont été utilisées pour déterminer la toxicité aiguë par la méthode modifiée de Lorke. De plus, vingt rats Wistar matures pesant entre 180 et 200 g ont été répartis en quatre groupes de cinq animaux chacun et ont été utilisés dans les études in vitro. Les procédures expérimentales standard ont été respectées. Le bain d'organes a été rempli de solution de Tyrode et recouvert d'une bandelette de tissu musculaire de l'iléon. Une analyse histologique a également été réalisée sur l'iléon prélevé par coloration à l'hématoxyline et à l'éosine de Mayer.

Résultats: La DL_{50} était de 2738,61 mg/kg, ce qui indique une toxicité aiguë relativement faible. L'extrait a montré des effets antispasmodiques dose-dépendants sur le muscle lisse de l'iléon, médiés au moins en partie par les récepteurs muscariniques, comme le démontrent les effets antagonistes de l'atropine. L'extrait de *C. odorata* (2 x 10?² mg/ml) a induit la réponse de relaxation maximale (-7,0 ± 0,58 mm, 100 % de la réponse maximale), bien que moins puissant que l'atropine, mais sans signification statistique à P<0,05. Les résultats histologiques suggèrent que les faibles doses sont sans danger, tandis que les doses plus élevées ont induit des lésions muqueuses modérées.

Conclusion: Les résultats suggèrent que l'extrait présente des effets antispasmodiques dose-dépendants sur le muscle lisse de l'iléon, médiés en partie par les récepteurs muscariniques. Les faibles doses sont sans danger, tandis que des doses plus élevées induisent des lésions muqueuses modérées, ce qui nécessite une optimisation prudente de la dose. Ces résultats valident l'utilisation traditionnelle de *C. odorata* dans le traitement des troubles gastro-intestinaux et suggèrent son potentiel en tant qu'agent antispasmodique naturel.

Mots clés: *Chromolaena odorata* ; antispasmodique; toxicité; iléon; méthanol

INTRODUCTION

Chromolaena odorata (L) King and Robinson (Asteraceae), commonly known as Siam weed, is a fast-growing perennial and invasive weed native to South and Central America. It has been introduced into the tropical regions of Asia, Africa and other parts of the world. *C. odorata* is also known by various other names such as Armstrong's Weed, Baby Tea, Bitter Bush, Butterfly Weed, Christmas Bush, Devil Weed, Eupatorium, Jack in the bush, King Weed, Paraffin Bush, Paraffin Weed, Turpentine Weed and Triffid Weed.[1] *Chromolaena odorata L.* has a short life cycle of approximately ten years. It occasionally reaches its maximum height of 6 m as climber on other vegetation. The root formation is fibrous and does not penetrate beyond 20-30 cm in most soil. The flowers are white or pale bluish-lilac.[2]

It is an aggressive competitor that occupies different types of lands where it forms dense strands that prevents the establishment of other flora. It is a menace in plantations and other ecosystems. It suppresses young plantations, agricultural crops and smothers vegetation as it possesses allelopathic potentialities and growth inhibitors.[3,4,5] The economic value of *C. odorata* is low. Consequently, there is relative paucity of research works on it.

In recent decades, it has become a serious weed in the humid tropics of South East Asia, Africa and Pacific Islands. Following its introduction to Nigeria, the weed quickly spread through eastern Nigeria in the 1940 s and was first reported by Ivens in 1974 to the west of the River Niger in 1955 and from Lagos and its environs in 1960, from where it might have spread into Benin Republic and other West African countries. By 1960, *C. odorata* had occupied the south-eastern states of Nigeria, and possibly spread from there into Cameroon.[6,7,8]

It spreads rapidly in lands used for forestry, pasture and plantation crops such as rubber, coffee, coconut, cocoa and cashew. The plant is poisonous to livestock as it has exceptionally high level of nitrate (5 to 6 times above the toxic level) in the eaves and young shoots; it can result in tissue anoxia when fed to cattle.[9] Despite the negative effects of the plant, it has patronage from practitioners of traditional medicine.

Figure 1. Leave and flowers of *Chromolaena odorata L .* Source: Field Source (2025)10

In the southern part of Nigeria, the leaves are used for wound dressing, skin infection and for haemostasis. The fresh leaves of *C. odorata* or the decoction has been used by practitioners of traditional medicine for the treatment of human burns, soft tissue wounds, ulcerated wounds, burn wounds, postnatal wounds and also for the treatment of leech bites, indigestion and skin infection. It is also used for the treatment of various ailments, such as amenorrhea, catarrh, cold-associated nasal congestion, diabetes, diarrhoea, fever, pertussis and rheumatism, and as a vermifuge.[11] Other pharmacological properties of this plant include anthelminthic,[12] antimalarial, analgesic,[13,14] anti-inflammatory, antipyretic, antispasmodic,[15-17] antimycobacterial, insecticidal, antioxidant, anti-gonorrheal), fungicidal, diuretic,[18,19] blood coagulating, and antimicrobial effects.[20]

The medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. Despite the widespread use of *C. odorata,* its impact on ileum smooth muscle remains poorly understood. While some studies explore the plant's anti-ulcer properties, a focused examination of its effect on the ileum is currently lacking. Given the increasing use of traditional medicine in the health sector, research is needed to determine whether *C. odorata* extracts have any direct effects on the contractility, relaxation and histology of the ileum smooth muscle; especially as it is often taken via the oral route when used as herbal mixture for treatments.

Phenols have been reported as one of the essential constituents in *C. odorata.* The structure contains a hydroxyl group, a property that is responsible for the scavenging effect of this plant.[21]

This study aimed at evaluating the effect of the methanol extract of *Chromolaena odorata* on the ileum smooth muscle mechanical activities and histology in Wistar rat model.

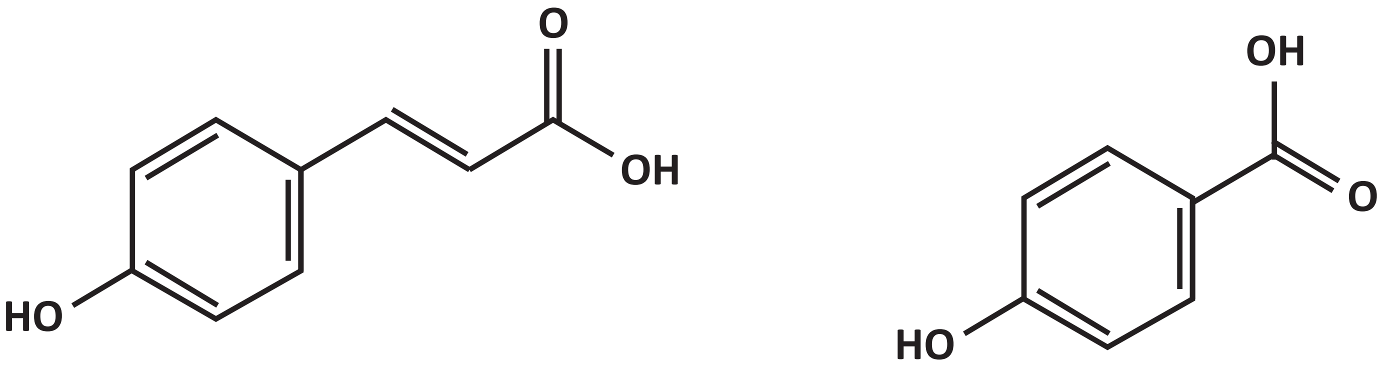

Figure 2: Chemical structure of p-coumaric acid (left) and p-hydroxybenzoic acid (right) from *C. odorata* leaves.
Source: Pisutthanan (2006)21

MATERIALS AND METHODS

Fresh leaves of *Chromolaena odorata* were collected from Ekebedi oboro, Ikwuano LGA, Abia State, Nigeria. The plant was identified and authenticated by a taxonomist of the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria, and deposited in the Faculty of Pharmacy Herbarium with a specimen No. UUPH No.10 (c).

Thirty (30) healthy albino mice weighing 20 g to 30 g and twenty (20) Wistar rats were obtained from University of Uyo animal house and the animals were allowed to acclimatize in the laboratory for a period of seven days. They were allowed free access to feed and water ad libitum throughout the period of the experiment. The mice and rats were kept in wooden cages furnished with hard wood chip beddings ambient temperatures of 28^{o} Celsius. Ethical standard and procedures were observed.

Aluminum foil, cotton wool, syringes (5 ml), cannula, masking tapes, disposable hand gloves and nose mask, breakable plates, beakers (250 ml, 1000 ml), surgical scissors, organ bottles, plain bottles, Electronic weighing balance, triple beam balance, stirring rods, pot, Wattman No. 1 filter paper.

Water, 10 % buffered formalin, ketamine, Atropine, tween 80 (polysorbate 80), 10 % formalin and other chemicals. All drugs and chemicals were of standard analytical grades and were purchased from the Pharmacy store of the University Uyo Teaching Hospital, Uyo and a few other chemicals gotten from the Laboratory of the Department of Pharmacology and Toxicology, University of Uyo, Uyo, Nigeria.

Preparation of drugs and extraction

Fresh leaves of *Chromolaena odorata* were carefully separated from the stalks, were washed free of sand and debris under a running water tap and rinsed with distilled water. The fresh leaves were shade-dried for a period of two weeks. The dried leaves were chopped into pieces and progressively turned into powder, with the use of an electric grinding machine. The resulting powder (400 g) was macerated exhaustively in 1500 ml of 80 % methanol for 72 hours, and was filtered through a filter paper (Whatmann no. 1) to obtain a methanolic crude extract.

The extract was concentrated by evaporation to dryness in a rotary evaporator at 40^oC to yield a dried methanolic extract of 96.2 g. The percentage yield was 24.05 %. This was appropriately labeled and stored in the refrigerator at - 20^oC for further use.

The volume of *Chromolaena odorata* extract administered to all the animals in the test group in the in vivo experiment using a 23 G stainless steel oropharyngeal cannula was calculated.

$$\text{Volume administered (mL)} = \frac{\text{weight of the animal (kg) x required dose (mg/kg)}}{\text{Concentration of test drug (mg/mL)}} \quad \ldots\ldots (1)$$

Preparation of atropine

An atropine injection of 1 mg/ml (ATOCAN INJ.) was used for this experiment. A four (4) fold serial dilution was carried out to produce different concentrations ranging from 10 $^{-2}$M Up to 10 $^{-6}$M atropine.

Preparation tyrode's solution

The following were used to make the solution: sodium chloride (8 g/L), potassium chloride (0.2 g/L), calcium chloride (0.1 g/L), sodium dihydrogenphosphate (0.05g/L), magnesium chloride(0.1 g/L), glucose(2 g/L). These salts were accurately weighed and dissolved in four liters of distilled water.

Experimental procedure

Study design for acute toxicity study (LD_{50})

Thirty (30) albino mice weighing between 20 g to 30 g were randomly selected from the animal house unit, Department of Pharmacology and Toxicology and the rats were subdivided into six (6) groups (with n=5 per group).

In the first phase of the acute toxicity study using modified Lorke's method, Group I, Group II and Group III mice were administered a single fixed dose of the respective *C. odorata* extract (1000 mg dissolved in 10 mL of distilled water) tested in a stepwise procedure (1000, 3000, and 5000 mg/kg body weight of mice). The animals were observed for any sign of toxicity such as rising fur, draping, tremors, excitability, twitching, salivation and mortality for the first 4 hours after the treatment period up to 24 hours.

The 1000 mg/kg body weight of the *C. odorata* extract was the highest dose determined not to induce acute toxicity in mice during the first phase of the present study.

The second phase was carried out with Group IV, Group V and Group VI mice (n=3 mice per group) by administering a single fixed dose of the *C. odorata* extract tested in a stepwise procedure, 1500 mg/kg for Group IV, 2000 mg/Kg for Group V and 2500 mg/Kg for Group VI. The animals were observed for any toxic effect such as rising fur, draping, tremors, excitability, twitching, salivation and mortality for period of 72 hours.

Study design for *in vivo* studies

Twenty (20) Albino rats weighing between 180 g to 200 g were randomly selected from the animal house unit, department of pharmacology and toxicology and the rats were subdivided into four (4) groups (with n=5 per group) of control, *Chromolaena odorata* extract (273.86 mg/Kg), *Chromolaena odorata* extract (547.72 mg/Kg), *Chromolaena odorata* extract (821.58 mg/Kg).

Treatments

The rats were divided into four (4) groups (n=5). Group 1, rats were administered distilled water (3 ml/kg) only as negative control; group 2 were also administered *Chromolaena odorata* extract (273.86 mg/kg) body weight; group 3 were administered *Chromolaena odorata* extract (547.72 mg/kg); group 4 were administered *Chromolaena odorata* extract (821.58 mg/kg). The period of treatment was twenty eight (28) days. The animals were then sacrificed and the ileum harvested and obtained respectively.

At the end of the 28 days of treatment, the animals were fasted for 24 hours after which, they were euthanized with 1.2 mg/Kg of ketamine as an anesthetic agent then on paralysis, they were sacrificed and the ileum harvested, washed with physiological saline solution to remove blood stains and then fixed into the organ bottle containing 10 % buffered formalin and sent for analysis and histological studies.

Experimental procedures in *in vitro* animal studies

Standard experimental procedures as described by Unekwe in 1990 and modified by Tologbonse , were carried out using an organ bath with a slow moving kymograph.[22,23] The organ bath was properly washed using distilled water after which it was sufficiently filled with physiological solution (Tyrode's solution). The rats were dissected via a V cut on the midline of the anterior abdominal and the ileum muscle was gotten at the hypogastric region.

The isolated ileum smooth muscle tissue was picked using sterile forceps; a needle was used to pass a thread through the tissue to form a loop through which the tissue holder was to be inserted. Another needle was used to pass a thread through the tissue and this was tied through the arm of the frontal writing lever. Subsequently, the tissue was aerated to ensure that it was alive for the experiment to be successful. A gum was used to maintain proper balance on both sides of the frontal writing lever. At this point, the tissue holder was placed in the organ bath tube containing the physiological salt solution. The tissue was allowed to stabilize for 30-60 minutes before the investigation commenced. The tissue in the physiological salt solution was closely observed for contractile response to ascertain that it was active.

Experimental protocols/ethical approval

Standard experimental protocols were observed, Faculty of Pharmacy, University of Uyo, ethical committee's clearance was obtained, in line with the Principle of Laboratory Animal care:[24] hence, standard protocols were used to carry out the following:

i. Determination of the intrinsic mechanical activity of the isolated ileum tissue in the presence of Tyrode's solution without the various contractile agents.
ii. Dose-response curves of Acetylcholine using isolated ileum smooth muscle preparation.
iii. Effect of *Chromolaena odorata* extract on the dose-response curve in step ii above,
iv. Effect of *Chromolaena odorata* extract on the relaxation action of Atropine.
v. Histological study of the effect of *Chromolaena odorata* extract in *in-vivo* studies using experimental rat models.

Statistical analysis

Results were presented as mean ± standard error of mean (SEM). Analysis of data was done using SPSS software package employing the one way ANOVA followed by Dunnett post Hoc test with significant differences at P<0.05 and P<0.01 respectively.

RESULTS

The results of this study revealed 24.05 % yield of the methanolic extract suggests that *C. odorata* contains a substantial amount of bioactive compounds: The LD_{50} of *C. odorata* methanolic extract was calculated and found to be 2738.61 mg/Kg, which indicates that *C. odorata* methanolic extract has low acute toxicity, as shown in the results below and Table 1 and 2 respectively.

Furthermore, the results of *in vitro* study on ileum contractility and relaxations are listed sequentially in Table 3 and Table 4: Also, the histological findings are also presented in Figures 3-6. They help to provide critical insights into the safety and potential toxicity of *C. odorata* extract on ileum tissues at the different treatment doses used. See detailed results below:

Calculation of percentage yield

Weight of beaker alone ……………………………….159.7g
Weight of beaker + Plant powder………………… 559.7g
Weight of plant powder ……………………………...559.7 - 159.7 = 400g
Weight of beaker + Methanolic extract……………255.9g
Weight of methanolic extract only…………………255.9 - 159.7 = 96.2g

$$\text{Percentage yield} = \frac{96.2}{400} \times 100 = 24.05\%$$

Calculation on LD_{50}

Table 1. Phase I (using modified Lorke's method)

Serial Number	Groups	Number of mice treated	Number of mortality	% Mortality
1	Control (Water only)	3	0	0
2	*C. odorata* extract (1000 mg/kg)	3	0	0
3	*C. odorata* extract (3000 mg/kg)	3	3	100
4	*C. odorata* extract (5000 mg/kg)	3	3	100

Table 2: Phase II (using modified Lorke's method)

Serial Number	Groups	Number of mice treated	Number of mortality	% Mortality
1	*C. odorata* extract (1500 mg/kg)	3	0	0
2	*C. odorata* extract (2000 mg/kg)	3	0	0
3	*C. odorata* extract (2500 mg/kg)	3	0	0

$LD_{50} = \sqrt{A\ B}$

$= \sqrt{2500 \text{ x } 3000}$

$= \sqrt{7{,}500{,}000}$

LD_{50} = 2,738.61 mg/Kg (Lorke's Method)

In the *in vitro* studies, *C. odorata* extract (2 x 10^{-2} mg/ml, - log [M] = 1.7) induced the maximum relaxation response (-7.0 ± 0.58 mm, 100 % of max response) as seen in the Table 3:

Table 3: Effect of *C. odorata* methanolic extract treatments on ileum smooth muscle / Atropine

Treatment	Final bath concentration (mg/ml)	log(m)	Height of concentration (mm)	% of maximum response
Atropine (Control)	0.00004	4.4	-9.2 ± 0.44	-
C. odorata extract	2 x 10^{-2}	1.7	-7.0 ± 0.58*	100
C. odorata extract	2 x 10^{-4}	3.7	-3.1 ± 0.10*	44.3
C. odorata extract	2 x 10^{-5}	4.7	-2.2 ± 0.17*	31.4
C. odorata extract	2 x 10^{-6}	5.7	-1.2 ± 0.17*	17.1

- *Values are Mean ± SEM*
- **The Mean difference is significant at *P<0.05*
- *The low SEM values (0.17–0.58) suggest high precision and reproducibility in the measurements.*
- *Dunnett t-tests treat Atropine (0.00004) as control and compare all other groups against it.*

The effect of atropine (4×10^{-3} mg/ml, fixed concentration) on *C. odorata* induced relaxation is shown in Table 4:

Table 4: Atropine effect on induced extract activity on ileum smooth muscle

Final bath concentration (mg/ml)	log(m)	Height of concentration (mm)	% of maximum response
4×10^{-2}	4.4	-9.5 ± 0.83*	100
4×10^{-3}	4.4	-4.0 ± 0.17	42.1
4×10^{-4}	4.4	-3.0 ± 0.17	31.6
4×10^{-5}	4.4	-2.5 ± 0.17	26.3

- *Values are Mean ± SEM, The Mean difference is significant at *P<0.05*
- *The highest concentration (2×10^{-1} mg) elicited the maximum response, while lower concentrations showed progressively reduced relaxation activity.*
- *The low SEM values (0.17− 0.58) suggest high precision and reproducibility in the measurements.*
- *Dunnett t-tests treat Atropine (0.00004) as control and compare all other groups against it.*

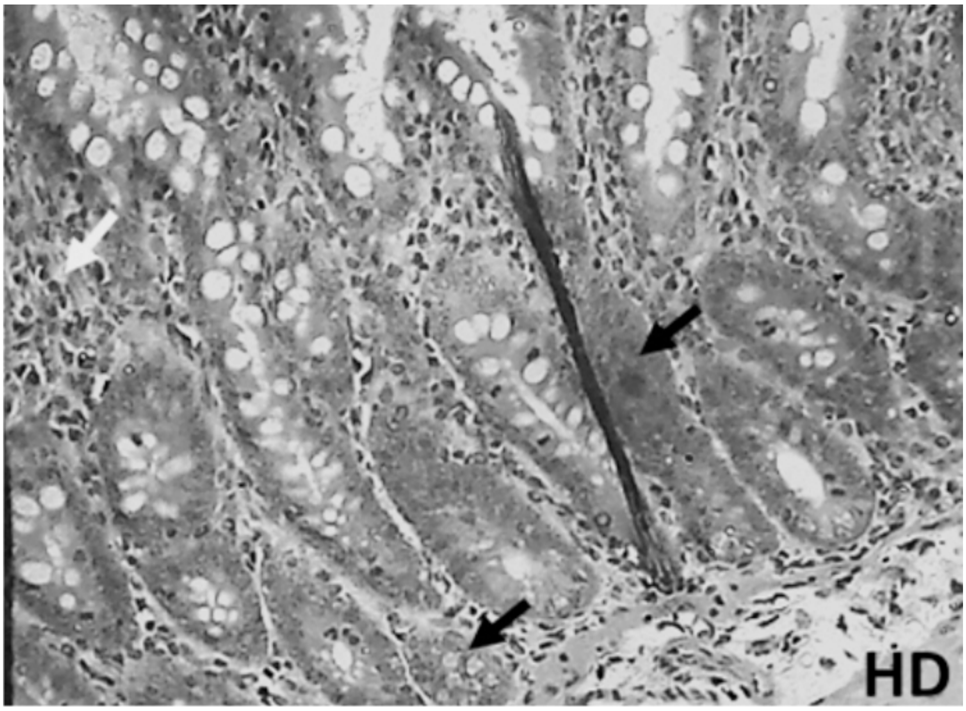

Figure 3: Photomicrograph of the longitudinal section of Group 3 (High Dose- 821.58 mg/Kg) treated small intestinal (ileum) mucosa showing a moderately affected digestive tissue with areas of hyperplasic crypt cells (black arrows), and proliferating fibrolysis of the lamina propria (yellow arrow) within the endometrial mucosa. (HandE x 100). Inference: Moderately ulcerated.

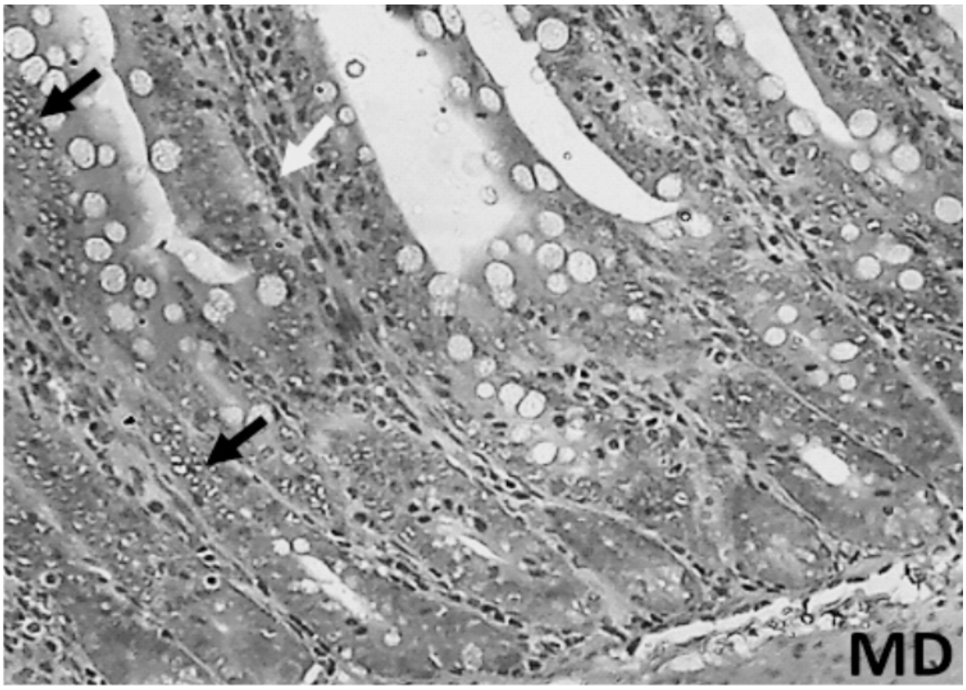

Figure 4 : Photomicrograph of the longitudinal section of Group 2 (Medium Dose- 547.72 mg/Kg) treated small intestinal (ileum) mucosa showing a moderately affected digestive tissue with areas of hyperplasic villi and crypt cells (black arrows), and proliferating fibrolysis of the lamina propria (yellow arrow) within the endometrial mucosa. (HandE x100). Inference: Moderately ulcerated.

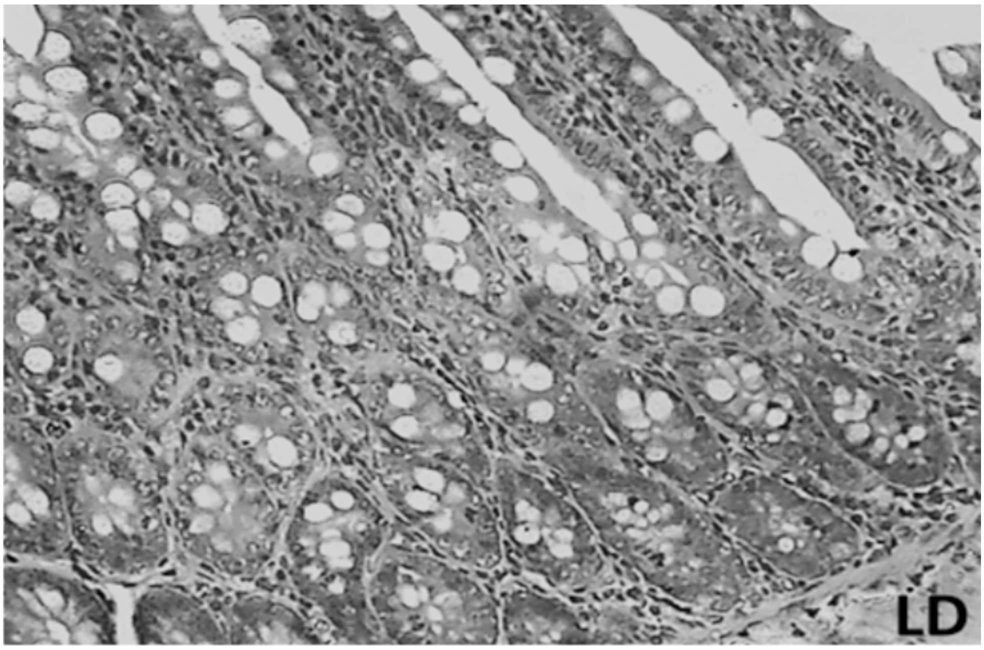

Figure 5: Photomicrograph of the longitudinal section of Group 1 (Low Dose- 273.86 mg/Kg) treated small intestinal (ileum) mucosa showing a normal digestive tissue with projecting villi having goblet cells and the crypts of leiberkuhn, laminar propria, presence of glandular cells, and an underlying submucosa. (HandE x100). Inference: Not ulcerated.

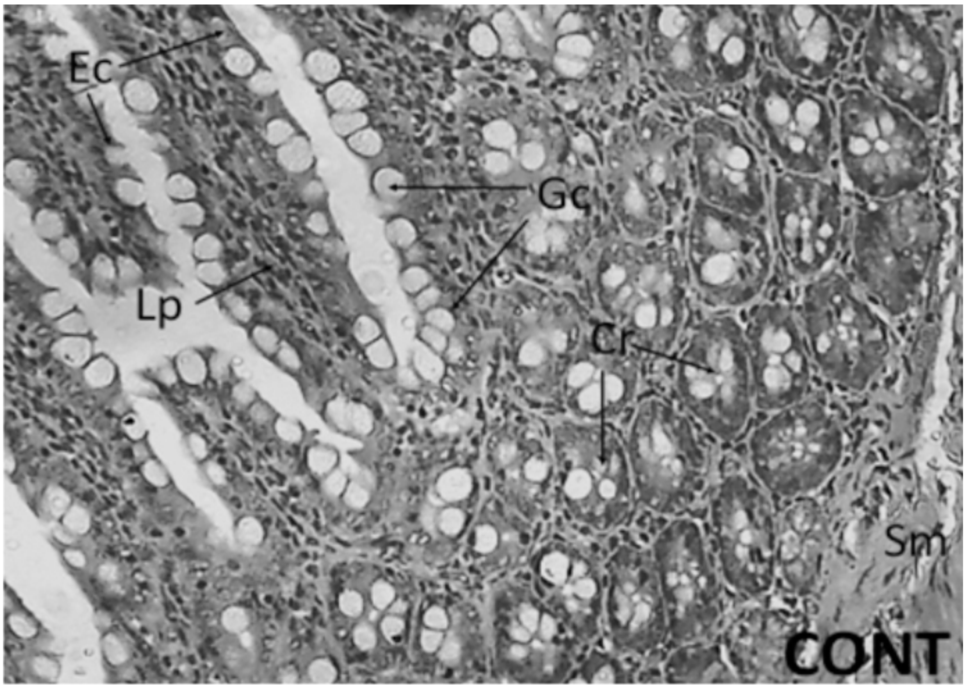

Figure 6: Photomicrograph of the longitudinal section of Group 4 Control treated with 10 mg/Kg of Distilled water: small intestinal (ileum) mucosa showing a normal digestive tissue with projecting villi having g oblet cells (Gc) and the crypts of leiberkuhn (Cr), laminar propria (Lp), presence of glandular cells (Glc), and an underlying submucosa (S m). (H and E x 100). Inference: Not ulcerated.

DISCUSSION

The 24.05 % yield of the methanolic extract suggests that *C. odorata* contains a substantial amount of bioactive compounds, such as phenolic compounds, flavonoids, and essential oils, which are likely responsible for its pharmacological effects. Previous studies have identified these phytochemicals as key contributors to the plant's antioxidant, anti-inflammatory, and antispasmodic properties.[25,21] The methanolic extraction method used in this study is effective for isolating polar and semi-polar compounds, which aligns with the observed pharmacological activity.

The LD_{50} of 2738.61 mg/kg indicates that *C. odorata* methanolic extract has low acute toxicity, as it is well above the threshold for substances considered highly toxic (< 50 mg/kg). This finding supports the traditional use of *C. odorata* in herbal medicine, as it suggests a wide therapeutic window. However, the 100 % mortality at 3000 mg/kg and 5000 mg/kg in Phase I highlights the need for careful dose selection in therapeutic applications to avoid toxicity.

The *in vitro* study evaluated the effect of *C. odorata* methanolic extract on isolated ileum smooth muscle, with atropine (0.00004 mg/ml, - log [M] = 4.4) as the control.

Atropine, a muscarinic receptor antagonist, is known to inhibit acetylcholine-induced contractions, leading to smooth muscle relaxation.[23] *C. odorata* extract induced the maximum relaxation response which was less potent than atropine, this effect was not significant (P<0.05) when compared to atropine.

The relaxation effect may be attributed to the phytochemical constituents of *C. odorata,* particularly flavonoids and phenolic compounds, which are known to modulate smooth muscle activity. Flavonoids, such as quercetin and kaempferol derivatives, have been reported to inhibit calcium influx or interact with G-protein-coupled receptors, thereby reducing smooth muscle contractility. The mechanism of action may involve inhibition of L-type calcium channels or enhancement of cyclic guanosine monophosphate (cGMP) pathways, similar to nitric oxide-mediated smooth muscle relaxation.[26] The dose-dependent inhibitory response suggests a concentration-dependent interaction with these pathways, with higher concentrations saturating the relevant receptors or ion channels.

When combined with atropine, a muscarinic receptor antagonist known to induce smooth muscle relaxation, *C. odorata* extract showed a synergistic effect. This suggests that *C. odorata* may enhance atropine's antispasmodic effects, possibly through complementary mechanisms, such as inhibition of muscarinic receptor-mediated contractions or modulation of intracellular calcium levels.

When atropine was applied to pre-relaxeded tissues by *C. odorata* extract, there was further relaxation, suggesting a synergistic or additive inhibitory effect. Lower responses in subsequent trials (- 4.0 mm, - 3.0 mm, - 2.5 mm) imply possible receptor desensitization or saturation.

These findings aligned with previous studies reporting antispasmodic properties of *C. odorata*[27] :supporting its potential use in treating gastrointestinal disorders characterized by spasms, such as irritable bowel syndrome.

The histological findings provide critical insights into the safety and potential toxicity of *C. odorata* extract on ileum tissue. The control group (treated with distilled water) and the low-dose group (273.86 mg/kg) exhibited normal histological features, including intact villi, goblet cells, crypts of Lieberkuhn, and lamina propria, with no signs of ulceration (Figures 5 and 6). These findings indicate that low doses of *C. odorata* extract are safe and do not induce structural damage to the ileum mucosa.

In contrast, the medium-dose (547.72 mg/kg) and high-dose (821.58 mg/kg) groups showed moderate ulceration, characterized by hyperplastic villi and crypt cells and proliferating fibrolysis in the lamina propria (Figures 3 and 4). These changes suggest that higher doses of the extract may induce mucosal irritation or damage, potentially due to cytotoxic compounds such as alkaloids or high nitrate levels.[9] The hyperplasia of crypt cells indicates an adaptive response to mucosal injury, while fibrolysis in the lamina propria suggests tissue remodeling, possibly as a repair mechanism. However, the absence of severe ulceration or necrosis indicates that the damage is moderate and potentially reversible.

The histological findings correlate with the acute toxicity results, where higher doses (3000 mg/kg and above) caused significant toxicity. The moderate ulceration observed at 547.72 mg/kg and 821.58 mg/kg suggests a dose-dependent toxic effect on the gastrointestinal mucosa, which may limit the therapeutic use of *C. odorata* at higher doses. The presence of bioactive compounds, such as phenolic acids and flavonoids, may contribute to both the therapeutic and toxic effects, as these compounds can have dual roles depending on concentration.[28]

CONCLUSION

The findings from this study suggest that *C. odorata* exhibit low acute toxicity, and the extract also exhibits dose-dependent antispasmodic effects on ileum smooth muscle, mediated at least in part through muscarinic receptors, as demonstrated by atropine's antagonistic effects. This result may give support to the safety of *C. odorata* for traditional medicinal use at moderate doses: indicating a need for careful dose optimization. However, lack of research data on chronic effect highlight the need for further research.

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