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Original Research Article

In vivo acute toxicity, analgesic and anti-inflammatory activities of phenolic extract of *Matricaria pubescens*

Hassiba Metrouh-Amir[1]*, Nadir Amir[2,3]

[1]Laboratoire de Biotechnologies Végétales et Ethnobotanique, Faculté des Sciences de la Nature et de la Vie Université de Bejaia, Route de Targa-Ouzemour, [2]Laboratoire de Maitrise des Energies Renouvelables, Equipe Biomasse et Environnement, Faculté de Technologie Université de Bejaia, Route de Targa-Ouzemour, [3]Département de Microbiologie, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, Route de Targa-Ouzemour, 06000 Bejaia, Algeria

For correspondence: **Email: hassiba.metrouh@univ-bejaia.dz; **Tel:** +213 77 45 77 04*

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Abstract

Purpose: *To evaluate the in vivo acute toxicity, analgesic and anti-inflammatory proprieties of the phenolic extract of Matricaria pubescens (Desf.) Schultz (Asteraceae).*

Methods: *Acute toxicity assessment was carried out on 18 mice that were divided equally into three groups and treated orally with saline, 2500 and 5000 mg/kg of M. pubescens extract, respectively. The evaluation of peripheral analgesic activity was done by applying acetic acid-induced contortion test on 40 mice. The mice were divided into 5 equal groups of 8 mice each. Negative and positive control groups were treated orally with saline (1 %) and acetylsalicylic acid (200 mg/kg), respectively. Other groups were treated with 50, 100 and 200 mg/kg of M. pubescens extract. Study groups were administered saline, diclofenac potassium (10 mg/kg) and M. pubescens extract orally at 50, 100, and 200 mg/kg. Central analgesic activity was carried out using the tail immersion test. The distribution and treatment of the mice was similar to the analgesic model. Anti-inflammatory effect was evaluated by carrageenan-induced mice paw edema.*

Results: *Acute toxicity results showed that the LD_{50} of M. pubescens phenolic extract is above 5000 mg/kg, which means that this extract is safe. Peripheral and central analgesic data revealed that different doses of extract significantly inhibited abdominal contractions and reduced pain caused by heat compared to control ($p < 0.05$). Anti-inflammatory activity data indicate significant inhibition of inflammatory edema at various doses compared to diclofenac potassium ($p < 0.05$).*

Conclusion: *The phenolic extract of M. pubescens exerts significant peripheral, central analgesic and anti-inflammatory activities in mice. However, further investigations are required to ascertain the potentials of the extract for its clinical development.*

Keywords: *Matricaria pubescens, Acute toxicity, Analgesic activity, Anti-inflammatory activity, Phenolic extract*

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INTRODUCTION

Inflammation is an adaptive response generated in response to various aggressions, which can be of physical, chemical or infectious origin. The inflammatory response causes the release of various inflammatory mediators. These mediators affect the development and resolution of inflammation by acting on various cells involved in the inflammatory reaction [1]. Pain results from complex physiological processes triggered when free peripheral nerve endings

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react to nociceptive stimuli of various kinds (mechanical, thermal or chemical) [2]. Nociception concerns the mechanisms that generate pain in response to a nociceptive stimulus, or described as such by normal subjects [3].

Anti-inflammatory drugs are the most commonly used pharmacological class in the treatment of inflammation, pain or fever. They are divided into two major groups – non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs (SAIDs). Non-steroidal anti-inflammatory drugs are an important class of drugs that exhibit different pharmacological activities such as anti-inflammatory, analgesic, antipyretic and antiplatelet type as well as gastrointestinal and renal side effects. The action of NSAIDs is mainly explained by the non-specific inhibition of the activity of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) with COX-1 inhibition by NSAIDs leading to gastritis and gastrointestinal ulceration [4].

Steroidal anti-inflammatory drugs are a large family of drugs derived from cortisol. They induce an increase in the transcription of inhibitor of NF-κB (I-κB) and consequently inhibit the activation of several genes by NF-κB, including those involved in the activation of T cells and the production of cytokines [5]. Glucocorticoids also reduce the ability of macrophages and neutrophils to phagocytose and kill microorganisms [5]. Adverse effects of corticosteroids are related to prolonged use of these products, increased dosage and discontinuation of corticosteroid therapy, leading to acute adrenal insufficiency. Various disorders arising from adverse effects of corticosteroids are observed including arterial hypertension, hyperglycemia, reduced immune defenses, osteoporosis, appearance of peptic ulcers and deregulation of the natural synthesis of glucocorticoids [6]. In this context, the use of natural resources and more particularly medicinal plants with fewer side effects becomes an important alternative.

Matricaria pubescens (*M. pubescens*) (Desf.) Schultz is an annual herbaceous plant, of the *Asteraceae* family. It reaches a height of 10 – 20 cm and possesses numerous thin stems and very little branches. The leaves are slightly fleshy and are between 10 – 20 mm long. The flowers are yellow and tubular and are grouped in a hemispherical discoid head. The capitula are fixed at the end of the stems. The flowering takes place in spring in the north of the Algerian Sahara and at any time after the rain in the center of the Sahara. In Algeria, the name of *M. pubescens* differs from one region to another; in Ouargla it is called *Guertoufa*, in Béchar it is called *Ouazouaza* or *Guertoufa* [7].

M. pubescens is a medicinal plant widely used in Central and Northern Sahara regions in Algeria, for the treatment of several diseases such as rheumatism, body aches, cough, allergies and gastrointestinal disorders. For children, it is used against measles, teething ailments, fever and dermatoses [7]. Among the chemical compounds isolated from *M. pubescens* are phenolic compounds, flavonoids and tannins [8]. The anti-inflammatory and analgesic properties of phenolic compounds have been demonstrated by numerous studies. However, phenolic compounds exert their anti-inflammatory effect by inhibiting certain enzymes involved in the inflammatory reaction such as inducible nitric oxide synthase, phospholipase A_2, cyclooxygenase and lipoxygenase [9]. In this study, the *in vivo* acute toxicity as well as the analgesic and anti-inflammatory properties of the phenolic extract derived from *M. pubescens* will be explored.

EXPERIMENTAL

Plant material

Matricaria pubescens plant was collected in March 2021 from the Ouargla area in the Algerian Septentrional Sahara and subsequently identified at the National Museum of Natural History, Paris. A voucher specimen (no. 644) was deposited at the Laboratoire de Botanique d'Alger, Algeria.

Animals

The *in vivo* study was carried out on mice of Swiss albino variety, with a weight range between 18 – 27 g, provided by Pasteur Institute (Algiers, Algeria). Animals were housed in a laboratory at 23 ± 2 °C, with a light-dark cycle of 12 ± 1 h. They were allowed access to standard food and water *ad libitum*. The mice were acclimatized for 15 days before the start of tests. Animal experiment protocols performed in this study were conducted according to the ethical guidelines, number 2010/63/EU [12] and the protocol was approved by the local Ethics Committee (approval no. CE-LBVE-2021-115).

Phenolic extract preparation

After harvesting, the *M. pubescens* plant was dried at room temperature in a ventilated place, protected from light. The dry matter obtained was

reduced to powder and then sieved. The preparation of the phenolic extract of *M. pubescens* was carried out with ethanol (50 %). A quantity (4 g) of the powder was added to 500 mL of the extraction solvent. After 3 h of maceration, the mixture was filtered, evaporated and lyophilized. The dry extract obtained was recovered in saline (1 %).

Determination of total phenolic content

The phenolic content was estimated according to the method of Goli *et al* [10]. Two hundred microliters (200 µL) of *M. pubescens* extract was added to 1 mL of Folin-Ciocalteu solution. After 3 min, 0.8 mL of sodium carbonate solution (7.5 %) was added. Following a 30-minute incubation period, the absorbance was read at 760 nm and the phenolic content was reported in grams of gallic acid equivalent (GAE) per 100 g of dry weight (DW).

Determination of total flavonoid content

The flavonoid content of the extract was evaluated by the method described by Bahorun *et al* [11]. An aliquot of the phenolic extract was mixed with the same volume of aluminum chloride (2 %). The absorbance was read at 410 nm after 10 min incubation. The results were expressed in gram quercetin equivalent per 100 g of dry weight (g QE/100 g DW).

Acute toxicity

In order to estimate the acute toxicity of *M. pubescens* extract, protocol 425 of the Organization for Economic Co-operation and Development was followed [12]. Eighteen (18) female mice were divided into 3 equal groups of 6 mice each. After an overnight fast (approximately 16 hours), 0.5 mL of the extract and the control solution were administered to each mouse orally, using a gavage. Control group received saline (1 %). The other two groups received 2500 and 5000 mg/Kg of *M. pubescens* extract respectively.

During the first four hours after the administration of the extract, the mice were deprived of food for two hours but had free access to water. These mice were observed for the possibility of immediate intoxication. Thereafter, the animals were then observed daily for 14 days. The observations focused on abnormal behavior such as changes in body weight, ingestion of water and food, tremors, convulsions, respiration, paralysis, diarrhea and sleep. Focus was also on changes in the skin, hair, eyes and especially mortality.

Peripheral analgesic activity

The peripheral analgesic effect of *M. pubescens* extract was evaluated via the acetic acid-induced mouse writhing test described by Koster *et al* with slight modifications [13]. The mice were divided into 5 groups of 8 mice with the first group treated with 0.5 mL saline (1 %), while those of the second lot were treated with 200 mg/kg bw of acetylsalicylic acid (ASA). The mice in the other groups were orally administered 50, 100 and 200 mg/kg bw of the plant extract, respectively. After 30 minutes, all animals were intraperitoneally injected with acetic acid (0.6 %) to induce contortions. Five minutes after the induction, the contortions were counted over 30 minutes and the percentages of inhibition (A) of abdominal contortions were calculated using Eq 1.

$A = \{(W_{control} – W_{test})/W_{control}\}100$…….. (1)

Where W is the mean writhing count.

Central analgesic activity

The central analgesic effect was carried out using the tail immersion test of D'Amour and Smith with modifications [14]. Here, the distribution and treatment of the mice was similar to the previous model. The lower tail section of the mice was introduced into warm water (55 ± 1 °C). The time taken for the mouse to withdraw its tail from the water is the reaction time and it was measured at 0, 30, 60, 90, 120 and 180 min, following administration of the different solutions tested. If the mouse does not react, the tail is removed after 15 sec. The percentage inhibition (B) against thermal stimulus was calculated using Eq 2.

$B = \{(L_n - L_0)/(15s - L_0)100\}$ ……………..(2)

Where L_0 and L_n are the latent time observed (in sec) before and after the different treatments (n = 30 to 180 min).

Anti-inflammatory activity

The anti-inflammatory effect of *M. pubescens* extract was evaluated by carrageenan-induced mouse paw edema according to the method of Winter *et al* [15]. Forty mice were separated equally into five groups. The negative control and positive control groups received 0.5 mL saline (1 %) and diclofenac potassium at 50 mg/kg bw, respectively. The other groups received doses of 50, 100 and 200 mg/kg bw of phenolic extract of *M. pubescens,* respectively. Saline, diclofenac potassium and different doses of *M. pubescens*

extract were administered orally. After one hour of treatment, 20 µL of a carrageenan solution (1 %) was injected into the sub-plantar tissue of the left hind paw, which induced paw edema. Mouse paw volume was measured using a plethysmometer. The percentage inhibition (I) of edema was calculated using Eq 3.

$I = \{((V_T - V_0)_{control} - (V_T - V_0)_{treated}) / (V_T - V_0)_{control}\}100$ ……………………….. (3)

Where V_0 and V_t, are the paw volume (mL) before and after treatment with carrageenan (at 1, 2, 3, 4, 5 and 6 hours), respectively.

Statistical analysis

Data are expressed as mean ± SD (for *in vitro* studies) and mean ± SEM (for *in vivo* experiments). Statistical analysis was investigated by using one-way analysis of variance (ANOVA), with significance set at $p < 0.05$.

RESULTS

Phenolic and flavonoid contents

The contents of phenolic compounds and flavonoids were determined for each dose studied (Figure 1). Analysis of the results indicates that the levels of phenolic compounds and flavonoids increased significantly ($p < 0.05$) with the dose. The contents of phenolic compounds in the doses of 50, 100 and 200 mg/kg are 1.30, 2.80 and 4.47 mg, respectively while the number of flavonoids is 0.43, 0.89, and 1.60 mg, respectively.

Acute toxicity

The result of the acute toxicity study to evaluate the toxic effects of oral administration of the phenolic extract of *M. pubescens* (2500 and 5000 mg/kg bw) over 14 days, shows that all mice survived after the 14th day of this study. This shows that the lethal median dose (LD_{50}) of the phenolic extract of *M. pubescens* is well above 2500 and 5000 mg/kg.

Analgesic activities

Peripheral analgesic activity

In this study, the peripheral analgesic propriety was determined by inducing abdominal pain in mice via intraperitoneal administration of acetic acid resulting in abdominal contortions. The obtained results revealed that the number of abdominal contortions recorded differs significantly ($p < 0.05$) from one group to another (Table 1). The highest number of contortions was recorded in control mice with 108.5 contortions. The lowest number was found in mice treated with acetylsalicylic acid (ASA) and *M. pubescens* extract (200 mg/kg) with 55 and 55.75 contortions, respectively.

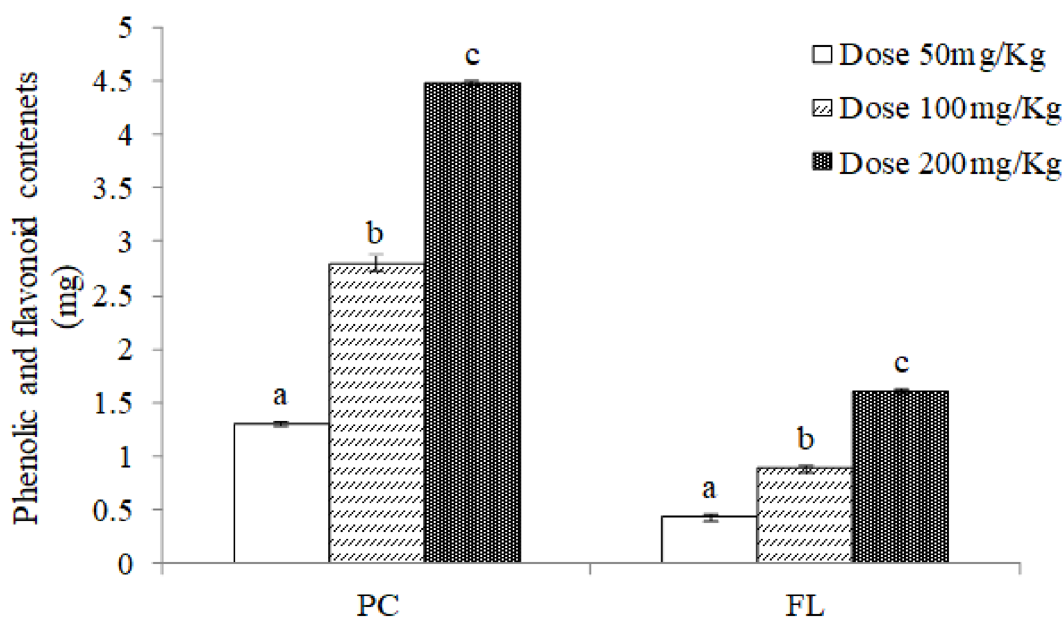

Figure 1: Phenolic and flavonoid contents of different doses of *M. pubescens.* Results with different letters are significantly different ($p < 0.05$). PC: Phenolic compounds FL: Flavonoids

Table 1: Effect of *M. pubescens* phenolic extract and acetylsalicylic acid (ASA) on acetic acid-induced writhing test in mice

Group	Contortions number	Percentage (%)
Control	108.50±2.31[d]	-
Standard drug	55.00±1.26[a]	49.31[c]
M. pubescens extract		
50 mg/kg	64.29±4.44[c]	40.75[a]
100 mg/kg	58.86±6.70[b]	45.74[b]
200 mg/kg	55.75±3.34[a]	48.62[b,c]

Results with different letters are significantly different ($p < 0.05$). Each value represents the mean ± SEM; n = 8

Table 2: Effect of *M. pubescens* phenolic extract and acetylsalicylic acid (ASA) on mouse tail withdrawal latency (seconds)

Group	0 min	30 min	60 min	90 min	120 min	180 min
Control	2.27±0.23[a]	2.25±0.21[a]	2.37±0.11[a]	2.45±0.11[a]	2.49±0.13[a]	2.73±0.10[a]
Standard drug	2.29±02.2[a]	3.15±0.27[b]	3.83±0.29[b,c]	4.60±0,25[d]	5.10±0.29[c]	6.12±0.44[c]
***M. pubescens* extract**						
50 mg/kg	2.07±0.23[a]	2.37±0.34[a]	2.52±0.41[a]	3.33±0.38[b]	4.11±0.52[b]	4.67±0.63[b]
100 mg/kg	2.29±0.23[a]	2.69±0.34[a,b]	3.32±0.,41[b]	4.09±0.40[c]	5.04±0.53[c]	6.16±0.68[c]
200 mg/kg	2.07±0.21[a]	2.98±0.34[b]	4.20±0.40[c]	5.69±0.38[e]	6.46±0.53[d]	7.42±0.66[d]

Results with different letters are significantly different ($p < 0.05$). Each value represents the mean ± SEM, n=8

The obtained results showed that the percentages of inhibition of abdominal contortions of the treated groups showed significant differences ($p < 0.05$) compared to those of the control group. Acetylsalicylic acid (ASA; 200 mg/kg bw) gave the highest percentage of inhibition (49.30 %), followed by *M. pubescens* extract administered at the same dose (48.62 %) while the extract at 50 mg/kg exerted the lowest percentage (40.75 %). These data indicate that the peripheral analgesic property of the extract studied is significantly dose-dependent ($p < 0.05$).

Central analgesic activity

Table 2 shows the result of tail immersion test, used to assess the central analgesic effects of drugs through opioid receptors. Throughout the experiment, the results obtained reveal that similar to acetylsalicylic acid, the phenolic extract at different doses prolonged the tail withdrawal latency of the mice compared to that of untreated mice (Table 2). Initially, no significant differences in latency times were detected among the groups studied. However, after 30 minutes, a significant difference ($p < 0.05$) was seen. At 180 minutes, the mice treated with the 200 mg/kg of *M. pubescens* extract displayed the most extended tail followed by the mice treated with acetylsalicylic acid. Also, the levels of heat-induced pain inhibition displayed notable variability ($p < 0.05$) depending on the time and the dose administered. After 30 minutes, the group that received the dose of 200 mg/kg bw of extract presented the strongest central analgesic power, followed by the groups treated with 100 mg/kg and ASA, while the group treated with 50 mg/kg of the extract showed the lowest central analgesic power (Table 3). This study revealed that the highest pain-inhibiting effect was exerted at the 180th minute by *M. pubescens* extract with a percentage of 38.23 %, followed by acetylsalicylic acid at the same dose (200 mg/kg) with a percentage of 27.59 %. In this work, the low central analgesic power exerted by acetylsalicylic acid compared to that of *M. pubescens* extract at the same dose, could be explained by the fact that acetylsalicylic acid is a peripheral analgesic.

Table 3: Percentage of inhibition of heat-induced pain exerted by *M. pubescens* phenolic extract and acetylsalicylic acid (ASA)

Group	Inhibition of heat-induced pain (%)				
	30 min	***60 min***	***90 min***	***120 min***	***180 min***
Standard drug	7.08	11.59	17.10	20.83	27.60
***M. pubescens* extract**					
50 mg/kg	0.93	1.18	7.02	12.97	15.83
100 mg/kg	3.46	7.50	13.07	20.41	27.92
200 mg/kg	5.74	14.45	25.80	31.76	38.23

Table 4: Anti-inflammatory effect of *M. pubescens* phenolic extract on carrageenan-induced paw edema

	Paw edema volume (mL)						
Group	***0h***	***1h***	***2h***	***3h***	***4h***	***5h***	***6h***
Control	0.17±0.00[a]	0.22±0.00[a]	0.25±0.01[a]	0.26±0.01[b]	0.23±0.01[b]	0.23±0.01[b]	0.22±0.00[b]
Standard drug	0.17±0.00[a]	0.22±0.00[a]	0.23±0.00[a]	0.24±0.01[a]	0.21±0.01[a,b]	0.19±0.01[a]	0.18±0.00[a]
Extract							
50 mg/kg	0.17±0.01[a]	0.21±0.01[a]	0.23±0.01[a]	0.24±0.01[a,b]	0.22±0.01[a,b]	0.21±0.01[a,b]	0.19±0.01[a]
100 mg/kg	0.17±0.00[a]	0.21±0.01[a]	0.23±0.01[a]	0.24±0.00[a,b]	0.22±0.01[a,b]	0.20±0.01[a]	0.19±0.01[a]
200 mg/kg	0.17±0.00[a]	0.21±0.01[a]	0.23±0.01[a]	0.24±0.01[a]	0.21±0.01[a]	0.20±0.01[a]	0.18±0.01[a]

Note: Values are expressed as the mean ± SEM (n = 8). Results with different letters are statistically different ($p < 0.05$)

Table 5: Percentage of edema inhibition exerted by *M. pubescens* phenolic extract and Diclofenac

	Inhibition of paw edema volume (%)					
Group	***1h***	***2h***	***3h***	***4h***	***5h***	***6h***
Standard drug	13.16	21.00	27.50	33.99	61.83	80.56
Extract						
50 mg/kg	5.26	8.71	13.00	16.95	28.43	52.78
100 mg/kg	6.43	14.17	21.37	24.29	40.63	62.96
200 mg/kg	10.53	17.49	23.14	29.73	45.13	72.22

Anti-inflammatory activity

The data in Table 4 reveals that the paw volume of all mice treated with carrageenan reached the maximum at the 3rd hour of the experiment, then decreased without reversing to the normal paw volume at the end of the experiment (6th hour). The volumes of treated mice paws increased from 0.17 to 0.24 mL at the 3rd hour and then decreased to values of 0.18 – 0.19 mL at the 6th hour. The results of this study showed that diclofenac (standard drug) and the different doses of *M. pubescens* extract inhibited the inflammatory edema from the first hour (Table 5).

Throughout the experiment, the highest percentage of edema inhibition was exerted by diclofenac at 50 mg/kg, with values ranging from 13.5 to 80.56 %, followed by that exerted by *M. pubescens* extract at 200 mg/kg, with inhibition percentages ranging from 10.63 to 72.22 %. This was followed by the 100 mg/kg dose with percentages ranging from 6.43 to 62.96 %, and finally the dose of 50 mg/kg with inhibitory percentages between 5.26 and 52.78 %.

DISCUSSION

This study revealed that the phenolic extract of *M. pubescens* is not toxic to mice, with an LD_{50} greater than 5000 mg/kg. According to the toxicity scale described by Hodge and Sterner an extract with an oral LD_{50} greater than 5000 mg/kg of body weight, is considered to be a practically non-toxic extract [16]. Throughout the study, the mice given the extract at both doses did not exhibit any abnormal behavior compared to the control mice. Statistical analysis did not show any significant difference ($p > 0.05$) between the volumes of water and the quantities of food consumed by the treated mice and those consumed by the control mice. Concerning body weight, the control and treated mice presented similar body weights. These data show that the phenolic extract of *M. pubescens* is not toxic at the doses administered.

The acetic acid-induced writhing test is a widely used model for its ability to reveal the antinociceptive effects of natural products. Acetic acid induces the release of endogenous mediators in peripheral tissues such as histamine, serotonin, bradykinin, acetylcholine, substance P, prostaglandins (PGE_2 and $PGF_{2\alpha}$), and pro-inflammatory cytokines such as TNF-α, IL-1β, IL-8 [17]. The peripheral analgesic effect exerted by *M. pubescens* extract at different doses could be associated with the inhibition of peripheral pain mediators such as prostaglandins by acting on visceral receptors sensitive to acetic acid [18]. The present study revealed that acetylsalicylic acid exerted analgesic potency similar to that of *M. pubescens* extract at the same dose (200 mg/kg). Acetylsalicylic acid is a nonsteroidal anti-inflammatory drug. It acts by inhibiting the synthesis of prostaglandins by cyclooxygenases (COX-1 and COX-2) in peripheral tissues [19]. The analgesic property exerted by the studied phenolic extract could be explained by the inhibition of prostaglandin synthesis arising from the inhibition of the cyclooxygenase enzyme.

This study revealed that the phenolic extract studied reduced the pain induced by heat significantly ($p < 0.05$) compared to the control and that the percentages of inhibition exerted are dose-dependent. The tail immersion method

involves a spinal reflex, which is modulated by a supraspinal inhibitory mechanism but could also involve neuronal structures. This method can measure μ2, κ1 and δ2 opioid receptors involved in nociception via spinal reflexes [20]. This suggests that the phenolic extract of *M. pubescens* exerts central analgesic activity by affecting opioid receptors. The analgesic activity revealed in the present study could be attributed to the phenolic compounds and flavonoids found in the extract of *M. pubescens*.

The anti-inflammatory activity of the phenolic extract of *M. pubescens* is dose-dependent. For all mice tested, the highest percentages of edema inhibition were recorded at the 5th and 6th hour of the experiment ($p < 0.05$). This could be due to the inhibition of prostaglandin synthesis via inhibition of cyclooxygenases by the phenolic extract of *M. pubescens.* The low anti-inflammatory activity of *M. pubescens* extract compared to that of Diclofenac, could be explained by the fact that diclofenac is administered in its pure form unlike the phenolic extract of *M. pubescens*, which contains other substances, such that the highest dose tested (200 mg/kg) contains only 4.47 mg of phenolic compounds. The anti-inflammatory activity revealed in the present study could be due to the high amount of phenolic compounds and flavonoids in the *M. pubescens* extract.

CONCLUSION

The phenolic extract of *M. pubescens* is non-toxic. It exerts significant peripheral and central analgesic activities as well as anti-inflammatory activity due to the high amounts of phenolics, in particular, flavonoids present in the extract. However, further investigations are required to ascertain the potentials of the extract for its clinical development.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. H MA and N A conceived and designed the study, collected and analysed the data and wrote the manuscript. All authors read and approved the manuscript for publication.

Use of Artificial Intelligence/large language models

No AI was used in this article

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