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ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF THE METHANOL LEAF EXTRACT OF *FICUS INGENS* (MORACEAE) IN RODENTS

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ABSTRACT

Ficus ingens is a medicinal plant used for haemorrhoids, in Borno State of Nigeria. In the present study, the analgesic and anti-inflammatory activities of methanol leaf extract of *Ficus ingens*, (Miq.) Miq., (at doses of 75, 150, and 300 mg/kg *i.p.*) were evaluated using acetic acid- induced writhing test and hot plate in mice, and carragenan- induced paw oedema in rats. The extract at all doses tested significantly ($P < 0.001$) inhibited acetic acid induced writhing and also significantly ($P < 0.05$) prolonged the reaction latency to pain thermally induced in mice by the hot plate. The extract at the doses (75, 150, and 300 mg/kg *i.p.*) tested afforded 61, 72, and 67% inhibition of paw oedema, respectively at the end of the third hour which implied that the extract inhibit the release of prostaglandins and lysosomal enzymes. The intraperitoneal median lethal dose (LD_{50}) value in mice was 1131.4 mg/kg suggesting that the extract is relatively non-toxic at doses used. The phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins and tannins which might be responsible for the observed analgesic and anti-inflammatory activity. This study showed that *Ficus ingens* possesses significant anti-inflammatory and analgesic properties in rodents which supported the folkloric claim for the use of the plant in the management of haemorrhoids.

KEY WORDS: *Ficus ingens*, Analgesia, Anti-inflammation, Phytochemical screening, Acute toxicity.

INTRODUCTION

Haemorrhoids (Piles) are varicose condition of the external or internal rectal veins causing painful swellings at the anus. Haemorrhoid is associated with pain and inflammation (Drickx, 2001). Currently available analgesics still produce enough side effects that the search for new types is justified (Sampson *et al.*, 2000). Plants which have been used traditionally as analgesics or have yielded compounds which are used in pain relief include *Cannabis sativa* (Cannabinaceae),

Mandragora officinarum (Solanaceae), *Papaver somniferum* (Papaverceae) and *Conium maculatum* (Umbelliferae). Plants which have been used as medicine, often over hundred of years, constitute an obvious choice for examination in the current search for therapeutically active drugs (Sampson *et al.*, 2000). According to an estimate made by the World Health Organisation (WHO) around 80% of the world's population in developing countries rely on traditional plant medicines for their primary health care needs, of which a major portion involves the use of plant extracts or their active

principles (Farnsworth, 1998). *Ficus ingens* is an ever green tree with a briefly deciduous period, is up to 10 m, occasionally higher, with a rounded or spreading crown and with a spread of up to 30 m wide. All the parts have milky latex when broken (Burkill, 1995). It occurs in the moist eastern subtropical or temperate regions of South Africa from the Albany district in the eastern cape northwards through Kwazulu Natal, the north provinces and further northwards into tropical Africa as far north as Ethiopia and Saudi Arabia and across into West Africa (Jordan, 2005). Ethnomedically, the bark is used in northern Nigerian local veterinary medicine, in the management of swollen feet in horses; a freshly collected bark is powdered, moistened and applied topically to the swollen surface, whereas in Ivory Coast, it is used for leprosy (Kerharo and Bouquet, 1950). Furthermore, the latex is applied to Guinea worm sore in Southern Nigeria. The leaves are powdered and applied topically to non-bleeding external injuries in humans (Kerharo and Bouquet 1950). In Borno, Nigeria, preparations of the bark, roots and leaves are used for piles (haemorrhoids) and diarrhea as a laxative and diuretic (Burkill, 1995). Present study is aimed at evaluating the analgesic and anti-inflammatory properties of methanol leaf extract of *Ficus ingens*.

MATERIALS AND METHODS

Test animals

Adult Wister rats, (127-146 g) and Swiss albino mice, (16-30 g) of either sex were used for the experiments. All animals were healthy and obtained from the Animal house facility of Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. They were housed in standard polypropylene cages and kept in a well ventilated area. The animals were fed on

standard laboratory diet (Vital Feeds, Jos, Nigeria) and water *ad libitum*. Food and water were withdrawn during the experimental hours. All experimental protocols were approved by the University animal ethics committee.

Preparation of Crude Extract

The leaves of *Ficus ingens* were collected from Ahmadu Bello University (A.B.U.) Farm, Zaria, Kaduna State, in January, 2009. The plant was identified and authenticated by U.S. Gallah and M. Musa, of the Herbarium section, Department of Biological sciences, Ahmadu Bello University, Zaria, Nigeria by comparing with existing specimen (voucher number: 890). The sample was air dried at room temperature and grounded into powder. 500g of the powder was macerated with four liters of methanol for 1 week with occasional shaking to obtain its methanol extract. The extract was concentrated on a water bath at a temperature of 40°C and this afforded a greenish mass of 53.92 g (10.78% w/w). Solutions of the extract were prepared freshly with distilled water for each study.

Drugs

The following chemicals and drugs were used: Piroxicam (Hovid, Malaysia), Morphine Sulphate (Martindale, Essex, England), Acetic acid solution (Searle Essex, England) and Carrageenan suspension (Sigma- Aldrich, USA).

Preliminary Phytochemical Screening

The methanol leaf extract of *Ficus ingens* was screened for the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins and tannins according to standard protocol (Trease and Evans, 1983).

Acute toxicity study

The method of Lorke (1983) was adopted. The study was divided into two phases. In the first phase, nine mice of either sex were

divided into three groups of three mice each. Group 1 received 10 mg/kg while group 2 and 3 received 100 and 1000 mg/kg extracts intraperitoneally (*i.p.*), respectively. The mice were observed for signs and symptoms of toxicity, and death for twenty four hours after treatment. In the second phase, based on the result of the first phase, 4 mice were divided into 4 groups of one mouse each, the first received extract at a dose of 200 mg/kg while the second, third and fourth group received the extract at doses of 400, 800, 1600 mg/kg respectively. The mice were observed for twenty four hours for signs and symptoms of toxicity and death. The final LD₅₀ was calculated as the square root of the product of the lowest lethal dose and the highest non lethal dose or the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

Acetic acid-induced writhing test in mice

The method described by Koster *et al.*, (1959), was employed to assess the analgesic activity of the methanol leaf extract of *Ficus ingens*. A total of 30 mice were divided into 5 groups of 6 mice each. The mice were treated with normal saline (10 ml/kg), *Ficus ingens* extract (75,150 and 300 mg/kg) or piroxicam (10 mg/kg) intraperitoneally.

30 minutes later, mice in all groups were treated with acetic acid 10 ml/kg of 0.6% v/v intraperitoneally. The number of writhes was counted 5 minutes after acetic acid injection for a period of 10 minutes. Percentage inhibition of writhing was calculated using the formula:

% inhibition =

Mean No. of writhes (control) – Mean No. of writhes (test) / Mean No. of writhes (control) X 100

Hot plate method

The test was carried out using a Gallenkamp (England) thermostat hot plate apparatus (cat no: HL-054), maintained at 50°C and the method described by Turner (1965) was employed. Only mice that showed initial nociceptive response within 30 seconds were selected for the experiment. The reaction time of the mice to the thermal stimulus, taken to be the interval between the instant the animal reached the hot plate to the time it licked its paw or jump off the hot plate.

30 mice were selected and divided into 5 groups of 6 mice each. The first group received normal saline 10 ml/kg intraperitoneally. The second, third and fourth groups were given the test extract at 300, 150 and 75 mg/kg via intraperitoneal route respectively. The fifth group received morphine sulphate 4 mg/kg intraperitoneally.

Thirty minutes after treatment, the reaction time of each mouse was again evaluated as above. The final test mean value (Ta) for each treatment group was calculated which represented the after treatment reaction time and was subsequently used to determine the percentage thermal pain stimulus or protection by applying the formula:

% protection against thermal stimulus = Test mean (Ta) – Control mean (Tb) / Control means (Tb) X 100

Carrageenan-induced paw oedema in rats

The test was conducted according to the method described by Niemegeer *et al.*, (1964). Wistar rats were divided into 5 groups of 6 rats each and treated with normal saline 1ml/kg, test extract 300, 150, 75 mg/kg and piroxicam 10 mg/kg via intraperitoneal route, respectively.

Thirty minutes later, 0.1ml of freshly prepared 1%w/v carrageenan suspension was injected into the sub plantar region of the left hind paw of each rat. The paw diameter was measured with the aid of vernier caliper at 0, 1, 2, 3, 4 hour after the injection of carrageenan.

Statistical analysis

The data was expressed as Mean \pm SEM (standard error of mean). Analysis of variance (ANOVA) followed by post hoc and Dunnet-t-test was used to statistically analyze data. P values less than 0.05 (P<0.05) were considered as significant.

RESULTS

The median lethal dose (LD₅₀) value of the methanol leaf extract of *Ficus ingens* in mice was 1131.4 mg /kg body weight intraperitoneally. The extract significantly attenuated the number of acetic acid induced abdominal writhes in mice, dose dependently. The highest percentage inhibition of abdominal constriction (80.5%) was observed at 300 mg/kg (P<0.001) and was greater than that of piroxicam (45.1%) at 10 mg/kg (P<0.05), the standard non-

steroidal analgesic and anti-inflammatory drug used. The extract at 75 and 300 mg/kg, significantly protected the mice against thermally induced pain stimulus. The extract (75 and 300 mg/kg), and morphine sulphate (4 mg/kg), significantly (p<0.05) increased pain latency thermally induced by the hot plate. The reaction time at the dose of 300 mg/kg (3.08) was found to be twice that of the control group (1.48). Morphine Sulphate, the standard drug afforded more than 400% protection against thermally induced pain stimulus in mice. In the normal saline treated rats, sub plantar injection of 1%w/v carrageenan produced a local oedema reaching its maximum at the third hour. The methanol leaf extract of *Ficus ingens* significantly inhibited the progressive increase in paw oedema produced by carrageenan. The anti-inflammatory effect of the extract at 150 mg/kg was comparable to piroxicam (10 mg/kg), the standard anti-inflammatory agent used. However, the extract (150 mg/kg) at the first hour, produced a greater inhibition than piroxicam (10 mg/kg). The preliminary phytochemical screening of the methanolic leaf extract of *Ficus ingens* revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins and tannins.

Table 1: Effect of methanol leaf extract of *Ficus ingens* on acetic acid- induced writhing in mice

Treatment (mg/kg)	Mean Number of Writhes	% Inhibition
Normal saline (10 ml/kg)	22.17 \pm 2.75	
Extract (75)	6.83 \pm 1.30 ^c	69.19
Extract (150)	6.67 \pm 1.05 ^c	69.91
Extract (300)	4.33 \pm 1.43 ^c	80.45
Piroxicam (10)	12.17 \pm 2.32 ^a	45.11

Each value is Mean \pm SEM of 6 mice; ^aP< 0.05, ^cP<0.001

One way ANOVA df=4, 25 f=14.421

Table 2: Effect of methanol leaf extract of *Ficus ingens* on thermally induced pain in mice

Treatment (mg/kg)	Mean Pain Latency (sec)	% Inhibition
Normal saline (10 ml/kg)	1.48 ± 0.17	
Extract (75)	2.08 ± 0.12 ^a	40.54
Extract (150)	1.98 ± 0.15	33.78
Extract (300)	3.08 ± 0.68 ^a	108.11
Morphine (4)	8.83 ± 1.88 ^a	496.62

Each value is Mean ± SEM of 6 mice; ^a P < 0.05

One way ANOVA df=4, 25 f=11.398

Table 3: Effect of methanol leaf extract of *Ficus ingens* on carrageenan induced paw oedema in rats

Groups (n=5)	Dose (mg/kg)	Mean Paw Diameter (cm)			
		1h	2h	3h	4h
Normal Saline	1 ml/kg	0.13 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.10 ± 0.01
Extract	75	0.08 ± 0.01 ^{NS} (38.46)	0.08 ± 0.01 ^c (52.94)	0.07 ± 0.01 ^c (61.11)	0.10 ± 0.02 ^{NS} (0)
Extract	150	0.04 ± 0.01 ^b (69.23)	0.05 ± 0.01 ^c (70.59)	0.05 ± 0.01 ^c (72.22)	0.05 ± 0.02 ^a (50)
Extract	300	0.05 ± 0.01 ^{NS} (61.54)	0.09 ± 0.02 ^b (47.06)	0.06 ± 0.02 ^c (66.67)	0.08 ± 0.02 ^{NS} (20)
Piroxicam	10	0.05 ± 0.01 ^{NS} (61.54)	0.05 ± 0.01 ^c (70.59)	0.04 ± 0.00 ^c (77.78)	0.05 ± 0.01 ^c (50)

Each value is Mean ± SEM of 6 rats; Figure in parentheses represents percentage inhibition of inflammation; ^a P < 0.05, ^b P < 0.01, ^c P < 0.001

DISCUSSION

This study showed that the methanol leaf extract of *Ficus ingens* possesses analgesic and anti-inflammatory activities. The acetic acid induced abdominal constriction method is widely used for the evaluation of peripheral antinociceptive activity (Gene' *et al.*, 1998). It is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods like the tail-flick test (Collier *et al.*, 1968; Bentley *et al.*, 1981). Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response (Bentley *et al.*, 1983). The method has been associated with prostanoids in general, e.g. increased levels of PGE₂ and PGF_{2α} in peritoneal fluids (Derardt *et al.*, 1980), as well as lipoxygenase products by some researchers (Levini *et al.*, 1984; Dhara *et al.*, 2000). Therefore the results of the acetic acid induced writhing; strongly suggest that the mechanism of action of this extract may be linked partly to lipoxygenases and/or cyclooxygenases pathways. The hot plate method is one of the most common tests of nociception that is based on a phasic stimulus of high intensity (Mandegary *et al.*, 2004). Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception (Parkhouse and Pleuvry, 1979). The ability of the extract to prolong the reaction latency to pain thermally-induced in mice by the hot plate further suggests central analgesic activity. The extract at the doses tested was shown to possess anti-nociceptive activity evident in all the nociceptive models, signifying it possesses both central and peripherally mediated activities. Carrageenan-induced hind paw oedema is the standard experimental model for acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is

none antigenic and is devoid of apparent systemic effect (Chakraborty *et al.*, 2006). Carrageenan model of inflammation is said to be biphasic, with the first phase attributed to the release of histamine, serotonin and kinins in the first hour; while the second phase is attributed to the release of prostaglandins and lysosome enzymes in the second to the third hour (Brooks and Day, 1991). The extract inhibited both the first and second phases of inflammation. The ability of the extract to inhibit carrageenan induced paw oedema suggests it possesses a significant effect against acute inflammation. The extract (150 mg/kg) also caused marked inhibition of carrageenan-induced hind paw oedema in rats as compared with piroxicam (10 mg/kg), the standard anti-inflammatory agent used. The co-existence of both antinociceptive and anti-inflammatory effect seen with this extract is well defined for various non steroidal anti-inflammatory drugs (NSAIDs), particularly the salicylates and their derivatives, since the cyclooxygenase enzyme which leads to the production of prostanoids is usually inhibited. It is therefore interesting that the extract behaved similar to NSAIDs in this study which correlates well with the traditional application of the plant in pain and inflammatory condition (Haemorrhoids). The phytochemical screening of the methanolic leaf extract of *Ficus ingens*, revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins and tannins. Flavonoids, saponins and tannins have been shown to exert analgesic effect on acetic acid induced writhing test (Calixto *et al.*, 2000 and Shin *et al.*, 1997). The flavonoids, saponins and tannins might be responsible in part for the observed analgesic and anti-inflammatory effect.

CONCLUSION

These findings suggest that the methanol leaf extract of *Ficus ingens* contain bioactive constituents with analgesic and anti-inflammatory activities, and further support the ethnomedical claim of the use of the plant in the management of pain and inflammatory conditions.

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