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ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF AQUEOUS LEAFEXTRACT OF*COMBRETUM MICRANTHUM*G. DON (*COMBRETACEAE*)

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ABSTRACT
The analgesic and anti-inflammatory effects of the aqueous leaf extract of Combretum micranthum were studied in mice and rats. The extract was screened for analgesic activity; using acetic acid induced writhing in mice and formalin induced paw licking test in rats. Anti-inflammatory effect was evaluated using formalin induced hind paw oedema in rats. Results showed that, at a dose of 200 mg/kg the extract significantly ($p < 0.05$) reduced the number of abdominal constrictions in mice and at doses of 100 and 200 mg/kg, the extract significantly ($p < 0.05$) reduced the licking time in rats in the formalin induced paw licking test. The extract at doses of 50,100 and 200 mg/kg significantly ($p < 0.05$) reduced hind paw oedema in rats from the first hour of formalin administration. The intraperitoneal LD_{50} value of the extract was found to be 2,154.1mg/kgin mice and 2,852.l mg/kg in rats. The analgesic and anti-inflammatory activities of the plant extract may probably be due to the presence of phytochemical contents.
Keywords: Combrentum micranthum, Analgesic, Anti-inflammatory, Mice, Rats.

INTRODUCTION

The practices of traditional medicine are based on beliefs that have been in existence for hundreds of years, but still prevalent today and people of all continents have this old tradition(Selby, 1998).The earliest use of plants for medicinal uses probably began as far back as 300BC, during this periodthe early Greek naturalist described plant and classified them by use as herbs, shrubs and trees (Klein, 1979).However, since ancient time, man was able to know that, some fruits, stem, and leaves of many plants can be used to cure some diseases including treatment of wounds as done by our rural folks (Sofowora, 1982). The knowledge that plants can cure diseases is probably instinctive because, even animals seek out appropriate herbs when they are ill. Herbalists used leaves, flowers, stems and roots of plant to prevent, relieve and treat illnesses.

Combretum micranthum of the Family *Combretaceae* is widely distributed in savannah regions and in some places near the coast as a shrub or a tree and its may grow up to 10 m in length, with opposite, ovate and acuminate leaves, the flowers are borne as auxiliary cluster on scaly stalks and the fruits are small with scaly and four winged (Burkill, 1985).*Combretum micranthum* is locally known as *farargeza* (Hausa), *Okan* (Yoruba) and *Nzaotego* (Igbo) (Burkill, 1985).Verbal discussion with herbalist revealed that, leaf of *C. micranthum* is used in the management of bleeding, fever, pain and tumor. The ashes of burnt wood of *C. micranthum* are used in Northern Nigeria, as a dehairing agent in preparation of skin for tanning (Burkill, 1985). It was also found that, *C. micranthum* plant contains potent anti-microbial constituents (Sofowora, 1993). Studies have shown *Combretum micranthum*to possess strong antimalarial activity against both chloroquine sensitive and resistant strain of *Plasmodium falciparium* (Kola and Benjamin, 2002).The methanol leaves extract of *C. glutinosum* was studied for anti-inflammatory activity in rats (Abdul-Fattah *et al.,* 2000). Tannins, flavones and amines were also reported (Burkill, 1985).*Combretummicranthum*is shown to contain tannins, carbohydrates, saponins, alkaloids, and flavonoids but other *Combretum* species are reported to contain anthraquinones, terpenoids and sterols (Trease and Evans, 1997).Kola and Benjamin (2002) reported that, the methanol leaves extract of *C.micranthum* contain alkaloids. Generally *Combretaceae* family is rich in tannins, saponins, sterols, carbohydrates, glycoside and trace of alkaloid (Trease and Evans, 1996). The objective of this study is to establish analgesic and anti-inflammatory activity of the aqueous leaf extract of *Combretum micranthum* in mice and rats.

MATERIALS AND METHODS

Plant collection and extraction

Fresh leaves of *Combretummicranthum* was collected from Malumfashi L.G.A, Katsina State. Plant was identified and authenticated in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, by comparing with voucher specimen number 900257. Leaves were air dried under the shade at room temperature (30^{o}C) for 28 days and then grounded into a fine powder using pestle and mortar. About 700 g of powdered material was soaked in water for two weeks and maceration method was used in the extraction. The extract was concentrated on water bath at temperature of 60^{o}C.

Animals
Mice (weighing 25 – 30 g) and rats (weighing150 – 180g) of either sex were used for the experiments. Animals were obtained from animal House of Faculty of Pharmaceutical sciences, Ahmadu Bello University Zaria. Animals were kept in a well-ventilated room, fed with a pelletized grower mash (vital) and water provided *ad-libitum*.

Acute toxicity study
The intraperitoneal median lethal dose (LD_{50}) determination was conducted using the method of Lorke (1983).

Acetic acid induced writhing test
Method of Koster *etal* (1959) was used. Thirty mice were divided into five groups of six mice each. Groups 1, 2 and 3 were treated with extract at doses of 50, 100, and 200 mg/kg body weight (*i.p.*) respectively. Group 4 was treated with piroxicam 10 mg/kg body weight intraperitoneally while Group 5 received normal saline 10 ml/kg body weight intraperitoneally. Thirty minutes after treatment, mice in all groups were administered 0.6% ($^{v}/_{v}$)freshly preparedacetic acid solution (*i.p.*) and the number of abdominal contractions were counted for each animal five minutes post acetic acid administration for 10 minutes.

Formalin induced paw licking test
Method of Hunskaar and Hole(1987) was adopted. In this test, licking time in seconds was registered from 0-5 minutes (first Phase) and 20 - 25 minutes (second phase) after subplantar administration of formalin (20 µl of 1% $^{v}/_{v}$) in the right hind paw of the rat. A total of 30 rats were divided into five different treatment groups with six rats in each group; Groups 1, 2 and 3 were treated with extract at doses of 50, 100, and 200 mg/kg body weight (*i.p.*) respectively. Group 4 was treated with pentazocin 10 mg/kg body weight intraperitoneally while Group 5 was administered with normal saline 10 ml/kg body weight intraperitoneally. Thirty minutes post-treatment 20 µl of freshly prepared1 % solution of formalin was administered at the subplantar region of right hind paw of each rat. Rats were placed individually in a transparent glass cylinder 20 cm in diameter and the time spent licking was recorded during 0 – 5 minutes and 20 – 25 minutes.

Formalin induced hind paw oedema test
This test was conducted according to Sayyah*et al*(2003).Thirty rats were divided into five different treatment groups with six rats in each group. Formalin solution was injected into subplantar of the right hind paw of the rat. Thirty minutes before injection of formalin, Groups 1, 2 and 3 were treated with extract at doses of 50, 100, and 200 mg/kg body weight (*i.p.*) respectively. Group 4 was treated with Diclofenac 25 mg/kg (*i.p.*) while Group 5 was administered with normal saline 10 ml/kg (*i.p.*). Increases in linear paw circumferences were taken as an index of increase in paw volume which is a measure of oedema. The paw volume (cm) was measured at 1, 2, 3, 4, and 5 hour after formalin injection using vernier caliper.

Statistical Analysis
The results were expressed as Mean ± SEM. The significance of difference between the means was determined by the student's *t*-test and results were considered significant when $p < 0.05$.

RESULTS
Acute Toxicity Study
The median lethal dose (LD_{50}) afterintraperitoneal (*I.P*) administration of the aqueous leaf extract is reported in Table 1. The LD_{50}value of the extract was found to be 2,154.1mg/kgin mice and 2,852.l mg/kg in rats

Analgesic Activities
Acetic acid induced writhing in mice
The anti-abdominal constrictions effect of aqueous leaf extract of *C. micranthum*in mice induced with acetic acid is presented in figure 1. The extract significantly ($P < 0.05$) reduced the number of abdominal constrictions at the dose of 200 mg/kg compared to group administered with normal saline while piroxicam (standard drug) significantly reduced abdominal constrictions in mice at $P < 0.01$.

Formalin induced licking in rats
The inhibitory effect of aqueous leaf extract of *C. micranthum* on formalin induced paw licking response in rat is presented in figure 2. At doses of 50, 100 and 200 mg/kg, the extract significantly ($P < 0.05$) reduced paw licking time in the second phase compared to group treated with normal saline. The reduction of paw licking time was dose dependent.

Anti-inflammationactivity
Formalin induced hind paw oedema in rats
The inhibitory effect of aqueous leaf extract of *C. micranthum* on formalin induced hind paw oedema in rat is presented in Table 2.The extract significantly ($P < 0.05$) inhibit the progressive increase hind paw oedema in rats at doses of 50, 100 and 200 mg/kg from the first hour and significantly ($P < 0.01$) showed inhibitory response similar to the standard drug (Diclofenac) in the fifth hour at the highest dose of 200 mg/kg while at lowest dose of 50 mg/kg showed no significant inhibition in the fourth and fifth hour.

Table 1: Acute Toxicity Studies of aqueous leaf extract of *C.micranthum* (LD_{50}) in mice and rats

Extracts	Route of administration	Animal species	LD_{50} values (mg/kg)
ALE	*I.P*	Mice	2154.1
ALE	*I.P*	Rat	2852.1

Table 2: Effect of aqueous leaf extract (ALE) of *C.micranthum* on formalin induced hind paw oedema in rats

Treatment	Dose (mg/kg)	Paw oedema (cm) at the 1hr	2hr	3hr	4hr	5hr
ALE	50	0.60±0.01[a]	0.61±0.01[b]	0.61±0.01[a]	0.64±0.01	0.67±0.02
ALE	100	0.59±0.02[a]	0.60±0.01[a]	0.61±0.01[a]	0.63±0.02[a]	0.65±0.01
ALE	200	0.57±0.02[a]	0.60±0.01[a]	0.60±0.01[a]	0.62±0.02[b]	0.64±0.02[b]
Diclofenac	25	0.59±0.02[a]	0.61±0.02[b]	0.61±0.01[a]	0.63±0.02[a]	0.64±0.02[b]
N/saline	0.2	0.67±0.01	0.76±0.02	0.74±0.05	0.79±0.03	0.84±0.02

[a]P< 0.05 and [b]p< 0.01, Students *t*-test, Mean ± SEM, n =6

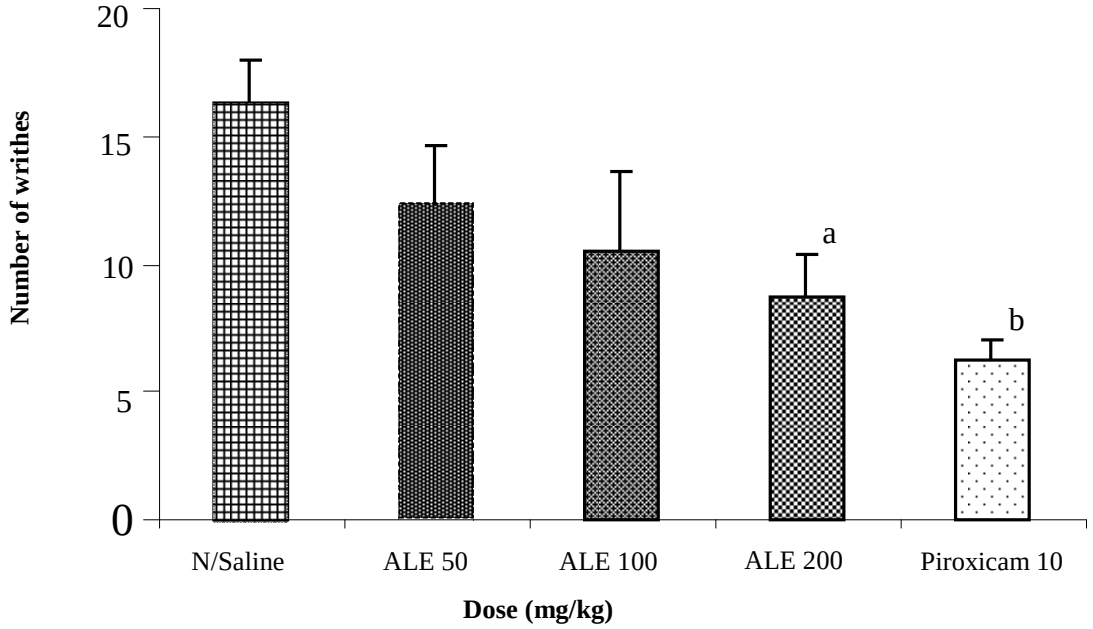

Fig. 1: The effect of ALE of *C. micranthum* on acetic acid induced writhing in mice. [a]p< 0.05; [b]p< 0.01 Student's *t*-test, Mean ± SEM for n = 6, ALE= Aqueous Leaf Extract

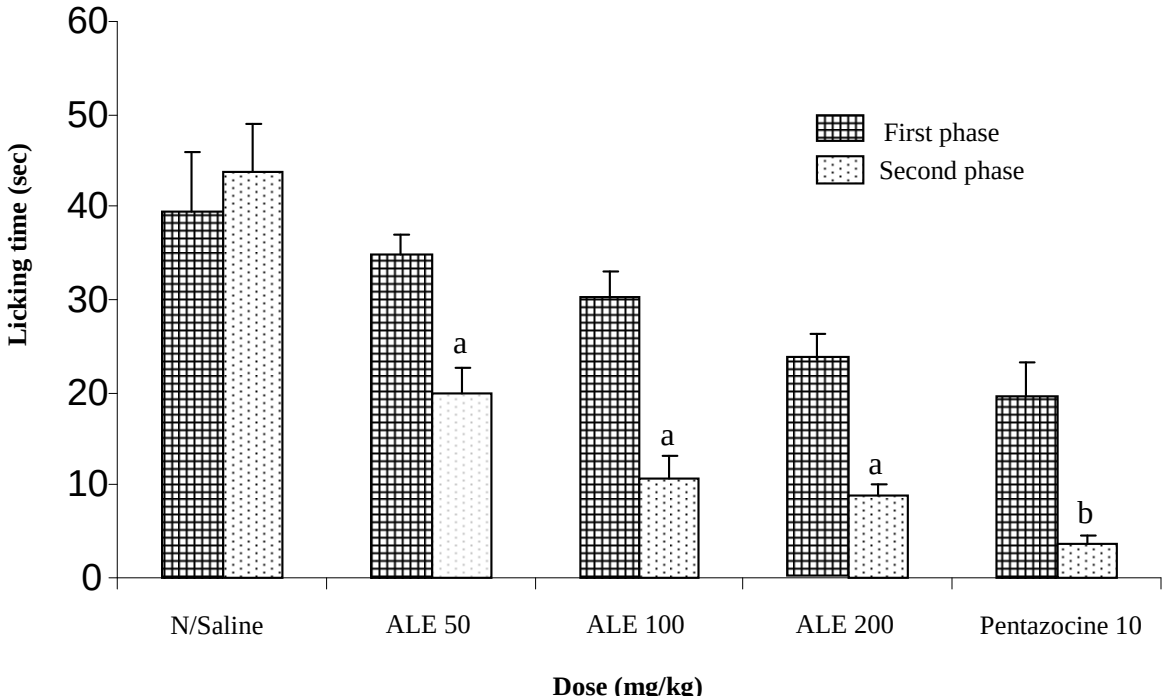

Fig. 2: The effect of ALE of *C. micranthum* on formalin induced licking in rats. [a]p< 0.05; [b]p< 0.01 Student's *t*-test, Mean ±SEM for n = 6, ALE= Aqueous Leaf Extract

DISCUSSION

In the present work, the aqueous leaf extract of *C. micranthum* was investigated for acute toxicity, analgesic and anti-inflammatory activities. The results of acute toxicity studies (LD_{50}) of aqueous leaf extract of *C. micranthum* in mice and rats showed that the extract was less-toxic via *I.P* administration and can be used for folkloric medicine, considering the LD_{50} by Lorke (1983); LD_{50}< 1.0mg/kg very toxic, LD_{50}< 10mg/kg toxic, LD_{50}up to 100 mg/kg less toxic, LD_{50}up to 1000 mg/kg slightly toxic and substances with LD_{50}values greater than 5,000 mg/kg are practically non-toxic. The same authors had earlier identified the following phytochemical constituents; alkaloids, flavonoids, glycosides, saponins, tannins and phlabotannins in the aqueous leaf extract of *C. micranthum* (Abdullahi *et al.,* 2014). Some chemical constituents found present in the aqueous leaf extract are known to have analgesic and anti-inflammatory activities.

The extract at a higher dose significantly reduced the number of acetic acid induced writhes in mice which revealed peripheral analgesic property of the extract in the peripheral tissue. The dose – dependent inhibitory activity of the extract on formalin induced paw licking in rats showed that the extract also possess central analgesic property comparable to the standard drug. The aqueous leaf extract of *C. micranthum* may therefore have both central and peripheral analgesic properties. The inhibitory activity of extract on formalin induced paw licking in rats was found to be greater in the second phase and it is a measure of the anti-inflammatory property of the extract. The analgesic and anti-inflammatory activities of theaqueous leaf extract of *C.micranthum*may be due to the presence of alkaloids and flavonoids as reported in the phytochemical constituents. Previous phytochemical screening of *C.micranthum* revealed the presence of flavonoids, tannins, alkaloids, phenanthrenes, dihydrophenanthrenes, gums and glycosides in the plant extract (Pazini, 1993). Flavonoids extracted from *Elaeagnusangustifolia* fruit have been reported to have analgesic and anti-inflammatory effects in rats (Ahmadiani *etal.,* 2000). Sawant *etal* (2004) reported that a total alkaloid fraction of *E. alba*, may be responsible for both central and peripheral analgesic activity in rats. Vougtau *etal*(2000) also reported that, flavonoids isolated from some medicinal plants have been proven to possess anti- nociceptive and anti-inflammatory effect. Flavonoids have been shown to inhibit prostaglandin biosynthesis, a group of powerful pro-inflammatory signaling molecules, and also phosphodiesterase involved in cell activation (Manthey *et al.*, 2001). The aqueous leaf extract of*C. Micranthum* may have produced its effects by inhibition of cyclo-oxygenase-1 (COX-1) or cyclo –oxygenase-2 (COX-2), but COX-2 has been found to be responsible for inflammatory conditions (Boxtel, 2001). This shows that, the extract might have produced analgesic or anti-inflammatory effects by possible inhibition of COX-2, an enzyme responsible for the conversion of arachidonic acid to prostaglandin.

CONCLUSION

TheCurrent result showed that the aqueous leaf extract of *C. micranthum* possesses antinociceptive and anti-inflammatory activities comparable to the standard drugs used in the research and this may account for use of the plant in the management of pain and inflammation in traditional medicine.

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