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# Antinociceptive activities with the possible mechanisms of action of hydroethanol leaf extract of *Eclipta prostrata* Hassk (Astraceae) in mice

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## Abstract

*Eclipta prostrata* belongs to the family of plants known as Astraceae. It is a species of plant in the sunflower family, popularly known as "false daisy" in English. The whole plant contains the alkaloids nicotine and ecliptine; and used locally for treating bruises, cuts, wounds, liver disorders and pain, among others. This study investigated the analgesic activities of hydroethanolic leaf extract of *Eclipta prostrata* in rodents, using standard laboratory protocols. Doses of 50, 100, and 200 mg/kg were separately administered via oral route to the animals used in the various models - acetic acid-induced mouse writhing test, formalin - induced pain, hot plate-induced pain, and tail clip test. The effect of the extract was comparable to that produced by peripheral analgesics like aspirin and centrally acting analgesics like morphine used as positive control in the various models employed. The mechanism of action was elucidated through pre-treatment with naloxone (1 mg/kg), yohimbine (1mg/kg), ondasetron (0.2 mg/kg), atropine (2 mg/kg), glibenclamide (2 mg/kg).

The extract activity was significant in all the models employed; however, it was more intense via the peripheral than the central pathway. Glibenclamide and atropine antagonized the activity of the decoction, which suggests that the herbal drug's antinociceptive effect is mediated via potassium ion channel opening and cholinergic receptor. Phytochemical analysis of the leaf extract indicated the presence of flavonoids, phenols, alkaloids, phlobatanins, steroids, cardiac glycoside, and which probably contributed to the analgesic activity of the extract.

**Keywords:** Antinociception; Pain; Medicinal plant; Extract; *Eclipta prostrate*; Mice

## 1. Introduction

Pain, an unpleasant experience, is evoked by different causes such as toothache, cancer, appendicitis, headaches, infections and surgery. It ensues when the pain receptors are stimulated by intense or damaging responses. Primarily, pain sensation is protective, as it indicates the presence of an environmentally unfriendly substance and the provoked tissue injury [1].

Pain is often scored as mild and severe, as well as acute and chronic, depending on the causative agents and its duration. The conventional therapies for treating pain are grouped as narcotics and non-narcotics. The latter are employed for treating mild and superficial pain, while the former is for visceral and severe pain. Most of these pharmaceuticals induce various side effects among which are constipation, peptic ulcer, respiratory depression, organ toxicity and physical dependence [2, 3]. Another setback to these therapies is their inability to treat the underlying cause of pain, which most often results in the use of multiple drugs and consequent drug-drug interactions [4]. The latter therefore calls for a continuous search for new analgesic drugs with promising pharmacological actions.

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Man has always discovered the great contributions of herbs in the management of almost every disease condition [5] and as a result, medicinal plants have often been employed as analgesic drugs in folk medicine, especially in developing countries, although the active principle of the herbal therapies have not been scientifically identified. According to the World Health Organization over 75 % of the world's population still depends on plant-derived medicines, usually obtained from traditional healers, for its basic health-care needs [6]. Worldwide, about 85 % of primary health care medications involve the use of natural products.

Among the plants used is *E. prostrata,* commonly known as false daisy, (*Arojoku;* Yoruba, and *Apa;* Igbo), which originated from Asia, but is widely distributed throughout the tropics and warm temperate zones. It is a much-branched, variable, prostrate, ascending or erect, rough-hairy annual herb, up to 90 cm tall with slender, reddish stems covered with short, stiff hairs, rooting at the lower nodes. It is a quantitative short-day plant, an abundant seeder, which flowers throughout the year.

The various Sanskrit names of *Eclipta prostrata* are literally translated as the 'King of Hair', clearly referring to its traditional reputation in Ayurveda as an herb [7].

*Eclipta* species (Astraceae) have been reported useful for treating liver diseases, microbial infections, pain, constipation, respiratory diseases, urinary infections, eye problems, cardiovascular diseases, and cancer, among other disease conditions [8]. The present study aimed at scientifically exploring the possible antinociceptive activity of *E. prostrata* and the possible pathway of the effect.

## 2. Material and methods

### 2.1. Plant Material

Fresh leaves of *Eclipta prostrata* were collected from swampy area at Idi-Araba, Lagos State of Nigeria. The plant was identified and authenticated by Mr. T.K. Odewo of the Department of Botany and Microbiology, University of Lagos, Lagos, Nigeria, and given a voucher number LUH 6535.

### 2.2. Experimental Animals

Healthy albino mice (20-25 g) of both sexes obtained from the Laboratory Animal Centre of the College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria were used. The animals were maintained under standard environmental conditions and were fed the standard laboratory diet, purchased from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, alongside with clean drinking water. The animals were kept in clean plastic cages, while they acclimatized for a period of one week and were fasted for 12 hours prior to experimentation. The protocol used in this study was in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biochemical Research [9]°.

### 2.3. Drugs and Chemicals

Formalin (Griffin and George, Leics, England), morphine (Martindale Pharma®, Essex, United Kingdom), acetylsalicylic acid (Reckitt Benckister Parkistan Ltd, Karachi), and acetic acid (Sigma, Chemical Company, England).

### 2.4. Preparation of Plant Extract

Fresh leaves of *Eclipta prostrata* collected, were rinsed under a running tap, and thereafter air dried and hand-shredded into coarse powder. A known weight, 130 g was macerated in 2 L hydroethanol (70 % ethanol and 30 % distilled water) for three days, and thereafter first filtered using clean white handkerchief to remove the shaft, and afterwards through a 25 mm Whatman filter paper. The filtrate was evaporated to dryness in an oven set at 40 °C.

### 2.5. Acute Toxicity Test

Groups of mice of both sexes fasted for 12 hours prior to the test were administered the extract orally at a dose of 5000 mg/kg, while the control group received 10 ml/kg of 1% tween 20. Employing the Miller and Tainter Method (1944) [10], with a slight modification, doses of 10, 100, 200, 400, 800 and 1000 mg/kg extracts were administered intra-peritoneally to different groups of three animals each, to determine the $LD_{50}$ of the plant extract. The control group was given 1% tween 20 (10 ml/kg). All animals were closely observed for toxic symptoms for the first 2 hours and the possible mortality within 24-hour post administration was recorded. Surviving animals were observed for further 14 days post treatment for any signs of delayed toxicity. The $LD_{50}$ was estimated by the log dose-probit analysis method [11].

## 3. Antinociceptive studies

### 3.1. Mouse Writhing Test

Albino mice of either sex weighing between 20- 25 g were divided into six groups (n = 5). The first four received doses of extract 50, 100, 200, and 400 mg/kg, selected based on the $LD_{50}$, while the remaining two groups received 1% tween 20 and 100 mg/kg aspirin respectively, one hour before intraperitoneal injection of 0.1 ml of 0.6% v/v acetic acid solution per mouse. The number of writhes/stretches was counted for 30 minutes [12].

$$\% \text{ Inhibition } = \frac{\text{No of writhes control } - \text{ No of writhes test}}{\text{No of writhes control}} \times 100$$

### 3.2. Formalin-induced Paw Licking

Overnight-fasted mice were divided into five groups of five animals each, and were treated as follows: group 1 (1% tween 20, 10 ml/kg), group 2 (morphine 10 mg/kg), group 3 (50 mg/kg extract), group 4(100 mg/kg extract) and group 5 (200 mg/kg extract). All treatments were given orally. One hour later, the mice were injected with 0.02 ml of 1 % formalin subcutaneously into the right hind paw of the mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Response was measured for 5 minutes (first phase) after formalin injection and 15- 30 minutes (second phase) after formalin injection [13].

$$\% \text{ Inhibition} = \frac{\text{reaction time control } - \text{ reaction time test}}{\text{Seconds of paw licking control}} \text{x } 100$$

### 3.3. Hot Plate Test

The pre-reaction time to the thermal stimulus was recorded as the time interval from introduction of the animals to the plate maintained at 55± 0.5 °C until the first lick of the limbs or the first jump of the animals. The animals were initially screened and those that responded to heat stimulus within the cut-off time were randomly batched for study.

Each group was administered via oral route, the test agents as follows: group 1 (10 ml/kg 1% tween 20), group 2 (10 mg/kg morphine), group 3 (50 mg/kg extract), group 4 (100 mg/kg extract) and group 5 (200 mg/kg extract). The subsequent reactions of the animals were recorded at 30 minutes intervals for 150 minutes following the administration of all treatments [12].

$$\% \text{ inhibition } = \frac{(\text{Post} - \text{ treatment Latency}) - (\text{Pre} - \text{ treatment Latency})}{(\text{Cut} - \text{ off Time } - \text{ Pre} - \text{ treatment Latency})} \text{ x } 100$$

### 3.4. Haffner's Tail Clip Test

Mice that were used in this experiment were screened initially by applying a metal artery clip to the root of the tail to induce pain and animal that failed to attempt to dislodge the clip in 10 seconds were discarded. Eligible mice were divided and treated as before, using a post-treatment cut-off time or reaction time of 30 s [12]. Reaction time of each mouse was determined 60 minutes post treatment for oral administration.

$$(\%) \text{ inhibition } = \frac{(\text{Post} - \text{treatment Latency}) \, (\text{Pre} - \text{treatment Latency})}{(\text{cut} - \text{off time} - \text{pre} - \text{treatment latency})} \text{x} 100$$

### 3.5. Elucidation of Mechanism of Antinociceptive Activity of *E. prostrata*

Randomly batched animals were respectively pre-treated with naloxone (1 mg/kg), yohimbine (1mg/kg), ondasetron (0.2 mg/kg), atropine (2 mg/kg), and glibenclamide (2 mg/kg).

Fifteen minutes post treatment, the test drug at 200 mg/kg was administered to the group of animals. Then, one hour post treatment, 0.6% v/v acetic acid solution was injected intraperitoneally into each mouse. The number of writhes/ stretches was counted as before [14].

### 3.6. Data analysis and interpretation

Data was presented as mean ± standard error of mean (SEM). Test of statistical significance was carried out using a one-way and two-way ANOVA. P value less than 0.05 ($p<0.05$) was considered statistically significant.

### 3.7. Phytochemical Screening

Phytochemical screening of the crude leaf extract was carried out using standard procedures and tests to identify the phytoconstituents both qualitatively [15] and quantitatively. Total phenols, alkaloids, tannins and flavonoids [16-20].

### 3.8. Physicochemical Properties of Extract

The extract was green in colour, sticky and with a pungent smell. It was readily soluble in 1 % tween 20; pH 7.0.

### 3.9. Acute Toxicity

Doses of 10 and 100 mg/kg produced zero percent mortality, while 200 g/kg recorded 33.3 %. Higher doses caused 100 percent deaths in the treated animals. $LD_{50}$ was extrapolated as 331 mg/kg. The hydroethanolic leaves extract of *Eclipta prostrata* was observed to cause moderate behavioural and sensory changes such as tachypnea, sedation, abdominal writhes, and itching.

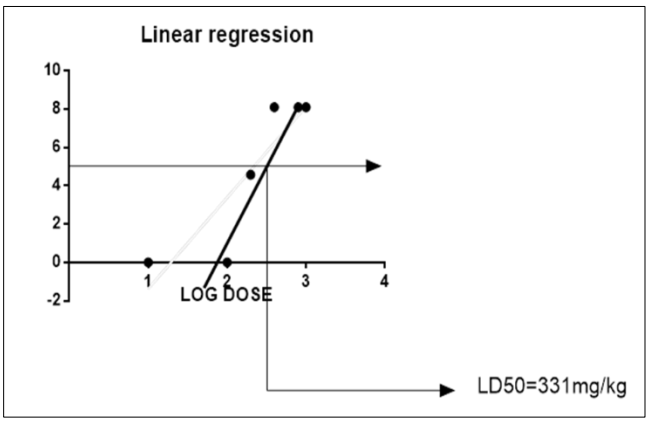


**Figure 1** $LD_{50}$ of *Eclipta prostrata*

### 3.10. Acetic Acid-induced Writhing

The aqueous extract of *Eclipta prostrata (*50, 100, 200 mg/kg,) produced a significant dose-dependent ($p<0.05$) reduction in the number of writhes, when compared with the control (Figure 2). Percentage inhibition by 200 mg/kg, 100 mg/kg, 50 mg/kg of extract were 64.28 %, 58.03 %, 42.85 % respectively, with ASA recording 71.96 %.

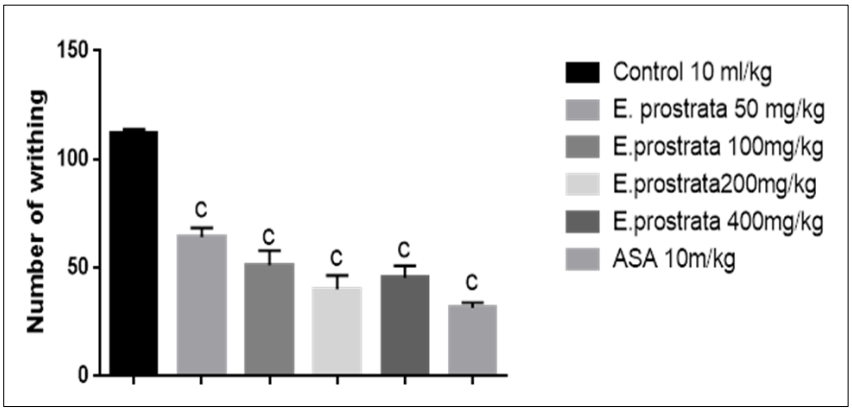


**Figure 2** Effect of *E. prostrata* on acetic acid-induced mouse writhing test. Data represented as mean ±S.E.M. (n= 5). $^{c}p<0.05$ statistically significant compared to control (one way ANOVA followed by Dunnett's multiple comparison test)

## 4. Formalin Test

*Eclipta prostrata* at all dose levels produced a significant dose-dependent ($p<0.05$) effect in both the phases. The highest percentage inhibition of phase one was 52.53% observed with 200 mg/kg, while 100 mg/kg produced 81.70% in the second phase. The standard drug, morphine produced an inhibition of 100% and 100% in the first and second phase respectively.

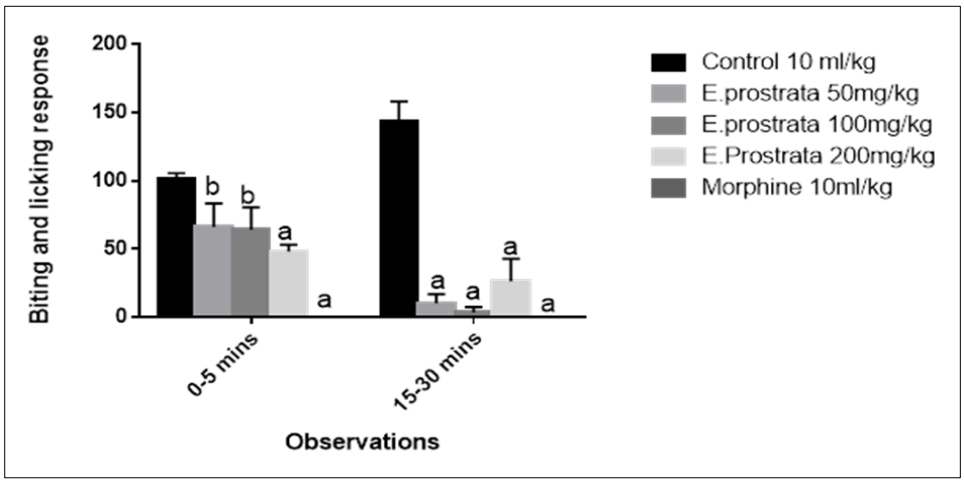


**Figure 3** Effect of *E. prostrata* on formalin test. Data represented as mean ±S.E.M. (n= 5). $^{c}p<0.05$ statistically significant compared to control (one way ANOVA followed by Dunnett's multiple comparison test)

### 4.1. Hot Plate Test

The extract of *Eclipta prostrata* produced a dose-dependent, but very mild action compared with the control (Table 1) and none of the dose levels of the extract showed any comparable effect with morphine, the standard drug used.

**Table 1** Effect of *E. prostrata* on Thermal Nociception

| | | **Reaction time (secs)** | | | | |
|---|---|---|---|---|---|---|
| | | **Pre-treatment** | **Post treatments** | | | |
| Treatments | Dose (mg/kg) | | **60 minutes** | **90 minutes** | **120 minutes** | **150 minutes** |
| Control (TW 20) | 10 ml/kg | 2.08±0.58 | 1.94±0.31 | 1.89±0.34 | 1.74±0.33 | 1.29±0.16 |
| *Eclipta prostrata* | 50 | 1.54±0.17 | 5.00±0.70$^{a,b}$ | 4.00±0.31$^{b}$ | 2.60±0.40$^{b}$ | 5.40±0.24$^{a,b}$ |
| | | Inhibition% | 12.15 | 8.64 | 3.72 | 13.56 |
| | 100 | 1.58±0.11 | 3.80±0.37$^{b}$ | 3.40±0.24$^{b}$ | 4.40±0.81$^{b}$ | 5.80±0.37$^{a,b}$ |
| | | Inhibition% | 7.81 | 6.40 | 9.92 | 14.84 |
| | 200 | 1.80±0.20 | 3.60±0.24$^{b}$ | 4.40±0.67$^{b}$ | 6.80±0.73$^{a,b}$ | 3.00±0.31$^{b}$ |
| | | Inhibition% | 6.38 | 9.21 | 17.73 | 6.91 |
| Morphine | 10 | 3.83±0.39 | 30.00±0.00$^{a}$ | 25.95±1.69$^{a}$ | 22.50±1.21$^{a}$ | 17.51±2.01$^{a}$ |
| | | Inhibition% | 100 | 84.54 | 71.29 | 52.30 |

TW 20: 1% tween 20±S.E M: Standard Error of Mean $^{a}p< 0.05$ statistically significant compared to control, $^{b}p< 0.01$ statistically significant compared to morphine (2 Way ANOVA followed by Turkey's multiple comparison tests).

### 4.2. Tail Clip Test

Use of the metal artery clip to the tail of animals in the control group showed reactions towards clip removal with the post-treatment latency being 10.1 ± 2.5s, 8.51 ± 4.38s, 4.00 ± 2.04 s and 8.12 ± 5.54 s measured at 60 minutes, 90

minutes, 120 minutes and 150 minutes respectively with a pre-treatment latency of 2.03 ± 1.01s. The percentage inhibition at 50 mg/kg are 7.54%, 17.80%, 17.09%, 13.83%. For 100 mg/kg, 2.47%, 6.83%, 13.98%, 2.75%. For 200 mg/kg, 3.9%, 14.59%, 16.3%, 28.41%. Morphine 10 mg/kg produced a peak inhibition (100 %) at 90 minutes post treatment. (Table 2).

**Table 2** Effect of Hydroethanolic Leaf Extract of *E. prostrata* on Tail Clip Test

| | | Post treatments Reaction time (secs) | | | | |
|---|---|---|---|---|---|---|
| **Treatments** | **Dose (mg/kg)** | | **60 minutes** | **90 minutes** | **120 minutes** | **150 minutes** |
| Control (TW 20) | 10 ml/kg | 2.30±1.01 | 10.10±2.55 | 8.51±4.38 | 4.00±2.04 | 8.12±5.54 |
| *Eclipta prostrata* | 50 | 1.47±6.08 | 3.62±1.56[b] | 6.55±4.11[b] | 6.35±2.04[b] | 5.42±2.67[b] |
| | | Inhibition% | 7.54 | 17.80 | 17.09 | 13.83 |
| | 100 | 1.51±0.24 | 2.22±0.36[b] | 3.44±1.78[b] | 5.50±1.79[b] | 2.30±0.51[b] |
| | | Inhibition% | 2.47 | 6.83 | 13.98 | 2.75 |
| | 200 | 1.44±0.05 | 2.57±0.15[b] | 5.61±2.19[b] | 6.10±1.96[b] | 5.69±1.94[b] |
| | | Inhibition% | 3.9 | 14.59 | 16.30 | 28.41 |
| Morphine | 10 | 2.21±0.05 | 27.08±1.64[a] | 30.00±0.00[a] | 26.74±1.36[a] | 23.12±1.31[a] |
| | | Inhibition% | 88.12 | 100 | 88.26 | 75.24 |

TW 20: 1% tween 20±S.E M: Standard Error of Mean [a]$p < 0.05$ statistically significant compared to control, [b]$p < 0.01$ statistically significant compared to morphine (2 Way ANOVA followed by Turkey's multiple comparison tests).

### 4.3. Mechanism of Action

The effect of the extract was reversed by glibenclamide and atropine. There was a significant increase in number of writhings when compared with the initial number recorded with the extract alone. Naloxone, yohimbine, and ondasetron did not affect the anti-nociceptive activity of the plant extract.

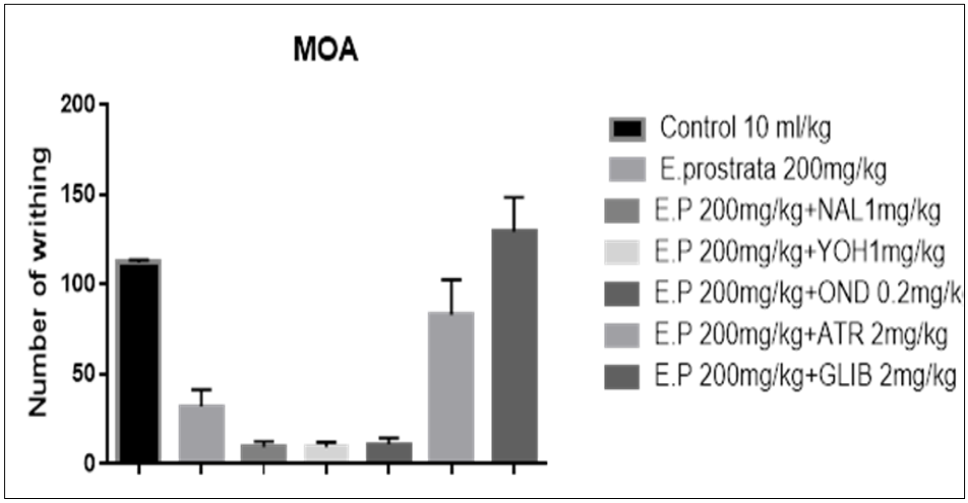


**Figure 4** Effect of various antagonists super-imposed with *E. prostrata* on acetic acid- induced mouse writhing test. Data represented as mean ±S.E.M. (n= 5)

**Table 4** Phytochemical Analysis Hydroethanolic Leaf Extract of *E. prostrate*

| Chemical constituent | Inference |
|---|---|
| Phenol | + |
| Tannin | - |
| Terpernoid | - |
| Steroid | + |
| Alkaloid | + |
| Anthraquinone | - |
| Cardiac glycoside | + |
| Phlobatanin | + |
| Flavonoid | + |
| Saponin | - |

**+ =** Present - =Absent

**Table 5** Quantitative Screening of *E. prostrate*

| Chemical Compounds | Quantity (mg/100g) |
|---|---|
| Phenol | 4.53 |
| Flavonoid | 11.76 |
| Alkaloid | 25.40 |

## 5. Discussion

Pain, the most common symptom of a disease is a protective mechanism by which the body responds to harmful stimulus. It is a subjective sensory and emotional experience, connected to the stimulus that it invokes, and is also based on the observation of psychological interpretation of the phenomena taking place. The feeling of pain (nociception) can be caused by irritation of pain receptors, which are located in the skin, joints, and on the internal organs. Nociceptive pain is the most common type of pain. Pain could also be neuropathic when there is a damage to, or dysfunction of the nervous system. The other type of pain that is noteworthy is psychogenic pain, caused by psychological factors such as anxiety and depression. Tissue damage is not causative in its case [21].

Antinociceptive activity of *E. prostrata* hydroethanol leaf extract was investigated by peripheral/non-narcotic models of nociception such as acetic acid writhing test useful for the evaluation of mild analgesic and peripherally acting drugs like aspirin (ASA), which have been reported to exhibit analgesic activity in the writhing test only. The mouse writhing test induced by acetic acid involves stimulation of the peripheral receptor system; the prolonged irritation of the peritoneal cavity by acetic acid results in increase in the levels of peritoneal fluid. The analgesic effect demonstrated by hydroethanolic leaf extract of *Eclipta prostrata* in the mouse writhing test in this study suggests a peripheral mechanism of action involving direct action on nociceptors, which could possibly be via direct inhibition of prostaglandin action or an indirect action mediated through inhibition of prostaglandin synthesis by inhibiting cyclo-oxygenase (COX) activity. The effectiveness of the extract in this test indicates its potential usefulness in the treatment of acute pain as the abdominal constriction test is said to be indicative of acute pain.

The formalin test is a more satisfactory model of clinical pain, useful for screening novel substances, as it encompasses inflammatory, neurogenic and central mechanisms of nociception [22]. It produces distinct biphasic nociceptive response; the first transient phase (early phase 0-5minutes) is caused by the direct effect of formalin on sensory C-fibers, and scores neurogenic nociception, while the second prolonged phase (late phase 15-30 minutes) which is associated with the development of the injury-induced spinal sensation scores inflammatory nociception [23, 24].

Results of the present model showed that the hydroethanolic leaf extract of *Eclipta prostrata* inhibit both the early and the late phases of formalin- induced pain, thus suggesting its central and peripheral anti-nociceptive actions. However, from results obtained there was greater inhibition at the late phase than the early phase and this effect was comparable to that produced by the standard drug (morphine). In agreement with this, morphine was effective in both phases of the formalin test. Data from the formalin test suggests that the hydroethanol leaf extract *of Eclipta prostrata* acts through peripheral mechanism as shown in the mouse writhing test and also via central mechanism because drugs which act mainly centrally, example is morphine which is a narcotic analgesic inhibit both early and late phase of the formalin test [12, 14] as demonstrated by the extract.

To further elucidate the possible antinociceptive potential of the herbal drug, central/narcotic models such as hot plate and tail-flick tests were also employed. Findings revealed an involvement of both μ-opioid and κ-opioid receptors. The use of different antagonists, with the aim of proposing the mechanism of antinociceptive activity of the herbal drug showed the latter to act via cholinergic pathway and potassium channel.

Potassium ion channels are widely distributed in neurons [25], and their opening causes repolarization or even hyperpolarization, which limits generation of action potential or rate of firing. The anti-nociceptive and analgesic effects mediated by cholinomimetics as well as anticholinesterases have been well documented [26; 27].

Cardiac glycosides and flavonoids were among the phytoconstituents found in the plant extract. It is projected that one or a combination of the phytochemical constituents may be responsible for the analgesic activity observed with extract. Analgesic activity of alkaloids has been demonstrated by morphine and its derivatives. Several reports have shown the analgesic properties of flavonoids, tannins and other polyphenolic compounds in different experimental animal models [28].

In the acute toxicity test carried out for the oral route of administration of the hydroethanolic leaf extract of *Eclipta prostrata,* no mortality was observed with the 5000 mg/kg, which records its relative safety. However, the intraperitoneal route recorded a median lethal dose ($LD_{50}$) of 331.5 mg/kg.

## 6. Conclusion

The study demonstrated that the hydroethanolic leaf extract of *Eclipta prostrata* possessess antinociceptive activity, mediated through peripheral and central pathways, using standard *in-vivo* analgesic models. Its peripheral activity compared well with conventional analgesics. The herbal drug's activity was antagonized by glibenclamide and atropine, which suggests that its antinociceptive effect is mediated via potassium ion channel and cholinergic receptor.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that there are no conflicts of interest.

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