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Acute Toxicity and Analgesic Effect of *Pentaclethra eetveldeana* De Wild & Th. Dur Leaves Aqueous Extract (Fabaceaee)

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Abstract

Toxicological and biological studies are essential to confirm the therapeutic or nutritional effects and assess the risks associated with the use of medicinal plants. The aim of this work was to study the acute toxicity and evaluate the analgesic and anti-inflammatory effects of the aqueous extract of *P. eetveldeana* leaves. The anti-inflammatory effect was assessed using the carrageenan edema induction test. The acute toxicity study, conducted according to OECD guideline no. 423 (2001), showed that this extract is mildly toxic, with an estimated LD50 of 2375 mg/kg. Exploratory behavioral and mobility disturbances, associated with dyspnea, were observed at doses of 2000, 3000, and 5000 mg/kg. At doses of 250 and 500 mg/kg, the aqueous extract of *P. eetveldeana* did not significantly reduce rat paw edema induced by 1% carrageenan. In the acetic acid-induced pain test, the aqueous extract showed a significant analgesic effect only at the 500 mg/kg dose. In contrast, in the hot water tail immersion pain test, a significant analgesic effect was observed at both 250 and 500 mg/kg doses. Furthermore, naloxone inhibited the analgesic effect of the extract only at the 250 mg/kg dose, in the hot water tail immersion pain test. The aqueous extract of *P. eetveldeana* exerts an analgesic effect involving both two mechanisms of action, central and peripheral.

Keywords

Pentaclethra eetveldeana, Acute Toxicity, Analgesic, Mechanism of Action

1. Introduction

Medicinal plants are part of alternative medicine. Their use by humans is an age-old practice. Increasingly, people around the world are using a wide variety of plants for their biological properties, as aromatic and culinary herbs, or as remedies [1]. Medicinal plants remain natural remedies for relieving and even preventing several illnesses, particularly in Africa where herbal treatment is favored by the cost of healthcare, accessibility, and cultural beliefs. However, the natural origin of these plants does not guarantee their safety or their therapeutic or nutritional properties [2] [3]. In recent decades, numerous studies have demonstrated the true potential of plants to effectively treat a variety of diseases such as diabetes, osteoarthritis, and asthma [4] [5], or to slow the progression of neurodegenerative diseases [5]. Examples also include multiple sclerosis, Alzheimer's disease, and Parkinson's disease [6]. In developing countries such as the Republic of Congo, people are turning to medicinal plants as an alternative to conventional medicine. The Congolese flora is very diverse in medicinal plants, thus facilitating research in several areas. We focused on the species *Pentaclethra eetveldeana*, belonging to the Fabaceae family. This choice is explained by the fact that this species has been the subject of few pharmacological studies. The aim of this work is to study the acute toxicity and evaluate the analgesic and anti-inflammatory activities of the aqueous extract of *P. eetveldeana* leaves.

2. Materials

2.1. Plant Material

Leaves of *P. eetveldeana* were collected in Makana II, Pool Department, in May 2024. Botanical identification was carried out by Dr. Miabangana Edmond Sylvestre of the National Institute for Research in Exact and Natural Sciences (IRSEN), and a specimen was deposited in the National Herbarium and registered under number PEFMP05/2024.

2.2. Animal Material

Male and female Swiss albino mice, weighing between 20 and 28 g and aged 4 months, and male and female Wistar albino rats, weighing between 150 and 200 g and aged 4 months, were used. These animals were provided by the animal facility of the Faculty of Science and Technology at Marien Ngouabi University. These animals were kept under standard conditions (12 hours of light and 12 hours of darkness at an ambient temperature of 26˚C ± 1˚C) and had free access to standard food and tap water. The ethical rules published by the International Association for the Study of Pain [7] were respected.

3. Methods

3.1. Aqueous Extract Preparation

The aqueous extract of *P. eetveldeana* leaves was prepared by decoction.

100 g of powdered *P. eetveldeana* leaves were placed in 1000 ml of distilled water. This mixture was placed in an oven for 30 minutes at a temperature of 70˚C. After heating, the mixture was left to cool and then filtered. The resulting filtrate was placed in the oven until the water evaporated. The extract was stored in a bottle in a refrigerator.

3.2. Evaluation of Acute Toxicity

To assess acute toxicity, we used the method described in OECD Guideline No. 423 [8], slightly modified, to determine the therapeutic dose. The method consists of administering the product once and determining the dose range in which the substance has adverse effects. In total, 24 male mice, divided into 6 groups of 4 mice, were used for the experiment. The animals were fasted for 2 hours before the experiment (with free access to water). The administration was oral. The extract was administered at the following doses: 500, 1000, 2000, 3000, and 5000 mg/kg body weight, and distilled water was administered at a dose of 0.5 ml/100 g body weight. Immediately after administration, each animal was placed in a cage to record immediate signs of toxicity (changes in skin, fur, eyes, motor activity, and behavior; tremors, convulsions, diarrhea, mortality, and drowsiness) for 2 hours. The body weight of the animals was recorded every three days for 14 days. The animals were fasted for 2 hours before the experiment (with free access to water). To calculate the lethal dose that kills half of the animals (LD50) we used the formula described by [9].

$$LD_{50} = LD_{100} - \sum \frac{a \times b}{n}$$

LD_{50}: lethal dose that kills the half animals; LD_{100}: lethal dose that kills all animals; a: average number of deaths between two successive doses; b: difference between two successive doses; n: number of animals per batch.

3.3. Motor Activity Assessment

We assessed motor activity using the method reported by Nsonde *et al.* [10], with slight modifications. We used a wooden grid cage with a length of 60 cm and a width of 40 cm. This cage contained 24 equally spaced squares, each measuring 10 × 10 cm.

Four (4) groups of five (5) mice were formed and treated orally as follows:

The first group received distilled water at 0.5 ml/100 g body weight;

The second, third, and fourth groups were treated with an aqueous extract of *P. eetveldeana* leaves at doses of 500, 1000, and 2000 mg/kg, respectively.

One hour after administration, each mouse was placed in the cage to count the number of squares it walked on during five (5) minutes.

3.4. Evaluation of Analgesic Activity

3.4.1. Acetic Acid-Induced Writhing Test

This method was described by Koster in 1959 and reported by Nsonde *et al.* [11].

It consists of inducing a pain syndrome by intraperitoneal (IP) injection of 0.6% acetic acid (0.1 ml/10 g body weight). The pain syndrome is characterized by torsion of the dorsoabdominal muscles and stretching of the hind legs.

The mice (n = 5) were fasted for 2 hours before administration of the test products.

The extract was tested at doses of 250 and 500 mg/kg body weight orally;

Distilled water was administered orally at a dose of 0.5 ml/100 g body weight;

Diclofenac sodium, the reference product, was also administered orally at a dose of 20 mg/kg body weight.

One hour after administration of the test products, each mouse was treated with 0.1 ml/10 g of 0.6% acetic acid intraperitoneally (IP) and placed in a cage. The number of cramps was assessed over 10 minutes by counting the number of cramps (torsoabdominal muscle torsion and leg stretching). An analgesic acts by suppressing or reducing the manifestations of pain.

The percentage of inhibition was calculated using the following formula:

$$\%I = \frac{\text{number of cramps in the control group} - \text{number of cramps in the treated group}}{\text{number of cramps in th control group}} \times 100$$

3.4.2. Tail Immersion Test in Hot Water

The study was conducted using the D'Amour and Smith test, modified by Nsonde *et al.* [11]. This test assesses the central analgesic effect. It involves inducing pain by immersing the animal's tail in a hot water bath at a temperature of 55˚C ± 2˚C. The animals were pre-treated orally with various test products. Four groups of five rats each were used for this purpose.

Distilled water was administered at a dose of 10 ml/kg body weight;

Tramadol, the reference product, was also administered orally at a dose of 30 mg/kg body weight;

The extract was tested at doses of 250 and 500 mg/kg body weight.

Thirty minutes after administration of the products, the animals were held in a vertical position while their tails were immersed to a depth of 5 cm in warm water maintained at 55˚C ± 2˚C. The reaction time (in seconds) it took the animal to withdraw its tail from the water was recorded at 30, 60, 90, and 120 minutes after administration. The maximum time the animal was allowed to remain in the warm water during this test was 30 seconds. Beyond this time, the animal was not included in the study, and its tail was voluntarily withdrawn to avoid injury to the tail tissue. The sensation of pain was characterized by the rapid withdrawal of the animal's tail. An analgesic would act by increasing the animal's reaction time. The percentage of increase in tail withdrawal latency time was calculated using the following formula:

$$\text{PILT}(\%) = \frac{\text{M2} - \text{M1}}{30 - \text{M1}} \times 100$$

PILT: Percentage of increase in latency time.

M1: Average withdrawal time of the control group.

M2: Average withdrawal time of the treated group.

3.4.3. Effect of the Extract on Hot Water-Induced Pain in the Rat Tail in the Presence of Naloxone

The study was conducted using the method described by Nsonde *et al.* [12]. This method illustrates a central mechanism of action of an analgesic. It consists of inducing pain by immersing the animal's tail in a hot water bath at a temperature of 55 ± 2˚C. The rats were pre-treated orally with various products. Thirty minutes later, 1 mL/kg of naloxone (1 mg/ml) was also administered subcutaneously to all the pre-treated rats. Four (4) groups of five (5) rats each were formed for this purpose.

Group 1 received distilled water (10 mL/kg body weight);

Group 2 received tramadol (30 mg/kg body weight) and naloxone (1 ml/kg body weight);

Groups 3 and 4 received the 250 mg/kg body weight extract with naloxone (1 ml/kg body weight) and the 500 mg/kg body weight extract with naloxone (1 ml/kg body weight), respectively.

Thirty minutes after administration, the animals were held upright while their tails were immersed to a depth of 5 cm in warm water maintained at 55˚C ± 2˚C. The reaction time (in seconds) the animal resisted before withdrawing its tail from the water was recorded at 30, 60, 90, and 120 minutes after administration. The maximum time the animal was allowed to remain in the warm water during this test was set at 30 seconds. Beyond this time, the animal is not taken into account, and its tail is voluntarily removed to avoid injury to the surrounding tissues. The sensation of pain is characterized by the rapid retraction of the animal's tail.

3.4.4. Edema Induction Test with Carrageenan

To evaluate the anti-inflammatory effect, we used the method reported by Boumba *et al.* [13], which involves inducing paw edema in rats using carrageenan, a polysaccharide that, once injected, causes acute inflammation 30 minutes later, characterized by a progressive increase in paw diameter.

The rats were randomly divided into four groups of five.

The extract was tested orally at doses of 250 and 500 mg/kg body weight.

Diclofenac sodium was administered orally at a dose of 10 mL/kg body weight.

Diclofenac sodium, the reference product, was also administered orally at a dose of 20 mg/kg body weight. One hour after treatment, 0.05 ml of a 1% carrageenan solution was injected into the animals' paws. The paw diameter was measured using calipers. Measurements were taken before and after carrageenan injection at 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours. Inhibition percentages were calculated using the following formula:

$$\%\mathrm{IP} = \frac{\left(D_t - D_0\right)t - \left(D_t - D_0\right)tr}{\left(D_t - D_0\right)t} \times 100$$

IP: percentage of inhibition; D_t: diameter of the paw after the induction of in-

flammation at time t; D_0: diameter of the paw before the induction of inflammation; $(D_t - D_0)t$: diameter of the edema of the control rat; $(D_t - D_0)tr$: diameter of the edema of the treated rat.

3.5. Statistical Analysis

Statistical analysis was performed using software (Graphpad Prism 8). Data entry and comparison of measurement means between groups were carried out using the ANOVA test and the Dunnett multiple comparison test. The significance threshold was set at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

4. Results

4.1. Acute Toxicity Assessment

4.1.1. General Observations on Animal Condition

The results of acute toxicity are recorded in Table 1. This table shows observations of the general condition and behavior of the animals.

Table 1. Effect of the aqueous extract from *P. eetveldeana* leaves on the general condition of mice.

Products	Dose	Nbrs An by group	Bhv expl (Nbrs An)	Mobilité (Nbrs Anx)	Dyspnée (Nbs Anx)	Number of deaths (72 hours)
Extract	5000 mg/kg	4	ABS (4)	A (4)	P(4)	4
	3000 mg/kg	4	ABS (3)	A (3)	P (3)	3
	2000 mg/kg	4	ABS (2)	A (2)	A(1)	2
	1000 mg/kg	4	p (4)	P(2)	A	0
	500 mg/kg	4	P (4)	P	A	0
Distilled water	0.5 ml/100g	4	P (4)	P	A	0

Nbrs: Numbers; An: animals; Bhv: Behavior; Expl: Explorer; A: absence; P: presence.

These results indicate that oral administration of the aqueous extract of *P. eetveldeana* leaves at doses of 500 and 1000 mg/kg did not result in the death of mice, while administration of the extract at doses of 2000, 3000, and 5000 mg/kg resulted in the death of mice. At the 2000 mg/kg dose, two deaths were recorded 24 hours after administration of the extract. At the 3000 mg/kg dose, three deaths were recorded 24 hours after treatment of the mice. Finally, at the 5000 mg/kg dose, all four mice in this group are dead, one was dead during the observation period (1 hour 30 minutes after administration of the product), one was dead after 24 hours, and two were dead after 72 hours from treatment. The aqueous extract of *P. eetveldeana* leaves at doses of 500 and 1000 mg/kg did not show any immediate signs of toxicity. However, we noted signs of toxicity at doses of 2000, 3000, and 5000 mg/kg (absence of exploratory behavior, motor impairment, and dyspnea).

4.1.2. Effect of Aqueous Extract of *P. eetveldeana* Leaves on Mouse Body Weight

The effect of the extract on weight gain during the fourteen (14) days of observation is shown in Figure 1. This figure illustrates the difference in body weight change compared to baseline (J0) over the course of the fourteen days. The aqueous extract of *P. eetveldeana* leaves at doses of 500 mg/kg and 1000 mg/kg did not show a significant effect on the animals' weight gain during the fourteen (14) days.

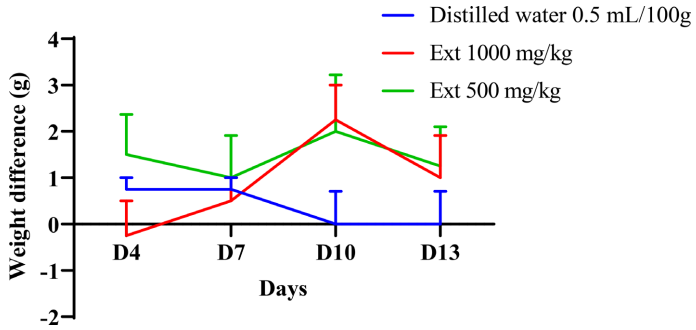


Figure 1. Evolution of the difference in body weight as a function of time, during the 14 days of the test, in mice receiving the aqueous extract of *P. eetveldeana* leaves at doses of 500 and 1000 mg/kg. Results expressed as average ± ES, n = 4, ext: extract.

4.1.3. Effect of *P. eetveldeana* Leaves Aqueous Extract on Mouse Motility

Figure 2 shows the effect of the aqueous extract of *P. eetveldeana* leaves on mouse motility. This figure shows that the aqueous extract of *P. eetveldeana* leaves at a dose of 2000 mg/kg significantly reduces ($p < 0.01$) the number of squares crossed by the mice compared to the control group. However, this reduction is not significant at doses of 500 and 1000 mg/kg compared to the control group.

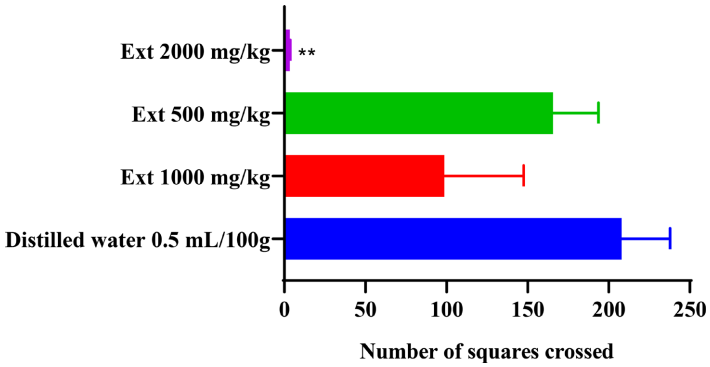


Figure 2. Effect of *P. eetveldeana* leaves aqueous extract on the motor skills of mice. Results expressed as average ± ES, n = 5; **$p < 0.01$; Ext: extract.

4.2. Analgesic Effect

4.2.1. Acetic Acid-Induced Dorsoabdominal Cramp Test

Figure 3 shows the effect of the aqueous extract of *P. eetveldeana* leaves on pain induced by 0.6% acetic acid. The extract at a dose of 500 mg/kg, as well as diclofenac, the reference molecule, significantly reduced ($p < 0.01$) the number of acetic acid-induced dorsoabdominal cramps compared to the control. The percentages

of inhibition corresponding to the extract at a dose of 500 mg/kg and diclofenac (20 mg/kg) were 52.63% and 43.15%, respectively. At a dose of 250 mg/kg, the extract does not significantly reduce the number of dorso-abdominal cramps compared to controls; the percentage of inhibition is 8.42%.

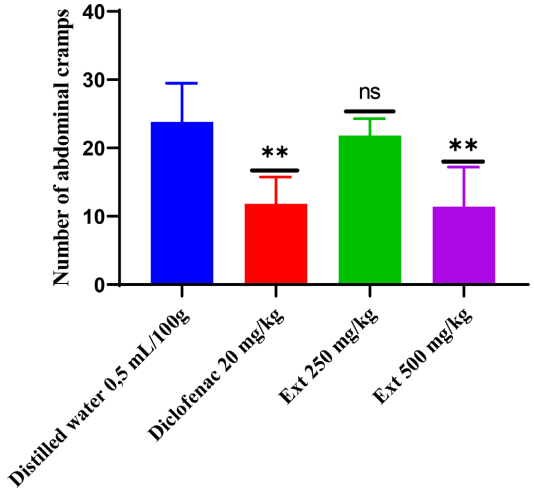


Figure 3. Analgesic effect of *P. eetveldeana* leaves aqueous extract on pain induced by 0.6% acetic acid. Results expressed as average ± ES, Ext: extract, n = 5, **p < 0.01.

4.2.2. Hot Water Tail Immersion Pain Test

Table 2 shows the tail withdrawal latency (in seconds) in rats exposed to hot water. These results demonstrate that the aqueous extract of *P. eetveldeana* at doses of 250 and 500 mg/kg body weight, as well as tramadol at a dose of 30 mg/kg body weight, significantly increased the tail withdrawal latency ($p < 0.0001$) at 60, 90, and 120 minutes post-treatment compared to controls. However, with tramadol, the effect was already highly significant after 30 minutes of administration. The increased tail withdrawal latency in rats treated with the extract compared to controls indicates that the extract possesses analgesic activity. Table 3 shows the percentage increase in tail retraction latency time. These results show that the aqueous extract and tramadol markedly increase the reaction latency time of rats from 90 minutes after treatment (with aqueous extract, tramadol and distilled water) with a percentage of 32% at the dose of 250 mg/kg, 40.63% at the dose of 500 mg/kg of aqueous extract, and 41.83% at the dose of 30 mg/kg of tramadol.

Table 2. Effect of the aqueous extract from leaves of *P. eetveldeana* on pain induced by immersion of the tail in hot water.

		Reaction time in seconds (s)			
Treatments	**Dose (mg/kg)**	**30 min**	**60 min**	**90 min**	**120 min**
Distilled water	10 ml/kg	4.47 ± 0.83	5.09 ± 0.56	3.97 ± 0.54	4.76 ± 0.70
Tramadol	30	10.75 ± 1.22***	10.95 ± 0.70***	14.86 ± 0.38***	19.41 ± 0.33***
Extract	250	7.81 ± 1.40*	10.72 ± 1.52***	12.37 ± 0.60***	10.90 ± 0.40***
Extract	500	4.47 ± 0.70 ns	10.47 ± 0.46***	14.55 ± 0.76***	17.27 ± 0.58***

Results expressed as average ± ES, Ext: extract, n = 5; *p < 0.05; **p< 0.01; ***p< 0.001. Ext: extract.

Table 3. Percentage increase in tail pull latency based on duration.

Time (minutes)	Tramadol 30 mg/kg	Ext 250 mg/kg	Ext 500 mg/kg
30	24.59	13.08	0
60	23.52	22.60	21
90	41.83	32	40.64
120	58.04	24.32	49.56

Ext: extract.

4.3. Effects of the Aqueous Extract of *P. eetveldeana* on Pain Induced by Hot Water Immersion in the Presence of Naloxone

Figure 4 shows the effect of the extract and tramadol on pain induced by hot water in the presence of naloxone. The latency of tramadol obtained in the presence of naloxone is substantially similar to that of animals that received distilled water at 30, 60, 90 and 120 min post-treatment. At a dose of 250 mg/kg, the aqueous extract of *P. eetveldeana*, in the presence of naloxone, shows no analgesic effect against pain induced by hot water at 30, 60, 90, and 120 min post-treatment. This indicates that naloxone inhibits the analgesic effect of the extract against pain induced by hot water. However, at a dose of 500 mg/kg, despite the presence of naloxone, the aqueous extract of *P. eetveldeana* significantly increases the tail withdrawal latency time in animals exposed to warm water at 30, 60, 90 and 120 minutes post-treatment. This demonstrates that naloxone does not influence the analgesic effect of the extract at a dose of 500 mg/kg body weight.

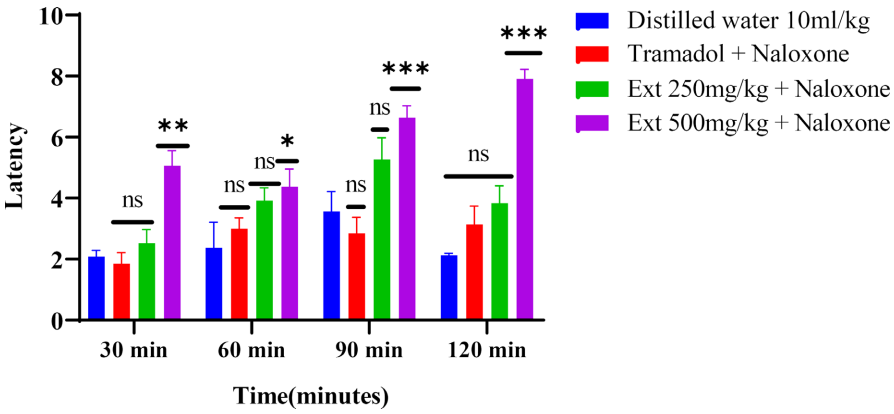


Figure 4. Naloxone inhibition of the analgesic effect of aqueous extract from *P. eetvelde ana* leaves on pain induced by immersion of the tail in hot water. Results expressed as average ± ES, Ext: extract, n = 5; *p < 0.05; **p < 0.01; ***p < 0.001.

4.4. Effect on Carrageenan-Induced Paw Edema in Rats

Aqueous extract of *P. eetveldeana* leaves at doses of 250 and 500 mg/kg did not significantly inhibit the progression of carrageenan-induced paw edema. Diclofenac at a dose of 20 mg/kg showed a significant effect on carrageenan-induced paw edema within the first hour (**Figure 5**). The percentage of hind paw edema

inhibition is shown in Table 4.

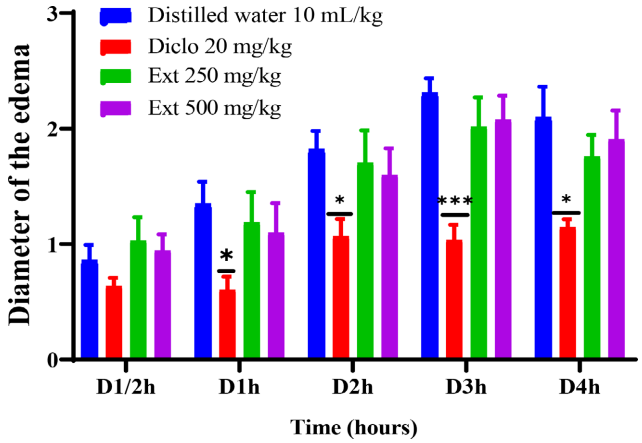


Figure 5. No anti-inflammatory effect of aqueous extract of *P. eetveldeana* leaves in albino wistar rats. Results expressed as average ± ES, Ext: extract, n = 5, Ext: extract.

Table 4. Percentage of inhibition of carrageenan-induced paw edema at 1%.

Time (hour)	Diclo 20 mg/kg	Ext 250 mg/kg	Ext 500 mg/kg
D 1/2h	26.70	-	-
D 1h	55.20	11.90	17.90
D 2h	41.80	6.60	12.60
D 3h	55.40	13.00	10.40
D 4h	45.50	15.80	9.10

Ext: extract.

5. Discussion

5.1. Acute Toxicity

The acute toxicity study conducted according to OECD guideline No. 423 [8] showed that the aqueous extract of *P. eetveldeana* leaves induces dose-dependent toxicity in mice. Although this guideline does not allow direct determination of the LD_{50}, its estimation using the Karber and Behrens method [14] [15] yielded an LD_{50} value of 2375 mg/kg. Whereas Nsonde Ntandou *et al.* [16] and Nkundineza *et al.* [17] observed no mortality at doses below 3000 mg/kg with polar extracts.

According to the Globally Harmonized System (GHS), this places the extract in toxicity category 5, indicating relatively low acute oral toxicity [8] [18].

No mortality or visible signs of toxicity were observed at doses of 500 and 1000 mg/kg, whereas deaths and toxic manifestations occurred at doses of 2000 mg/kg and above. These findings suggest a satisfactory safety margin at low doses but significant toxicity at higher concentrations. Similar observations have been reported for medicinal plant extracts rich in secondary metabolites, whose toxic effects generally appear only at elevated doses [19]. The dose-dependent mortality

observed is characteristic of acute toxicity and reflects the increasing biological burden of the tested substance [20].

Clinical signs observed at high doses, including reduced exploratory activity, motor impairment, and dyspnea, indicate possible alterations affecting the central nervous, neuromuscular, and respiratory systems. Such manifestations are recognized indicators of systemic toxicity and may result from the action of phytochemicals such as alkaloids, flavonoids, saponins, and terpenoids [21] [22]. Most deaths occurred within the first 24 hours after administration, suggesting rapid toxic effects following absorption, although delayed mortality observed at 5000 mg/kg indicates the possible persistence or accumulation of toxic constituents.

Assessment of locomotor activity using the open-field test [23] confirmed the behavioral observations recorded during the toxicity study. The extract significantly reduced the number of squares crossed only at the dose of 2000 mg/kg, while doses of 500 and 1000 mg/kg produced no significant effect. This decrease in spontaneous locomotion suggests a depressant effect on the central nervous system, sedation, or impaired motor coordination. Similar findings have been reported for the aqueous extract of Cassia alata another fabaceae [24] and *Vitex madiensis* [25] and have been described as an early manifestation of neurotoxicity induced by certain plant extracts [26]. This effect may be attributed to flavonoids and other secondary metabolites capable of modulating GABAergic neurotransmission.

Body weight monitoring over the 14-day observation period showed no significant differences between treated and control animals, despite a slight increase in body mass at doses of 500 and 1000 mg/kg. Since all animals received the same amount of food, the results indicate that the aqueous extract of *P. eetveldeana* does not significantly affect body weight under the experimental conditions used.

Overall, the aqueous extract of *P. eetveldeana* leaves appears relatively safe at doses of 1000 mg/kg or less but exhibits significant toxic and neurobehavioral effects at higher doses. These effects may result from individual toxic constituents, synergistic interactions among compounds, or changes occurring during extract preparation. Further subacute and chronic toxicity studies, including biochemical and histopathological investigations, are required to better characterize its safety profile and identify potential target organs.

5.2. Analgesic Activity

Pain induced by acetic acid is a key experimental model for studying peripheral nociception and evaluating substances with analgesic potential. Intraperitoneal injection of 0.6% acetic acid induces dorsoabdominal cramps in mice through the release of pain-inducing mediators such as prostaglandins, bradykinin, histamine, serotonin, and acetylcholine [27]. This phenomenon results in particular from the activation of phospholipases A_2, leading to the formation of arachidonic acid, a precursor of endogenous nociceptive mediators [11].

In this study, the aqueous extract of *P. eetveldeana* leaves at a dose of 500 mg/kg significantly reduced the number of acetic acid-induced cramps ($p < 0.01$), with

an inhibition percentage of 52.63%, higher than that obtained with diclofenac (43.15% at 20 mg/kg). In contrast, the 250 mg/kg dose did not produce a significant effect (%IP = 8.42%), suggesting a dose-dependent analgesic activity.

The observed effect could be attributed to an inhibition of the synthesis or release of inflammatory mediators involved in peripheral pain, similar to non-steroidal anti-inflammatory drugs (NSAIDs), which act primarily by inhibiting cyclooxygenase and/or lipoxygenase pathways [28].

These results support the traditional use of *P. eetveldeana* in the treatment of painful conditions and provide a scientific basis for its use in traditional medicine. However, further studies are needed to identify the active principles responsible for this effect, elucidate their mechanisms of action, and assess their long-term safety.

5.3. Hot Water-Induced Pain

The hot water tail immersion test is a commonly used experimental model for evaluating centrally mediated analgesic activity. It relies on measuring the tail retraction latency after noxious thermal stimulation; an increase in this latency indicates central antinociceptive activity.

Results show that the aqueous extract of *P. eetveldeana* leaves significantly increases tail retraction latency in rats at doses of 250 and 500 mg/kg, with a more pronounced effect at 500 mg/kg. This increase appears at 60 minutes and is maintained up to 120 minutes, indicating decreased sensitivity to thermal pain and dose-dependent central analgesic activity. At 90 minutes, the increases reach 32% and 40.63% for 250 and 500 mg/kg, respectively.

Tramadol, used as a reference, induces a more rapid effect within 30 minutes, with latency increases of 41.83% at 90 minutes and 58.04% at 120 minutes, which is explained by its well-known central opioid action. The later onset of the extract's effect suggests a gradual absorption or biotransformation of its active constituents.

The observed activity could be linked to the presence of secondary metabolites such as flavonoids, alkaloids, tannins, and saponins, known to modulate nociceptive transmission centrally via the opioid, or serotonergic, or GABAergic systems.

These results complement those of the acetic acid-induced dorso-abdominal cramp test, which explores peripheral nociception, thus suggesting both central and peripheral action of the *P. eetveldeana* extract. These results are in agreement with those obtained by Nsonde Ntandou *et al.* [11] [12] [29], who worked on *Cassia siamea* with the same botanical family.

5.4. Naloxone Antagonist Test Analgesic Effect

The effect of an aqueous extract of *P. eetveldeana* and tramadol on pain induced by tail immersion in warm water, in the presence of naloxone, an opioid receptor antagonist.

The results show that, in animals receiving tramadol in the presence of nalox-

one, the tail withdrawal latency is comparable to that of the control group (distilled water) at different observation times (30, 60, 90, and 120 minutes). This observation indicates that naloxone abolishes the antinociceptive effect of tramadol, thus confirming that its analgesic action is primarily mediated by the opioid system.

At a dose of 250 mg/kg, the aqueous extract of *P. eetveldeana* do not showed significant antinociceptive effect in the presence of naloxone at any of the observation time points. This suggests that the analgesic activity observed at this dose is also dependent on opioid receptors. Indeed, blocking these receptors with naloxone abolished the protective effect of the extract, implying the likely involvement of the endogenous opioid system in its mechanism of action as in *Cassia siamea* sterm bark extract [12].

In contrast, at a dose of 500 mg/kg, the extract retained significant antinociceptive activity despite the presence of naloxone, with an increase in tail retraction latency at all observation time points. This resistance to antagonism by naloxone suggests that, at high doses, the analgesic effect of the extract is not exclusively dependent on opioid receptors. Other mechanisms could therefore be involved, including the modulation of non-opioid pathways such as the serotonergic and noradrenergic systems, as well as through a peripheral mechanism, or even the direct inhibition of nociceptive transmission at the spinal level.

These results highlight a dual dose-dependent mechanistic component of the aqueous extract of *P. eetveldeana* a predominant opioid component at low doses, and a more pronounced non-opioid component at high doses. This diversity of action could be explained by the presence of different secondary metabolites capable of acting on several pharmacological targets. These results are similar to those obtained on *Cassia siamea* another species of Fabaceae [29].

5.5. Anti-Inflammatory Activity

The induction of edema by carrageenan in the rat paw is a well-established animal model for evaluating the anti-inflammatory effect of natural products as well as synthetic chemical compounds [30]. The temporal evolution of the development of this carrageenan-induced edema is generally represented by a biphasic curve; the first phase lasts approximately 2 hours after carrageenan injection and is partly due to the trauma associated with the injection as well as the action of histamine, serotonin, and bradykinin released during this phase, which affect vascular permeability [31]. The second phase, which extends from 2.5 to 6 hours, is correlated with the rise in the activity of oxygen-derived free radicals, prostaglandins, as well as the infiltration and activation of neutrophils at the inflammatory site [3]. The aqueous extract of *P. eetveldeana* at doses of 250 and 500 mg/kg did not significantly prevent carrageenan-induced rat leg edema compared to the control group. However, it showed an edema inhibition rate of 17.90% at 1 hour (500 mg/kg) and 15.80% at 4 hours (250 mg/kg) after carrageenan administration. In contrast, diclofenac, the reference molecule, significantly inhibited edema compared to the

control group from the first hour, with an inhibition rate of 55.20% and 45.50% at the fourth hour. This demonstrates that the extract has no anti-inflammatory effect. These results contradict those obtained by N'Goka [32], who used in vitro models to evaluate the anti-inflammatory effect of *Pentaclethra eetveldeana*. Using the same acute inflammation study assay on two other Fabaceae species, *Cassia siamea* and *Cassia* al*ata*, Nsonde Ntandou *et al.* demonstrated anti-inflammatory activity, which contradicts these results [12] [29]. In light of the above, diclofenac appears to act by inhibiting the production of bradykinin, serotonin, and histamine in the first phase, and by inhibiting the cyclooxygenase (COX) enzyme responsible for prostaglandin (PG) synthesis in the second phase.

6. Conclusion

This study aimed to evaluate the analgesic and anti-inflammatory effects of the aqueous extract of *P. eetveldeana* leaves, a plant in the Congolese pharmacopoeia traditionally recognized for its therapeutic properties. The toxicity study revealed that the aqueous extract of *P. eetveldeana* leaves is of low toxicity, with an LD50 of 2375 mg/kg. This allows it to be classified in category 5 according to the Globally Harmonized System of Drugs [8]. The extract did not affect the body weight of the mice. It caused respiratory problems (dyspnea), inhibited mobility, and led to the death of the animals at doses of 2000, 3000, and 5000 mg/kg. The study concluded that the aqueous extract of *P. eetveldeana* leaves exhibits significant peripheral and central analgesic activity. The central analgesic effect is partially mediated by opioid receptors and also utilizes other mechanisms of action. This extract has no anti-inflammatory effect on carrageenan-induced rat paw edema.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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