Anti-nociceptive and anti-inflammatory activities of the methanol extract of *Waltheria americana* Linn. leaf in experimental animals

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**ABSTRACT**

**Background:** *Waltheria americana* has been used in African folklore for the treatment of minor and complicated ailments like pain, fever, and rheumatism.

**Aim:** To investigate the analgesic and anti-inflammatory potentials of *Waltheria americana* and its possible mechanism of action in Swiss mice and Wistar rats, respectively.

**Materials and Methods:** Methanol extract of *Waltheria americana* leaf was evaluated for anti-nociceptive activities (using tail flick, formalin-induced paw licking, and acetic acid-induced writhing tests in mice) and possible mechanisms of action using atropine (5 mg/kg ip), prazosin (1 mg/kg po), glibenclamide (8 mg/kg po), propranolol (40 mg/kg po), naloxone (2 mg/kg ip), nifedipine (10 mg/kg po) using acetic acid, and tail-flick models of analgesia. The anti-inflammatory effect was evaluated in carrageenan-induced paw edema and cotton pellet-induced granuloma tests.

**Results:** Acute toxicity revealed no mortality in the mice up to a dose of 2,000 mg/kg. The phytochemistry revealed the presence of alkaloids, flavonoids, saponins, and terpenoids. The extract (100 or 200 mg/kg) produced inhibition \((p < 0.05)\) both in the formalin-induced paw licking and acetic acid-induced writhing tests. In the thermal test, the extract (200 mg/kg) significantly increased the withdrawal latency. There was a reversal of the analgesic effect of the extract with the cholinergic and opioidergic blockers that significantly produced changes in the analgesic effects of the extract. The extract produced dose-related inhibition of cotton pellet-induced granuloma in rats and carrageenan-induced paw edema in rats.

**Conclusion:** The extract produced anti-inflammatory potentials and anti-nociception through mechanisms that may depend on the cholinergic and opioidergic system.

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**KEYWORDS**

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**Introduction**

Medicinal plants are relied upon for the prevention and treatment of many health problems among rural populations globally, which improves the quality of life. The cost and access to modern drugs among the rural populations of tropical Africa made a large proportion of rural people to depend on traditional herbal drugs [1].

*Waltheria americana* L. belongs to the family *Sterculiaceae*, also known as velvetleaf, marshmallow, monkey bush, boater bush, etc [2]. It is found throughout the tropics and warmer subtropics. *Waltheria americana* grows on disturbed areas, roadside weed, old pastures, cotton fields, rock crevices on top of plains, inundated savannas, riverbanks, forests borders or slopes, impoverished soils, on limestone, or basalt rock outcrops [3]. In traditional medicine, *Waltheria americana* is used for the treatment of minor ailments (e.g., sore throat, cough) and complicated ailments (e.g., inflammation, asthma). Its roots were used in the treatment of wounds [4], leprosy [5], fever and pain [6].
rheumatism [4], night blindness, gum and teeth disease, diarrhea, and dysentery [7]. Preliminary phytochemistry has revealed the presence of alkaloids [8], terpenoids and saponins [9], and flavonoids [10].

Several flavonoids were isolated from *Waltheria indica* as reviewed by Zongo and co-workers [11]. These include (-)-epicatechin, tiliroside [12], quercetin [12,13], and kaempferol [13,14]. A rare acylated flavonol glycoside—Kaempferol-3-O-β-D-(6"-E-p-coumaryl)-glucopyranoside was isolated from the whole plant [13].

Flavonoid derivatives from *Waltheria indica* were evaluated in vitro and showed that tiliroside, (-)-epicatechin, and quercetin induced a dose-dependent inhibition of the production of the inflammatory mediators, including nitric oxide, tumor necrosis factor-α, and interleukin-12 [12]. It is noteworthy that most of the drugs used presently for the management of pain possess more toxic effects [15]. On the contrary, many medicines of plant origin are economical in the treatments of different ailments. However, there are few reports on the analgesic and anti-inflammatory activities of *Waltheria americana* leaf. Therefore, the purpose of this study is to evaluate the analgesic and anti-inflammatory effects of the methanol extract of *Waltheria americana* (MEWA) leaf to support the folkloric claim, and the possible mechanisms of action using different experimental models of analgesia and inflammation.

**Materials and Methods**

**Animals**

A total of 135 male Swiss mice (22–30 g) were used for the acute toxicity and analgesic studies and 40 Wistar rats (150–200 g) were used for the inflammatory study. They were acclimatized for 2 weeks, kept under standard laboratory conditions, and fed on rodent cubes (Ladokun Feeds, Ibadan, Nigeria). All experimental procedures on rodents were conducted in accordance with established protocols under the guidelines of the Principle of Laboratory Animal Care (National Institute of Health publication No. 85-23) [16] and ethical guidelines for investigation of experimental pain in conscious animals by Zimmerman [17].

**Collection and extraction of Waltheria americana leaf**

*Waltheria americana* leaves were obtained around College of Agriculture, Kabba, Kogi state, and authenticated by Mr. Odewo S. A. and Mr. Adeniji K. A. of the Herbarium, Forestry Research Institute of Nigeria, Ibadan, Nigeria where the plant was kept with a voucher number FHI: 111064. The leaves were air-dried at room temperature and finely powdered with a blender. Four hundred grams of the pulverized plant was macerated in methanol for 72 hours. After the extraction, the extract was sieved and filtered. The filtrate was concentrated in the oven at 40°C [18]. The dried extracts were stored at 4°C until needed. Appropriate dose dilutions were made with distilled water.

**Acute oral toxicity test**

Twenty-five adult male Swiss mice (20–30 g) were divided into five groups. Each group consists of five mice [19]. The mice fasted for 24 hours. Groups 1–5 received 50, 100, 200, 1,000, and 2,000 of MEWA leaf. The mice were observed continuously for 2 hours daily for behavioral and autonomic profiles, and for any sign of toxicity or mortality, up to a period of 14 days.

**Phytochemical screening**

The extract was screened to detect the presence of some phytochemicals according to the methods described by Sofowora [20].

**Drugs and chemicals**

The following drugs and chemicals were used: prazosin (alpha-adrenergic blocker), atropine (a non-selective muscarinic receptor antagonist), indomethacin (Cyclooxygenase Cox inhibitor), propranolol (Beta-adrenergic blocker), nifedipine (L-type voltage-gated calcium channel blocker), acetic acid, ketamine (anesthesia), formaldehyde, methanol, naloxone (a non-selective opioid receptor antagonist), glibenclamide (an Adenosine triphosphate (ATP)-sensitive K⁺ channel inhibitor). They were of high analytical value.

**Analgesic screening**

Chemical and thermal models were used in this study. All tests were conducted under the ethical guidelines of the International Association for the Study of Pain [21].

**Acetic acid-induced writhing test in mice**

The acetic acid-induced abdominal constriction test was carried out with minor modifications [22]. In order to induce pain in mouse peritoneal cavity, 0.6% of acetic acid (10 ml/kg) was injected
intraperitoneally 60 minutes after the administration of MEWA leaf (50, 100, and 200 mg/kg po). The number of abdominal constrictions was counted cumulatively between 5 and 15 minutes after acetic acid administration. Anti-nociception of MEWA leaf was indicated by the reduction in the number of abdominal constrictions in the test groups compared to the control group. Indomethacin (10 mg/kg po) was used as reference drug while the control group received distilled water (10 ml/kg po).

**Formalin-induced paw licking test in mice**

The procedures used in the formalin-induced paw licking test was similar to [23] with minor modification. Animals were pre-treated with MEWA leaf (50, 100, and 200 mg/kg po) 60 minutes before the formalin injection. Control animals received only distilled water (10 ml/kg po) while (indomethacin 10 mg/kg po) was used as the reference drug. After 60 minutes, the intraplantar area of the right hind paw of the mice was injected with 20 μl of 2.5% of formalin. The animals were then immediately placed individually in a transparent observation chamber and the time the animal spent licking the injected paw was recorded for 30 minutes following formalin injection (first 5 minutes after formalin injection (early phase) and 15–30 minutes after formalin injection (late phase)).

**Tail-flick latency assay in mice**

Ugo Basile tail-flick 37360 model maintained at 55°C was used and the time taken for the animal to withdraw its tail was recorded as withdrawal latency [24]. The animals were grouped as above, the control received (10 ml/kg po) distilled water; the reference group received (10 mg/kg po) indomethacin, and the other groups received (50, 100, and 200 mg/kg po) of the extract.

**Mechanism of anti-nociception of the methanol extract of Waltheria americana leaf**

The possible mechanism of action of *Waltheria americana* leaf was investigated using the acetic acid-induced abdominal writhing test and tail-flick test. The animals were pre-treated with *Waltheria americana*, distilled water (10 ml/kg) 60 minutes before acetic acid (0.6%, ip), and before the tail-flick test. Alpha-adrenergic blockers (prazosin, 1 mg/kg po); beta-adrenergic blocker (propranolol, 40 mg/kg po); muscarinic cholinergic blocker (atropine, 5 mg/kg ip); L-type voltage-gated calcium channel blocker (nifedipine, 10 mg/kg po); non-selective opioidergic receptor blocker (naloxone, 2 mg/kg ip); and ATP-sensitive K+ channel blocker (glibenclamide, 8 mg/kg po) were given before the extract, against the probable analgesic potential of MEWA leaf.

**Anti-inflammatory effects of methanol extract of Waltheria americana leaf in carrageenan-induced paw edema model of inflammation**

Carrageenan-induced rat paw edema was done by the method of Winter and a co-worker with a slight modification [25]. Inflammation was induced by the injection of 0.1 ml of freshly prepared carrageenan (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats. The different groups of rats were administered with MEWA (100 and 200 mg/kg po) and indomethacin (10 mg/kg po). The control group received vehicle (distilled water, 10 ml/kg po). One-hour after treatment, paw edema was induced by the injection of edematogenic agent carrageenan. The paw volume was measured by a Plethysmometer (Ugo Basile model: 37140). The measures were determined at 0 hour ($V_0$; before edematogenic agent injection) and 1, 2, 3, 4, and 5-hour intervals later ($V_t$). The difference between $V_t$ and $V_0$ was taken as the change in paw volume (edema value).

**Anti-inflammatory effects of methanol extract of Waltheria americana leaf in cotton pellet-induced granuloma model of inflammation**

The effect of MEWA leaf on the chronic phases of inflammation was assessed in the cotton pellet-induced granuloma rat model [26]. Sterilized cotton pellets weighing 30 mg was implanted subcutaneously through a small ventral incision in anesthetized rats. The different groups of rats were treated with MEWA leaf (100 and 200 mg/kg po) and indomethacin (10 mg/kg po) once daily for seven consecutive days from the day of cotton pellet insertion. The control group received vehicle (distilled water, 10 ml/kg po). On the eighth day, the animals were sacrificed and the cotton pellets were removed; dried at 60°C for 24 hours, and their mass was determined. Mean weight of the granuloma tissue formed around each pellet was obtained and percentage inhibition was expressed as compared with the control group.

**Statistical analysis**

Data were presented as mean ± Standard Error of Mean (SEM). Comparisons between groups were made using the one-way analysis of variance (ANOVA) followed by Dunnett’s post-hoc test, 95%
confidence level, and at $p < 0.05$ was considered statistically significant.

**Results**

**Acute toxicity**

There was no mortality recorded in any of the animals treated with varying doses 50, 100, 200, 1,000, and 2,000 mg/kg of the MEWA leaf. During the observation period of 14 days, all the treated animals were found to be healthy and normal without any apparent symptoms of adverse effects.

**Preliminary qualitative phytochemical analysis of methanol extract of Waltheria americana leaf**

The yield was 2.4%, and the phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, and terpenoids in the MEWA leaf as shown in Table 1.

**Effects of methanol extract of Waltheria americana (MEWA) leaf on acetic acid-induced writhing test in mice**

Figure 1 shows that the extract (100 and 200 mg/kg) produced a significant ($p < 0.05$) reduction in writhing movements caused by acetic acid. The reference drug indomethacin 10 mg/kg also produced a significant reduction in writhing movements caused by acetic acid. The extract dose (200 mg/kg) has the highest inhibition which is comparable to the reference drug indomethacin (10 mg/kg).

**Effects of methanol extract of Waltheria americana leaf on tail-flick latency in mice**

Figure 2 shows the effects of graded doses of the MEWA leaf on tail-flick latency(s). The extract (200 mg/kg) tested showed significant ($p < 0.05$) increase in the tail-flick latency(s), the extract (50 and 100 mg/kg) were not significant compared with the control (distilled water 10 ml/kg). The potency of the 200 mg/kg of the extract was comparable to the reference group (indomethacin, 10 mg/kg).

**Effects of Waltheria americana on formalin-induced paw licking test in mice**

From Figure 3, extract doses (200 and 100 mg/kg) significantly ($p < 0.05$) and dose-dependently decreased the early and late phases. All the extract doses (50, 100, and 200 mg/kg) and the reference drug indomethacin (10 mg/kg) seem to exert an effect on the early phase than on the late phase.
Analgesic and anti-inflammatory properties of *Waltheria americana* leaf

**Figure 3.** Effects of graded doses of MEWA leaf on formalin-induced paw-lick in mice. Data represent means ± SEM of five mice. Comparisons were made using one-way ANOVA, followed by Dunnett’s post-hoc test. *p < 0.05 compared to control is significant.

**Effects of pre-treatment with adrenergic, cholinergic, L-Type voltage-gated calcium channel blocker, opioidergic receptor, and ATP sensitive K\(^+\) channel blocker in the tail flick and acetic acid-induced writhing in mice**

From table 2, the pretreatment with propranolol, prazosin, nifedipine, and glibenclamide did not significantly affect the tail-flick latency and acetic acid-induced writhing in mice. But pre-treatment with atropine and naloxone significantly reversed the effect of the extract as compared with the control (200 mg/kg *Waltheria americana* leaf).

**Anti-inflammatory effects of methanol extract of *Waltheria americana* leaf in carrageenan-induced paw edema in rats**

The results of carrageenan-induced inflammation in Table 4 showed a decrease in paw size of all the treated groups compared with that of the control group. At 2, 4, and 5 hours post-carrageenan administration, the paws of the extract (100 and 200 mg/kg) with the indomethacin-treated animals decreased significantly (*p < 0.05*) compared with the control group. After 1 hour of carrageenan administration, the paws of all the treated groups decreased significantly compared with the control group. The percentage change produced by the extract 100 mg/kg, 200 mg/kg, and indomethacin 10 mg/kg are 39.13%, 56.52%, and 52.17%, respectively.

**Table 2.** Effects of pretreatment with adrenergic, muscarinic cholinergic receptors, L-Type voltage-gated calcium channel blocker, Opioidergic receptor blocker, and ATP sensitive K\(^+\) channel blocker followed by 200 mg/kg *Waltheria americana* leaf on tail flick test and acetic acid-induced writhing in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Latency (sec)</th>
<th>Numbers of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Waltheria americana</em>  (control)</td>
<td>4.18 ± 0.14</td>
<td>15.00 ± 1.71</td>
</tr>
<tr>
<td>Prazosin + extract</td>
<td>3.94 ± 0.44</td>
<td>16.00 ± 0.68</td>
</tr>
<tr>
<td>Propranolol + extract</td>
<td>4.40 ± 0.48</td>
<td>14.00 ± 0.93</td>
</tr>
<tr>
<td>Atropine + extract</td>
<td>2.66 ± 0.17*</td>
<td>26.00 ± 2.00*</td>
</tr>
<tr>
<td>Nifedipine + extract</td>
<td>5.20 ± 0.51</td>
<td>16.00 ± 1.6</td>
</tr>
<tr>
<td>Naloxone + extract</td>
<td>2.70 ± 0.20*</td>
<td>29.40 ± 1.81*</td>
</tr>
<tr>
<td>Glibenclamide + extract</td>
<td>4.04 ± 0.43</td>
<td>18.20 ± 1.46</td>
</tr>
</tbody>
</table>

Data represent means ± SEM (n = 5 mice. Comparisons were made using one-way ANOVA, followed by Dunnett’s post-hoc test. *p < 0.05 compared to control.

**Table 3.** Anti-inflammatory effects of *Waltheria americana* leaf in cotton pellet-induced granuloma in rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose (mg/kg)</th>
<th>Pellet weight (g/100g b.w)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>0.023 ± 0.001</td>
<td>-</td>
</tr>
<tr>
<td>MEWA</td>
<td>100</td>
<td>0.014 ± 0.002*</td>
<td>39.13</td>
</tr>
<tr>
<td>MEWA</td>
<td>200</td>
<td>0.010 ± 0.001*</td>
<td>56.52</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>0.011 ± 0.001*</td>
<td>52.17</td>
</tr>
</tbody>
</table>

Data represent means ± SEM of five mice. Comparisons were made using one-way ANOVA, followed by Dunnett’s post-hoc test. *p < 0.05 compared to control is significant.

**Table 4.** Anti-inflammatory effects of MEWA leaf in carrageenan-induced paw edema in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Change in paw volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 0 hour</td>
</tr>
<tr>
<td>Control</td>
<td>1.30 ± 0.15</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>0.69 ± 0.10*</td>
</tr>
<tr>
<td>MEWA (200 mg/kg)</td>
<td>0.74 ± 0.18*</td>
</tr>
<tr>
<td>MEWA (100 mg/kg)</td>
<td>0.70 ± 0.11*</td>
</tr>
</tbody>
</table>

Data represent means ± S.E.M of five mice. Comparisons were made using one-way ANOVA, followed by Dunnett’s post-hoc test. *p < 0.05 compared to control is significant.
administration, the reference drug (indomethacin, 10 mg/kg) was the only group that showed a significant decrease in paw size. Meanwhile, at 3 hours post-carrageenan administration, the extract (100 mg/kg) did not show a significant decrease in paw size compared to the control group.

Discussion

This study was carried out to investigate the anti-nociceptive and anti-inflammatory effects of the MEWA leaf. Phytochemical analysis of Waltheria americana leaf revealed the presence of alkaloids, flavonoids, saponins, and terpenoids. The alkaloids, the largest single class of secondary plant substances, have been reported by researchers to exhibit a remarkable range of pharmacological activities. These include analgesic, anti-inflammatory, and anti-cancer activities [27–29], and this may be responsible for the analgesic and anti-inflammatory effect of the extract. Chemical and thermal models were employed to check the analgesic potentials of Waltheria americana while the cotton pellet-induced granuloma and the carrageenan-induced paw edema were used for the anti-inflammatory study.

The acetic acid-induced writhing is normally used to test for new analgesic agents [30]. The intraperitoneal injection of acetic acid usually leads to the release of inflammatory mediators like substance P, bradykinin, serotonin, histamine, and prostaglandins which stimulate the primary afferent nociceptors to send a nerve impulse to dorsal horn neurons of the Central nervous system (CNS) leading to abdominal constrictions [31]. There was an inhibition of this abdominal constrictions in animals treated with the extract as compared with the control, suggesting that the extract possibly prevents the release of inflammatory mediators. This was also supported by comparing the potentials of the cyclooxygenase (COX) inhibitor, indomethacin with 200 mg/kg of the extract which were comparable. This test suggests that the extract may act by the inhibition of COX and other inflammatory mediators. Further tests were employed as muscle relaxants could cause data misinterpretation in the acetic acid-induced writhing test [32].

The formalin test has two phases; the early phase is the neurogenic phase as a result of direct stimulation of sensory nerve fibers while the late phase referred to as the inflammatory phase is caused by the release of chemical mediators including histamine, bradykinin, serotonin, and substance P [33]. This study showed that both phases were significantly reduced by the extract as compared with the control which was similar to the observation of Onasanwo and Elegbe [34]. Conventionally, centrally acting drugs are well known to inhibit both early and late phases significantly [35]. Therefore, the inhibition of both the early and late phases in this test together with the reduction of abdominal constrictions in the acetic acid-induced writhing test showed that the extract of Waltheria americana leaf acts centrally and peripherally.

The tail-flick test is based on a phasic stimulus of high intensity; the nociceptive experience is short-lasting and they are thus, models of acute pain [35]. As investigated in this study, the MEWA leaf produced increased withdrawal latency, and this result shows that the extract has anti-nociceptive activity in acute pain. Thermally-induced nociceptive responses in mice could be inhibited by narcotic agents as centrally acting analgesics [36]. This result implies that the analgesic effect of the extract is centrally mediated. Thus, the increased nociceptive threshold in this test together with the reduction of nociception in the formalin-induced paw licking test (early and late phases) also supports the evidence of centrally mediated anti-nociceptive activity of MEWA leaf.

Chronic inflammation leads to the development of proliferative cells. These cells are either spread or in granuloma form, in this study the anti-inflammatory activity of the MEWA leaf has been established using the cotton pellet granuloma model. The dry weight of the pellet correlates with the amount of granuloma tissue [27]. The extract showed a significant decrease in the weight of the pellet. This reflects the efficacy of the extract in chronic inflammatory conditions.

Carrageenan has been used as an agent for inducing experimental inflammation, which is a screening tool for agents with the anti-inflammatory property. Carrageenan-induced rat paw edema is suitable in vivo model to predict the value of anti-inflammatory agents which act by inhibiting the mediators of acute inflammation [37]. The early phase of carrageenan-induced paw edema is due to the release of serotonin, histamine, and similar substances while the late phase leads to the activation of kinin-like substances such as prostaglandins, proteases, and lysosomes [38]. The MEWA leaf inhibits both early and late phase, which shows that the effect may be by preventing the release of inflammatory mediators, mostly the COX products.
More so, arachidonate COX inhibitors due to its COX-dependent mechanism control this test effectively. Thus, the extract of Waltheria americana leaf may possess arachidonate COX inhibitory property. In the investigation of the mechanism of the anti-nociceptive activity of MEWA leaf, administration of adrenoceptors blockers (prazosin and propranolol), L-type voltage-gated calcium channel blocker (nifedipine) and ATP-sensitive K⁺ channel did not affect the anti-nociception of Waltheria americana leaf except for the pretreatment with muscarinic cholinergic blocker (atropine) and non-selective opioidergic blocker (naloxone) which significantly abolished the anti-nociception of Waltheria americana leaf, showing that the analgesic activity of MEWA leaf is non-adrenergic dependent, does not involve L-type voltage-gated calcium channel and ATP sensitive K⁺ channel. But the cholinergic and opioidergic systems might be involved in the mechanism of action of the extract of Waltheria americana leaf.

Conclusion
In conclusion, this study clearly revealed the analgesic and anti-inflammatory activity of the MEWA leaf, and showed that the mechanism of action of anti-nociception may not be due to the adrenergic system, ATP sensitive K⁺ channel and does not involve L-type voltage-gated calcium channel; but the cholinergic and opioidergic systems may be involved in the anti-nociceptive activity of the MEWA leaf.

References


