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RESEARCH ARTICLE

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Anti-nociceptive activities of the ethanolic stem bark extract of *Cordia Africana* (Boraginaceae) in rats and mice

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ABSTRACT

Cordia africana (Boraginaceae), a widely known shade tree, is used in Northern Nigeria and East Africa for management of piles, headache and fatigue. In this study, the ethanolic stem bark extract of Cordia africana was screened for acute toxicity and analgesic activities. Acute toxicity profile of the plant extract was studied, via intraperitoneal and oral routes, in rats and mice using Lorke's method while analgesic activities were investigated using formalin, acetic acid and thermal-induced pain models. Results of acute toxicity study revealed LD_{50} values of greater than 5 g/kg ($LD_{50} \geq 5$ g/kg) for oral route and greater than 1 g/kg ($LD_{50} \geq 1$ g/kg) for intraperitoneal route. In formalin-induced pain model, the extract significantly ($P < 0.05$) reduced pain in the second phase of the test at 3.2 g/kg and 4.8 g/kg oral doses while, in acetic acid-induced abdominal writhings and hot plate-induced pain models, the extract at oral doses of 1.6 g/kg and 3.2 g/kg body weight significantly ($P < 0.05$) reduced abdominal writhes and pain respectively. These results revealed that ethanolic stem bark extract of Cordia Africana is relatively orally non-toxic and only slightly toxic via intraperitoneal route. In addition, it possesses significant analgesic activity at 1.6 g/kg, 3.2 g/kg and 4.8 g/kg oral doses. These findings support the ethnomedical use of Cordia africana stem bark in the management of pain associated with medical conditions such as plies in Northern Nigeria and East Africa.

Keywords: Pain, Acute toxicity, Analgesia, Piles, Inflammation.

INTRODUCTION

In the developing countries many people rely on traditional healing practices and medicinal plants for their daily health care needs. In some developing (Asian and African) countries, 80% of their population depends on traditional medicine for primary health care. This is due to the fact that traditional medicine is a more affordable and accessible health care system when compared to modern medicine [1].

Cordia africana (family: Boraginaceae) is a small-to-medium sized tree widely distributed throughout Africa where it is commonly planted as a shade or roadside tree and also used for wood or demarcation. In Northern Nigeria, the Stem bark powder is used traditionally to treat pain and inflammation associated with piles (Mal. Garba L. personal communication, 4th January, 2013) while in East Africa it is used for general healing of open wound, treatment of schistosomiasis, skin troubles and jaundice, and as stimulating tonic for fatigue, pain, headache and exhaustion [2].

Due to the limitations of opioid and NSAID drugs used in the management of pain, there is need for continuous search for new and better analgesics. In view of this limitation and the fact that claims of usefulness of *Cordia africana* in traditional management of some painful conditions have not been scientifically evaluated, this current study is aimed at investigating the acute toxicity and anti-nociceptive activities of the ethanolic stem bark extract of *Cordia africana* in mice and rats.

MATERIALS AND METHODS

2.1 Collection of plant material

The stem bark of *Cordia africana Lam.* was collected from Malali Village, Igabi L.G.A. Kaduna-Nigeria. The plant was identified and authenticated at the herbarium section in the Department of Biological Sciences, Ahmadu Bello University (A.B.U), Zaria-Nigeria, where it was compared with an existing voucher specimen number, 14666. The plant material was then dried under shade until constant weight was obtained before it was crushed into coarse powder using wooden pestle and mortar.

2.2 Preparation of extract

1.5kg of the powdered plant was extracted with 2 L of N-hexane. The marc (N-hexane residue) was extracted with 3 L of aqueous ethanol (70/30% v/v) for 72 hours at room temperature using a percolator. The solvent was evaporated over a water bath at temperature 45 degree Celsius (45℃). The extract was then stored in a desicator and only prepared freshly for each study.

2.3 Animals

Adult Wistar rats and albino mice of both sexes weighing 150-200 g and 18-25 g respectively were obtained from the Animal House of Department of Pharmacology and Therapeutics, A.B.U Zaria, after obtaining animal ethical committee approval (ABU/AEC/Pharm-Sc/7610). The animals were maintained on standard animal feed and tap water *ad libitum* in well-ventilated plastic cages under natural light/dark cycle and allowed to acclimatize to the laboratory environment for a period of 24 hrs before the commencement of each experiment.

2.4 Drugs and Chemicals

The following drugs and chemicals of analytical grade were used: ethanol, N-hexane, acetylsalicylic acid (M&B), tramadol (Interbact), Acetic acid (Sigma Aldrich USA), Morphine (Sigma Aldrich USA).

2.5 Equipment's

Hot plate, UV spectrophotometer, electronic weighing balance, stop watch, needles and syringes, spatula, animal cages, pestle and mortar, test tubes, beakers, digital calliper, gloves, cotton wool, labelling tape, red and blue markers.

2.6 Phytochemical screening

Standard tests as described by Evans [3] were employed in screening the ethanolic stem bark extract of *Cordia africana* for phytochemicals.

2.7 Acute toxicity studies in rats and mice (LD_{50}).

Testing for median lethal dose (LD_{50}) was conducted in both rats and mice via oral and intra-peritoneal routes as described by Lorke [4]. The method was divided into two phases: In the first phase, for both routes, 3 groups of animals (n = 3) received the ethanolic stem bark extract at doses of 10, 100 and 1000 mg/kg body weight, respectively and signs of toxicity and death was observed for 24 hours. In the second phase: for the oral route, 3 groups of animals (n = 1) received 1,600 mg/kg, 2,900mg/kg and 5000 mg/kg doses of the extract respectively. For the intraperitoneal route in rats, four groups of animals (n = 1) received 200 mg/kg, 400 mg/kg, 800 mg/kg and 1600 mg/kg doses while in mice, the groups received 140 mg/kg, 225 mg/kg, 370 mg/kg and 600 mg/kg doses respectively. The LD_{50} was determined by calculating the geometric mean of the lowest lethal dose and highest non-lethal dose (1/1 and 0/1) as shown below:

$$\mathbf{LD50} = \sqrt{\boldsymbol{highest\ non\ lethal\ dose} \times \boldsymbol{lowest\ lethal\ dose}}$$

2.8 Formalin induced pain model in rats

The method described by Dubuisson and Dennis [5] was adopted. 30 rats were divided into five groups (n = 6) and treated orally with normal saline (1 ml/kg), three different doses of the extract (1.6 g/kg, 3.2 g/kg and 4.8 g/kg) and morphine (5 mg/kg), respectively. Thirty minutes later, 50µl of a freshly prepared 2.5% solution of formalin was injected subcutaneously under the plantar surface of the left hind paw of each rat. The rats were monitored for one hour and the severity of pain was recorded for each rat based on the following scale: **0**: rat walked or stood firmly on the injected paw; **1**: the injected paw was favoured or partially elevated; **2**: the injected paw was clearly lifted off the floor; **3**: the rat licked, chewed or shook the injected paw. The anti-nociceptive effect was determined in two phases: the early phase (phase I) was recorded during the first 5minutes after formalin administration, while the late phase (phase II) was recorded during the last 45 minutes with 10 minutes lag period in-between both phases.

2.9 Acetic acid-induced abdominal writhes model in mice

The method described by Koster *et al.*, [6] was adopted. 24 Swiss albino mice were divided into 4 groups of 6 animals each. Group 1 received normal saline (10 ml/kg) orally, group 2 and 3 received the extract at doses of 1.6 and 3.2 g/kg orally respectively while group 4 received acetylsalicylic acid (ASA) 100 mg/kg orally. Sixty minutes later, the mice were treated with 0.6% acetic acid intraperitoneally. After 5 minutes (latency) following acetic acid administration, the mice were observed for number of abdominal writhes for a period of 10 minutes. The percentage inhibition of writhes was calculated using the formula below.

$$\textbf{Percentage Inhibition} = \frac{\textbf{MeanNo.ofwrithes (control)} - \textbf{MeanNo.ofwrithestest}}{\textbf{MeanNo.ofwrithescontrol.}} \times \mathbf{100}$$

2.10 Hot plate-induced pain model in mice

With slight modification, method described by Woolfe and Macdonald [7] was used. Using a reaction time of 2-3 seconds, 24 pre-screened mice were divided into 4 groups (n = 6). Group 1 received oral normal saline 10 ml/kg body weight, group 2 and 3 received 1.6 and 3.2 g/kg of the ethanolic stem bark extract orally, respectively while group 4 received oral tramadol (10 mg/kg). The reaction time which is the time it takes the animal on hot plate to lick its paw or jump out was recorded at 0, 30, 60, 90 and 120 minutes following treatments. Throughout the test, temperature of hot plate was maintained at 55 ± 2℃ and a cut-off time of 20 seconds was set to prevent injury to the animal. The percentage (%) analgesia was calculated thus.

$$\textbf{Increase in response latency (\%)} = \frac{\textbf{latency test - latency control}}{\textbf{Latency control.}} \times \mathbf{100}$$

2.11 Statistical Analysis

All results obtained were expressed as mean ± SEM and analysed by analysis of variance using SPSS software, version 19. Results obtained from thermally-induced pain and acetic acid-induced abdominal writhing models were analysed using one-way ANOVA and Tukey's post hoc test while results obtained from formalin induced pain model were analysed using Kruskal-Walis and Mann Whitney's test. At $p < 0.05$, differences in means were considered significant. Results were also presented and analysed in the form of tables and graphs.

RESULTS

3.1 Preliminary phytochemical screening

Phytochemical screening of the extract revealed the presence of alkaloids, tannins, flavonoids and triterpenes (Table 1).

3.2 Median Lethal Dose, LD_{50}

The oral median lethal dose (LD_{50}) of the ethanolic stem bark extract of *Cordia africana* Lam. was found to be greater than 5000 mg/kg in both rats and mice. But via intraperitoneal route, the median lethal dose of was found to be 1265 mg/kg and 1131 mg/kg for rats and mice respectively (Table 2).

3.3 Formalin-induced Pain in Rats

In the first phase (phase I), the ethanolic stem bark extract of *Cordia africana* did not show any statistically significant reduction in pain at all oral doses when compared to both controls (normal saline and morphine). In the second phase, 3.2 g/kg and 4.8 g/kg oral doses showed significant ($P < 0.05$) reduction in pain when compared to normal saline group. However, morphine (5 mg/kg) inhibited pain significantly ($p < 0.05$) in both phases (Figure 1).

3.4 Acetic Acid-induced Writhes in Mice

The number of writhes induced by acetic acid was significantly ($p < 0.05$) reduced by oral administration of the ethanolic stem bark extract of *Cordia africana* at doses of 1.6 g/kg and 3.2 g/kgwhen compared to negativecontrol (normal saline, 10 ml/kg *p.o.*). The extract at oral doses of 1.6 and 3.2 g/kg showed 31.1% and 48.5% maximum writhes inhibition respectively as compared to oral aspirin which showed 57.3% maximum writhes inhibition (Figure 2).

3.5 Hot Plate-induced Pain in Mice

The ethanolic stem bark extract of *Cordia africana* at 1.6 and 3.2 g/kg oral doses significantly ($p < 0.05$) increased the reaction (latency) period to pain induced by thermal stimulus using hot plate 120 minutes after treatments. Tramadol (10 mg/kg) showed significant increase in pain reaction (latency) time 30-120 minutes after treatment (Figure3).

Table 1: Preliminary Phytochemical Screening Result of Ethanolic Stem Bark Extract of *Cordia africana* Lam.

Test	Constituents	Results
Dragendoff's, Meyer's and Wagner's	Alkaloids	+
Shinoda's, Dragendoff's and NaOH	Flavonoids	+
Frothing/Haemolysis	Saponins	+
LibermanBuchard	Triterpenes	+
Molisch and Fehling's A&B	Carbohydrates	+
Borntrager's Test	Anthraquinone	-
Lead acetate/Ferric Chloride	Tannins	-

+ = present, - = absent.

Table 2: Median Lethal Dose (LD_{50}) Values of Ethanolic Stem Bark Extract of *Cordia africana*Lamin Rats and Mice.

Route of Administration	Animal Species	LD_{50} Values (mg/kg)
Oral	Rats & Mice	>5000
i.p.	Rats	1265
i.p.	Mice	1131

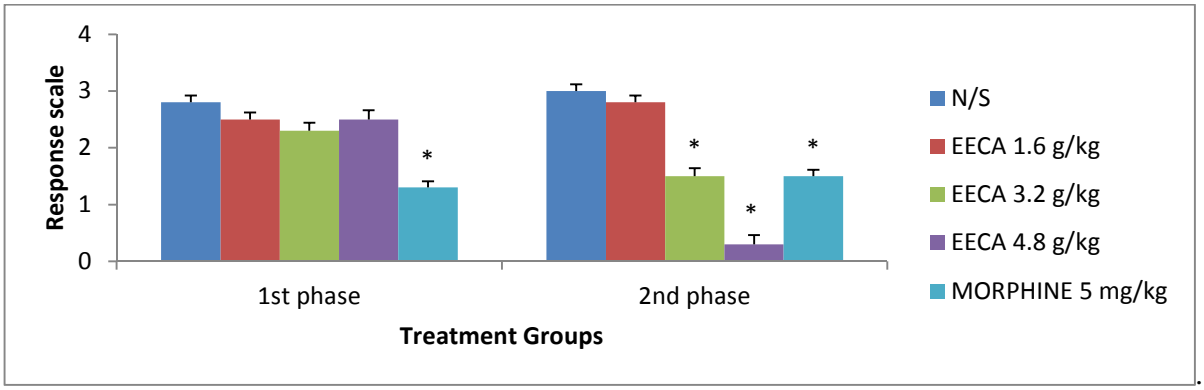

Figure 1: Effects of oral administration of ethanolic stem bark extract of *Cordiaafricana* Lam. and morphine on formalin-induced pain in rats.

*Each value represents Mean ±SEM for six rats (n=6). *P < 0.05 significant compared to negative control (normal saline) using one-way ANOVA. EECA = ethanolic stem bark extract of Cordia africana. N/S = normal saline.*

Figure 2: Effects of oral administration of ethanolic stem bark extract of *Cordia africanaLam*. on number of acetic acid-induced abdominal writhes in mice.

*Each value represent Mean ±SEM (n=6). *P < 0.05 when compared to group that received normal saline using one-way ANOVA. EECA = ethanolic stem bark extract of Cordia africana. N/S = normal saline.*

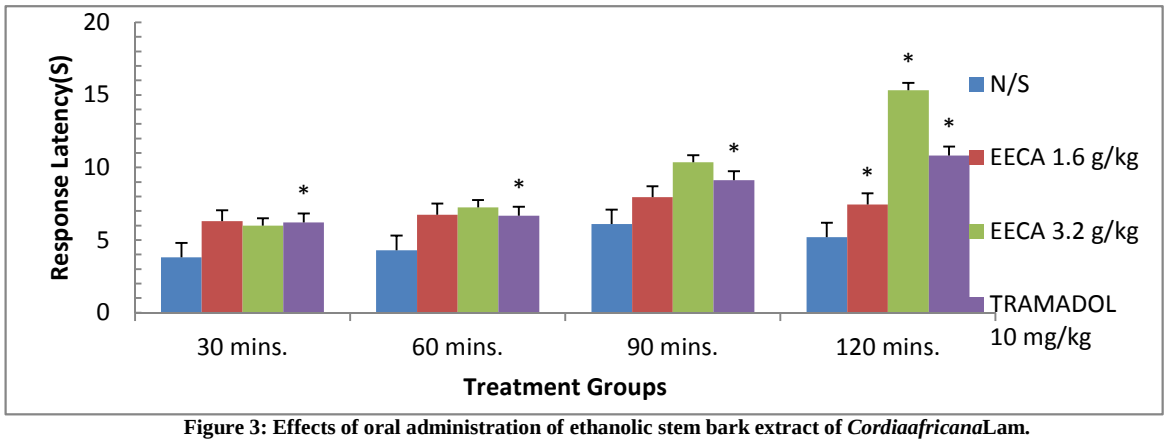

Figure 3: Effects of oral administration of ethanolic stem bark extract of *Cordiaafricana*Lam. and tramadol on pain response time (latency) in mice on hot plate.

*Each value represent Mean ±SEM (n = 6). *P < 0.05 compared to negative control group (normal saline) using one-way ANOVA. EECA = ethanolic stem bark extract of Cordia africana, N/S = normal saline.*

DISCUSSION

4.1 Discussion

Phytochemicals such as alkaloids, saponins, glycosides and tannins have been shown to be bioactive and possess desirable pharmacologic activities such as anti-nociceptive, anti-diabetic, anti-inflammatory and anti-oxidant activities [8–14]. Thus, owing to the phytochemical constituents detected in the ethanolic crude extract, *Cordia africana* bark may serve as valuable starting materials for drug development.

Investigation into the acute toxicity profile of the EECA, using Lorke's method [4] revealed LD_{50} values of greater than 5000 mg/kg in both rats and mice respectively via oral route, and 1265 mg/kg and 1131 mg/kg in rats and mice respectively via intraperitoneal route. According to Lorke's postulate, lethal doses of substances are classified according to their LD_{50} values: for $LD_{50} \geq$ 1mg/kg, substance is highly toxic; $LD_{50} \geq$ 5mg/kg, substance is toxic; $LD_{50} \geq$ 100 mg/kg, substance is moderately toxic; $LD_{50} \geq$ 1000 mg/kg, substance is slightly toxic; and $LD_{50} \geq$ 5000 mg/kg, substance is non-toxic. Therefore, ethanolic crude extract of *Cordia africana* bark may be said to be orally non-toxic and slightly toxic when administered through intraperitoneal route. This result could be a logical explanation as to the history of safety of the crude plant extract in traditional medicine [2].

Preliminary pilot study revealed a minimum effective oral dose of the extract as 1.6 g/kg body weight. This, coupled with the LD_{50} values (for safety reasons) of the plant, informed the choice of 1.6, 3.2 g/kg and 4.8 g/kg body weight oral doses used for the investigation.

Acetic acid-induced abdominal writhings model in mice, formalin induced pain model in rats and hot plate induced pain model in mice were primarily employed to investigate whether anti-nociceptive effects of extract, where present, is as a result of central or peripheral mechanism [15]. Central pain is known to be caused by a direct stimulation of nociceptors whereas peripheral pain is as a result of release of pain mediators such as histamine, serotonin and prostaglandins, which result in inflammation and subsequent stimulation of pain receptors [16-18].

Formalin induced pain model is employed to screen drugs for both central and peripheral anti-nociceptive activities. It consists of two phases of nociceptive response termed early phase (phase I) and late phase (phase II). In phase I (0-5minutes after drug administration), induced pain is as a result of stimulation of nociceptive receptors in the paw depicting neurogenic pain caused by the direct effect of formalin on the sensory C fibres. In phase II (15-60minutes after drug administration), induced pain is as a result inflammatory response and the release of nociceptive mediators such as serotonin, histamine, bradykinin and prostaglandin [5]. It is well known that drugs such as morphine that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs such as NSAIDs inhibit the late phase [19]. Using this model, results obtained showed that the extract significantly ($p < 0.05$) reduced pain in the second phase (phase II) at oral doses of 3.2 g/kg and 4.8 g/kg while morphine (5 mg/kg) significantly ($p < 0.05$) reduced pain in both phases (phase I & II). The ability of the extract to significantly ($p < 0.05$) inhibit pain in the second phase shows that it may possess the ability to manage peripheral pain due to inflammation caused by release of inflammatory mediators such as serotonin, histamine, bradykinin and prostaglandin. Morphine's ability to inhibit both phases of formalin induced shows it has the ability to control both centrally and peripherally mediated pain.

Acetic acid induced abdominal writhing model in mice is a visceral pain model employed as a screening tool for the assessment of anti-nociceptive activity of new analgesic agents for peripheral nociception [15]. This method is simple and reliable and affords rapid evaluation of peripheral type of analgesic action with the ability to detect dose levels that may appear inactive in other methods like the tail-flick test [20, 21]. In this model, animals respond with characteristic stretching behaviour called writhings as a result of an increase in levels of cyclo-oxygenase (COX) and lipo-oxygenase (LOX) products such as PGI_2, $PGF_{2\alpha}$ and leukotrienes as well as the release of many inflammatory mediators such as bradykinin, substance P, TNF-α, IL-1_β andIL-8 in the peritoneal fluid caused by acetic acid. This consequently excites the primary afferent nociceptors entering dorsal horn of the central nervous system [22]. The reduction in abdominal writhes caused by ethanolic stem bark extract of *Cordia africana* at oral doses of 1.6 g/kg and 3.2 g/kg showed the extract possess analgesic activity against peripheral pain when compared to normal saline. However, compared to aspirin (57.3% inhibition), the extract (48.5% inhibition) did not show any better inhibition in the number of acetic acid induced abdominal writhings. Therefore, in-line with results obtained, the extract possesses anti-nociceptive property against peripherally mediated pain but to a lesser extent when compared to a prototype NSAID such as aspirin.

Hot plate-induced pain model, as originally described by Woolfe and MacDonald [7], is suitable for the evaluation of centrally acting anti-nociceptive agents [23]. The validity of this model has been shown even in the presence of substantial impairment of motor performance [24]. Oral doses of 1.6 g/kg and 3.2 g/kg of ethanolic stem bark extract of *Cordia Africana* showed significant ($p < 0.05$) increase in pain response time (latency) at 120minutes after treatment. Therefore, the extract significantly reduce centrally controlled pain two hours after oral administration and can be said to possess a weak central analgesic activity as compared to the standard reference drug, tramadol, which showed significant increase in pain response time all through the study. The delay in the onset of action of the extract can possibly be explained by understanding the pharmacokinetic properties of the extract.

CONCLUSION

Results obtained from this research work revealed that the ethanolic stem bark extract of *Cordia africana* is relatively non-toxic at high oral doses and possess strong peripheral analgesic properties in laboratory animals. These findings support the ethnomedical use of *Cordia africana* bark for the relief of pain associated with haemorrhoids and other medical conditions in Northern Nigeria and East Africa.

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