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Preliminary Studies on the Anti-Inflammatory and Analgesic Effects of Methanol Leaf Extract of *Ficus asperifolia* Miq

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ABSTRACT

Ficus asperifolia is used traditionally in most African countries for the treatment of inflammatory diseases, pain, tumours and infertility. This study was carried out to evaluate the anti-inflammatory and analgesic properties of methanol leaf extract of *Ficus asperifolia* in rodents. The extract was subjected to phytochemical and acute toxicity tests. Anti-inflammatory (using carrageenan-induced paw oedema test) and analgesic studies (using acetic acid-induced writhing, hot plate and formalin tests) were conducted on the extract at doses of 250, 500 and 1000 mg/kg. The intraperitoneal median lethal dose of *Ficus asperifolia* was estimated to be 3800 mg/kg in mice. The extract at all the tested doses significantly (p<0.05) decreased the mean paw oedema induced by carrageenan when compared to control. It also offered 20.98% inhibition of writhing behaviour above piroxicam (positive control) at 1000 mg/kg. In the hot plate test, *Ficus asperifolia* extract significantly (p<0.05) increased the mean reaction time at all the tested doses. Similarly, the extract significantly (p<0.05) reduced the paw licking time in both phases of the formalin test with a peak activity (>50% inhibition of pain) at 1000 mg/kg. In conclusion, the results obtained revealed that methanol leaf extract of *Ficus asperifolia* possesses anti-inflammatory and analgesic properties.

Keywords: *Ficus asperifolia*, Anti-inflammatory, Analgesia, Nociception.

Introduction

Herbal medicine forms an important component of the health care delivery system in African countries.[1] Researches on herbal products have led to the discovery of novel lead compounds for potential development as drugs that function on established or new therapeutic targets.[2,3] Herbal products are extensively used in the management of many inflammatory and painful conditions and recently there is a renaissance of interest in medicinal plants with anti-inflammatory and analgesic properties.[4] This could be attributed to not only the accessibility, acceptability, affordability and safety profiles of the medicinal plants[4,5] but also to the adverse effects associated with the currently used orthodox drugs.[6]

Ficus asperifolia Miq. is a variable plant that belongs to the family Moraceae. It is widely distributed across Africa and is abundant mainly in damp areas and river banks of the savannah regions.[7,8] *Ficus asperifolia* is commonly known as Sandpaper tree in English and is known in Nigerian local languages as "*Ibbi gorki"* (Fulfulde), "*Kawusa*" (Nupe and Hausa) "*Ipin"* (Yoruba), and "*Anmerenwa* or *Asesa"* (Igbo). Other names are *Tiãgtiãgad, Safen* (Senegal) *Kagami, Kamakor, Nyoin* (Sierra Leone) *Niénié, Sutro, Nioyeniye* (Guinea).[7] In African Traditional Medicine, the latex, leaves, bark and roots of *Ficus asperifolia* are generally used to alleviate menstrual pain and liver problems. In addition, the leaf extract is used in the treatment of headache, tumors and inflammation of the gums while the root extract is used in the treatment of diabetes mellitus, hypertension, dysentery, diseases of the kidneys, urinary and respiratory tract infections.[7, 9] Previous studies conducted on *Ficus asperifolia* showed that it possesses gastroprotective,[10] uterotonic,[11] hypoglycaemic [12] and hypolipidaemic properties.[13] The leaves of the plant have been found to possess antioxidant activities[14] as well as ameliorative effects against testicular problems.[8] Essential oils obtained from *Ficus asperifolia* were found to possess antimicrobial properties[15] and according to Watcho *et al*.,[16] the fruit extracts possess androgenic-like activities. However, there are relatively scarce reports on the anti-inflammatory or analgesic properties of the plant despite its wide usage in ethno-medicine against inflammatory diseases and pain management. This study, therefore, was aimed at evaluating the anti-inflammatory and analgesic activities of methanol leaf extract of *Ficus asperifolia* on mice and rats.

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Materials and Methods

Drugs and chemicals

The drugs and chemicals used for the studies include: Morphine sulphate (Martindale Pharma., U.K.); Piroxicam capsules (Pfizer, U.S.A.); Acetic acid (Sigma-Aldrich, U.S.A.), Carrageenan (Sigma Aldrich, U.S.A.) and Formalin (Sigma Aldrich, U.S.A.).

Experimental animals

Wistar strain rats (220-240 g) and Swiss albino mice (18-25 g) of either sex were obtained from the Animal Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. The animals were housed in standard propylene cages with saw dust beddings and kept under natural day and light cycle at the Department of Pharmacology and Therapeutics, Bayero University, Kano. They were fed on standard rodent feed (Vital Feeds, Nigeria) and were allowed access to water *ad libitum*. The experimental protocol was approved by the Bayero University Research and Ethical Committee (BUK/CHS/REC/VII/54).

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Plant collection and authentication
Fresh leaves of *Ficus asperifolia* were collected from Toro district, Toro Local Government Area of Bauchi State, Nigeria in the month of November, 2017. The plant specimen was authenticated by Mr. Chris Abok, a taxonomist at the Herbarium of Federal College of Forestry, Jos, where a specimen was deposited under voucher number FHJ 234. The identification of the specimen was further confirmed at the Herbarium of Biological Sciences Department of Bayero University, Kano, by Bahauddeen Said Adam and was assigned a voucher number (BUKHAN 106).

Preparation of plant extract
Fresh leaves of *Ficus asperifolia* were rinsed with clean water and shade-dried. The dried leaves were pulverized into fine powder, and the powdered plant material (850 g) was macerated with 7 L of 70% methanol at room temperature for 7 days with occasional agitation of the mixture. At the end of the extraction, the crude methanol extract was filtered using Whatman filter paper (Grade 1) and concentrated on a water bath (maintained at 45°C) for 12 hrs to obtain the residue. The extract was weighed and then stored in a desiccator until further use.

Phytochemical screening
Standard phytochemical tests were employed in screening the *Ficus asperifolia* leaf extract.[17] The extract was screened for the presence or absence of secondary metabolites including alkaloids, flavonoids, saponins, cardiac glycosides, tannins, triterpenes, steroids and anthraquinones.

Preparation of stock solutions of extract and drugs
Stock solutions of *Ficus asperifolia* leaf extract were prepared by dissolving 1000 mg in 10 ml of distilled water to give 100 mg/ml solution. This was followed by serial dilution to obtain the appropriate concentrations for the studies. Correspondingly, stock solution of the positive controls (piroxicam, 1 ml/kg and morphine, 0.4 ml/kg) were prepared using distilled water to obtain appropriate concentrations. The drug solutions were freshly prepared before each experiment to maintain their stability.

Acute toxicity studies
The acute toxicity of methanol leaf extract of *Ficus asperifolia* were investigated in mice using the method described by Lorke.[18] This was conducted in two phases where in the first phase, 3 groups of three mice each were used. The 1st, 2nd and 3rd groups were treated intraperitoneally (*i.p.*) with the extract at doses of 10, 100 and 1000 mg/kg body weight respectively. The animals were then observed for 24 hours for signs of toxicity and death. In the second phase, three mice were administered more specific doses (1600, 2900 and 5000 mg/kg) of the extract *i.p.* (which depended on the result of the first phase) and also observed for 24 hours for signs of toxicity and death. Thereafter, the median lethal dose (LD50) was estimated using the relationship below:

$LD_{50} = \sqrt{\text{lowest lethal dose} \times \text{highest non-lethal dose}}$

Anti-inflammatory studies
Carrageenan-induced rat paw oedema test
The method described by Winter et al.[19] was employed to evaluate the anti-inflammatory activity of *Ficus asperifolia* using thirty rats which were divided into five groups of six rats each. The first group received 1 ml/kg of distilled water (*i.p.*) and served as negative control. Groups 2, 3 and 4 received 250, 500 and 1000 mg/kg of leaf extract of *Ficus asperifolia i.p.*, while group 5 rats received piroxicam (10 mg/kg, *i.p*) which served as positive control. Thirty minutes after treatment, oedema was induced in all the rats by injection of carrageenan (0.1 mL of 1% $^{w}/_{v}$ solution) into the sub-planter tissue of the right hind paws. The paw diameters were measured using digital vernier caliper at 0, 0.5, 1, 2, 3 and 4 hours post carrageenan injection. The increase in paw diameter (oedema index) was calculated for each rat as the difference in paw diameter before and after carrageenan injection,[20] while the percentage inhibition of oedema was calculated using the relationship below:

$$\% \text{ Inhibition} = \frac{\text{Oedema index (negative control)} - \text{Oedema index (test)}}{\text{Oedema index (negative control)}} X\ 100$$

Analgesic studies
Acetic acid-induced writhing test
The method described by Koster et al.[21] was employed in this test. Thirty mice were divided into five groups of six mice each. The first group received 10 ml/kg of distilled water *i.p.* which served as negative control. Groups 2, 3 and 4 received 250, 500 and 1000 mg/kg of *Ficus asperifolia* methanol leaf extract while mice in group received piroxicam (10 mg/kg) as the positive control. Thirty minutes after treatment, mice in all groups were treated with acetic acid (0.6% $^{v}/_{v}$, 10 ml/kg, *i.p.*) and then placed in individual observation cages. The number of abdominal writhes (stretching of abdomen with involvement of the hind limbs) was counted for each mouse for a period of 10 minutes after a 5 minutes latency period and the percentage inhibition of writhes was calculated using the relationship below:

$$\% \text{ Inhibition} = \frac{\text{Mean no.of writhes (negative control)} - \text{Mean no.of writhes (test)}}{\text{Mean no.writhes (negative control)}} X\ 100$$

Hot plate test in mice
The method described by Eddy and Leimback,[22] was employed for the study. Thermo-sensitive mice were grouped into five groups of six mice each. The first group served as negative control and received 10 ml/kg distilled water. Groups 2, 3 and 4 received 250, 500 and 1000 mg/kg of methanol leaf extract of *Ficus asperifolia* (*i.p.*), while the fifth group (positive control) received 10 mg/kg morphine (*i.p.*). Thirty minutes after treatment, each mouse was placed on a digital electronic hot plate which was set and maintained at 55 ± 1°C. Thereafter, the reaction time (the time until either licking of the paws or jumping off the plate) for each mouse was recorded using a stopwatch at 30, 60, 90 and 120 min after treatment.

Formalin-induced pain test in mice
The method of Dubuisson and Dennis[23] as modified by Tjølsen et al.[24] was adopted in this study using six groups of six mice each. The first group served as negative control (administered 10 ml/kg of distilled water *i.p.*), the 2nd, 3rd and 4th groups were treated with *Ficus asperifolia* extract (250, 500 and 1,000 mg/kg *i.p.*, respectively), while the 5th and 6th groups (positive controls) were treated with piroxicam (10 mg/kg, *i.p*) and morphine (4 mg/kg, *i.p*) respectively. Thirty minutes post treatment, 20 µl of freshly prepared 2.5% $^{v}/_{v}$ formalin in water was injected subcutaneously into the right hind paw of each mouse. The mice were placed individually in an observation chamber and monitored. The time (sec) spent licking the injected paw, indicative of pain, was recorded. The responses of the mice were observed for the first 5 min (early phase) and 15-40 minutes (late phase) post formalin injection.[24, 25, 26] The percentage inhibition was also evaluated using the relationship below:

$$\% \text{ Inhibition} = \frac{\text{Mean licking response (negative control)} - \text{Mean licking response (test)}}{\text{Mean licking response (negative control)}} X\ 100$$

Statistical analysis
Data obtained for acetic acid and formalin tests were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett post hoc test, while data for hot plate and carrageenan tests were analyzed by repeated measure ANOVA followed by Bonferroni post hoc tests. Values of $p \leq 0.05$ were considered significant. The results were expressed as mean ± standard error of the mean (S.E.M.).

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Results and Discussion

The extraction of 850 g of *Ficus asperifolia* powdered leaves provided a yield of 10.5%$^{w}/_{w}$. The extract obtained was greenish-black in colour with a sticky nature, while preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, anthraquinones, cardiac glycosides, tannins, saponins, steroids and triterpenes. Several plant extracts that showed anti-inflammatory and analgesic activities in animal models have been attributed to the presence of their secondary metabolites.[2] Phenolic compounds (phenolic acids, flavonoids and other polyphenolics) are well known for their ability to inhibit pain perception as well as inflammatory processes.[27, 28] Alkaloids present a valuable source of pharmaceuticals because they show a variety of biological activities.[29] Gelsemine and Bullatine A are alkaloids with established anti-nociceptive and anti-inflammatory activities respectively.[30,31] Euphol is a tetracyclic triterpene alcohol and is the main constituent found in the sap of *Euphorbia tirucalli*. This compound was able to reduce the pain model induced by B16F10 melanoma cell injection and model of inflammatory pain in rats.[32] In this study, phytochemical screening of methanol leaf extract of *Ficus asperfolia* showed the presence of flavonoids, alkaloids and triterpenes amongst other secondary metabolites and plausibly, the anti-inflammatory and analgesic actions produced by the extract could be attributed to these phyto-constituents.

The administration of *Ficus asperifolia* leaf extract (10 to 1000 mg/kg) did not produce any sign of toxicity or death in the first phase of the study. However, mortality was observed with a single mouse that was given 5000 mg/kg of the extract in the second phase (Table 1). The intraperitoneal LD_{50} in mice was thus estimated to be 3800 mg/kg body weight. Acute systemic toxicity evaluates the adverse effects that occur following exposure of organisms to a single or multiple doses of a test substance within 24 hours by a known route.[33] Assessment of the acute toxic potential of substances is required to determine the adverse effects that might occur due to inadvertent or deliberate short-term exposure.[34] In addition, results from acute toxicity test serves as a guide in dosage selection for long term toxicity studies as well as other studies that involve the use of animals.[35] Based on the estimated intraperitoneal LD_{50}, the methanol leaf extract of *Ficus asperifolia* is considered as slightly toxic in mice.[36]

Table 1: Median lethal dose determination of methanol leaf extract of *Ficus asperifolia alismifolium* in mice

Phase one doses (mg/kg)	Number of mice used	Mortality
10	3	0/3
100	3	0/3
1000	3	0/3
Phase two doses (mg/kg)	**Number of mice used**	**Mortality**
1600	1	0/1
2900	1	0/1
5000	1	1/1

Table 2: Effect of methanol leaf extract of *Ficus asperifolia* on acetic acid-induced writhes in mice

Treatment (mg/kg)	Mean number of writhes	% Inhibition
DW 10 mL/kg	23.83 ± 0.65	–
MEFA 250	2.17 ± 0.17***	90.89
MEFA 500	1.33 ± 0.21***	94.42
MEFA 1000	1.00 ± 0.00***	95.89
PRC 10	5.98 ± 0.41***	74.91

Values are presented as Mean ± S.E.M., *** = significant difference at $p < 0.001$ as compared to DW group – One-way ANOVA followed by Dunnett post hoc test, n=6, DW = Distilled water, MEFA = Methanol leaf extract of *Ficus asperifolia*, PRC = Piroxicam.

Injection of carrageenan into the hind paws of the negative control rats caused oedema development that peaked at the 4th hour post injection. However, the administration of methanol extract of *Ficus asperifolia* significantly ($p<0.05$) decreased the mean paw oedema at all the tested doses (250, 500 and 1000 mg/kg) when compared to the negative control (Figure 1a). The extract (1000 mg/kg) offered the highest inhibition of inflammation (35.2%) at the 3rd hour. Similarly, the positive control (Piroxicam, 10 mg/kg) significantly inhibited the oedema during the four hours observation period with maximum inhibition (34.8%) at the 4th hour (Figure 1b). The carrageenan test is commonly used for evaluation of acute inflammation in animals.[37] The development of oedema induced by carrageenan is a two-phase event; the first phase (first hour after carrageenan injection) is attributed to the release of histamine, serotonin and bradykinins which increase vascular permeability; while the second phase (2-3 hrs after carrageenan injection) is attributed to the release of prostaglandins (produced by tissue macrophages), proteases, lysosomal enzymes, leukotrienes and poly-morphonuclear cells.[38, 39] The results obtained showed that *Ficus asperifolia* exerted anti-inflammatory activity in both phases of the test and this suggest it possibly acts by inhibiting the release and or action of histamine, serotonin, kinins and prostaglandins.

The analgesic activity of *Ficus asperifolia* in this study was investigated using the mouse writhing, hot plate and formalin tests. In the acetic acid test, administration of *Ficus asperifolia* produced a dose-dependent and significant ($p<0.001$) reduction in the mean number of writhes when compared to the distilled water group. The extract also offered 15.98%, 19.51% and 20.98% inhibition above piroxicam at doses of 250, 500 and 1,000 mg/kg respectively (Table 2). The acetic acid-induced writhing test is a classical model of chemical/inflammatory pain largely employed in screening analgesic drugs in mice.[40] Although the test is sensitive to centrally acting analgesics, it is generally employed in screening of peripherally acting analgesics.[41] It is well known that the observed abdominal constrictions is said to involve local peritoneal receptors where the injected acetic acid into the peritoneal cavity increases the level of cyclooxygenase and lipoxygenase enzymes in the peritoneal fluid and indirectly leads to the release of endogenous mediators of pain thereby stimulating the neurons responsible for pain sensation.[20, 42] In this study, the methanol leaf extract of *Ficus asperifolia* produced significant reduction in the mean number of writhing which suggests its possible peripheral analgesic activity.

The hot plate test was carried out to evaluate the central analgesic activity of *Ficus asperifolia* leaf extract. This test produces paw licking and jumping behavioural responses, both of which are considered to be supraspinal responses.[43] Increment in the mean response time was utilized in evaluating the central analgesic activity. Narcotic analgesics like morphine and pentazocine have been shown to prolong the mean response time by interacting with the opioid receptors to increase the pain threshold.[43, 44] In this study, the methanol leaf extract of *Ficus asperifolia* produced a significant ($p<0.05$) increase in the mean reaction time at all the tested doses when compared with the negative control (distilled water, 10 mL/kg). A significant increase in the mean reaction time was also produced by the positive control (Morphine, 4 mg/kg) (Figure 2). This suggests the extract possesses centrally mediated analgesic properties. Considering the two experimental results above (writhing and hot plate tests), it is evident that the extract has both peripheral and central acting analgesic properties.

In the first phase of formalin test, pretreatment of mice with *Ficus asperifolia* extract (250, 500 and 1,000 mg/kg) produced a dose-dependent and significant ($p<0.05$) reduction in the mean paw licking time with a peak activity (59.71% inhibition) at 1000 mg/kg. A significant ($p<0.05$) reduction in the duration of licking response was also produced by morphine but not with piroxicam (Figure 3b).

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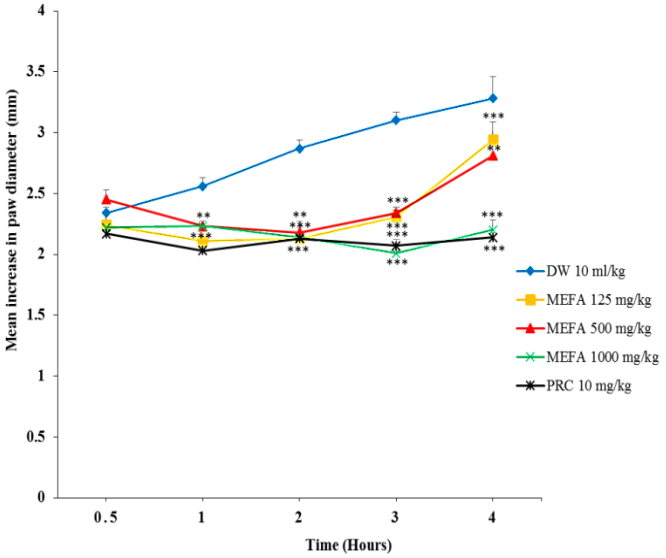

Figure 1a: Effect of methanol leaf extract of *Ficus asperifolia* on carrageenan-induced paw oedema in rats. Values are Mean ± S.E.M., ** and *** = significant difference at $p<0.01$ and $p < 0.001$ respectively compared to DW group - Repeated measure ANOVA followed by Bonferroni post hoc test, n = 6, MEFA = Methanol leaf extract of *Ficus asperifolia*, PRC=Piroxicam, DW= distilled water.

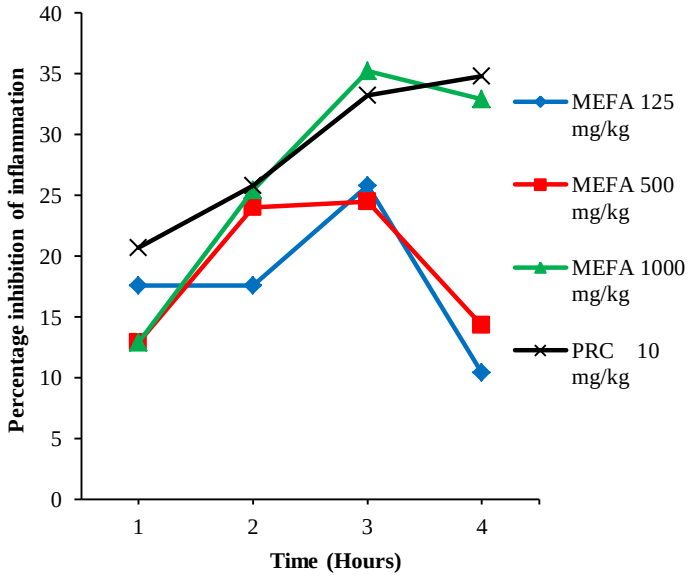

Figure 1b: Percentage inhibition of inflammation by methanol leaf extract of *Ficus asperifolia* on carrageenan-induced paw oedema test. MEFA = Methanol leaf extract of *Ficus asperifolia*, PRC = Piroxicam, n = 6.

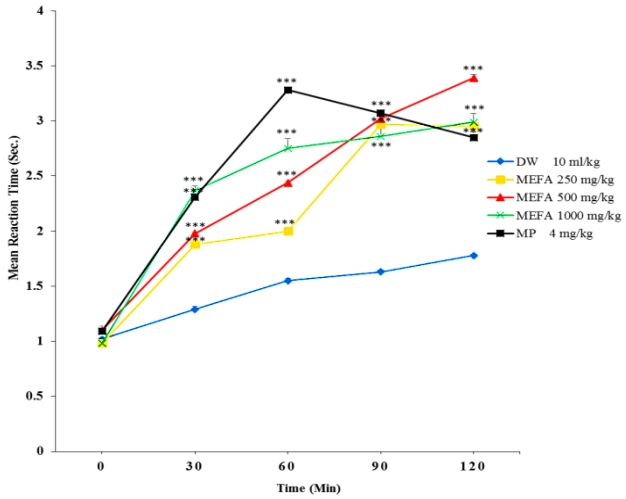

Figure 2: Effect of methanol leaf extract of *Ficus asperifolia* on hot plate test in mice. Values are presented as Mean ± SEM, ***= significant difference at $p<0.001$ compared to DW group - Repeated measure ANOVA followed by Bonferroni post hoc test, n= 6, MEFA = Methanol leaf extract of *Ficus asperifolia* MP = Morphine, DW = Distilled water.

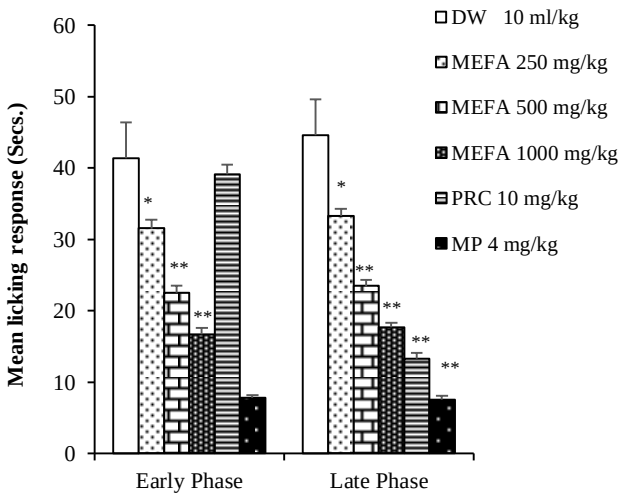

Figure 3a: Effect of methanol leaf extract of *Ficus asperifolia* on formalin-induced pain in mice. Values presented as Mean ± SEM,* and ** significant difference at $p<0.05$ and $p<0.01$ respectively compared DW group – One-way ANOVA followed by Bonferroni test, MEFA=Methanol leaf extract of *Ficus asperifolia*, PRC=Piroxicam, MP=Morphine, DW=Distilled water, n = 6.

In the second phase, the extract treated groups as well as the positive controls (piroxicam and morphine) all produced significant ($p<0.05$) reduction in the paw licking time when compared to the negative control (Figure 3a and 3b). The formalin test is a useful model for analgesia research, particularly for the screening of novel compounds, since it encompasses inflammatory, neurogenic, and central mechanisms of nociception.[24, 45] It differs from most other nociceptive tests in that it requires little or no restraining of experimental animal during testing and the nociceptive stimulus and responses are persistent rather than transient. The bi-phasic nociceptive behaviour of the test is well established; the first phase been attributed to transient receptor potential ankyrin 1 (TRPA1)-mediated excitation of nociceptors, while the second phase to their inflammatory and/or spinal sensitization.[46]

Centrally acting analgesics inhibit both phases, while peripherally acting analgesics like NSAIDs inhibit only the chronic phase.[20, 47] In this research, the ability of methanol leaf extract of *Ficus asperifolia* to inhibit both phases of the formalin-induced pain further support its peripheral and central acting analgesic properties.

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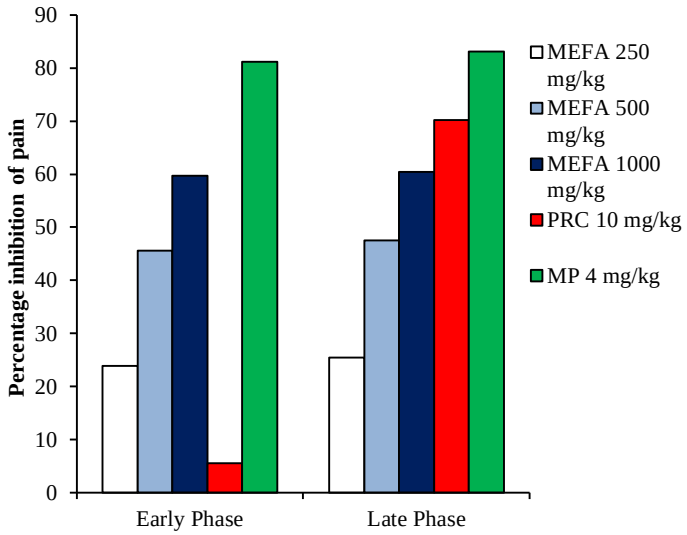

Figure 3b: Percentage inhibition of pain by methanol leaf extract of *Ficus asperifolia* on formalin-induced pain test. MEFA=Methanol leaf extract of *Ficus asperifolia*, PRC=Piroxicam, MP=Morphine, n = 6.

Conclusion

The results obtained demonstrated that methanol leaf extract of *Ficus asperifolia* possesses anti-inflammatory and analgesic properties. This provides scientific credence for its use in ethno-medicine against inflammatory conditions and pain.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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