Antipyretic and Antinociceptive Effect of the Methanol Leaf Extract of *Ficus asperifolia* in Murine Models

Ibrahim Doma Abdullahi[*1], Ibrahim Haruna Sani[2], Mustapha Ibrahim Gudaji[3], Abdullahi Hamza Yaro[4]

[1]Department of Pharmacology and Therapeutics, Bayero University, Kano, Nigeria
[2]Department of Clinical Pharmacology and Therapeutics, College of Health Sciences, Yusuf Maitama Sule University, Kano, Nigeria
[3]Department of Psychiatry, Faculty of Clinical Science, Bayero University, Kano
[4]Department of Pharmacology and Therapeutics, Bayero University, Kano, Nigeria

Submitted: 18th Oct., 2023; Accepted: 14th Feb., 2024; Published online: 29th Feb., 2024
DOI: https://doi.org/10.54117/jcbr.v4i1.2
**Corresponding Author: Ibrahim Doma Abdullahi; ibdomaa@yahoo.com; +2348037019253*

Abstract

Pyrexia occurs as a result of the resetting of the hypothalamic set-point. Non steroidal anti-inflammatory drugs used for the treatment of fever and related illnesses are associated with side effects including gastrointestinal irritation, bleeding, ulcers and perforation. In traditional medicine, many plants have been found to possess antipyretic activity. *Ficus asperifolia* (Miq), family Moraceae has been traditionally used to treat pain and fevers. The aim of the study was thus to evaluate the antipyretic and antinociceptive activity of the methanol leaf extract of *Ficus asperifolia* (FME) in murine models. Preliminary phytochemical screening and oral acute toxicity studies were conducted using standard protocols. Antipyretic activity was evaluated using the Brewer's yeast induced pyrexia model. Antinociceptive effects were investigated using the acetic acid and thermal induced models. The mechanism of action of the plant was also evaluated against several antagonists. FME significantly ($p<0.05$) and dose dependently reduced yeast induced pyrexia. FME at doses 125, 250 and 500 mg/kg significantly ($p<0.05$) decreased acetic acid induced abdominal writhes and increased the mean reaction time of mice to thermal-induced pain stimulus. Pre-treatment of mice with naloxone (a non-selective opioid receptor antagonist), glibenclamide (a potassium channel blocker) and L-NNA (a nitric oxide synthase inhibitor) significantly reversed the analgesic action of the extract suggesting that the activity is likely mediated via the involvement of these pathways.

Keywords: Antipyretics, Aspirin, Pain, Brewer's yeast, *Ficus asperifolia,* Temperature.

Introduction

Fever, also termed pyrexia, denotes an abnormal rise in body temperature due to various factors like infections, tissue damage, and inflammatory conditions (Sultana *et al*., 2015; Nock *et al*., 2022). Internal factors, known as endogenous pyrogens, such as pyrogenic cytokines (e.g., interleukin-1β, tumor necrosis factor, interleukin-6), directly stimulate the hypothalamus, triggering a fever

response (Croft *et al*., 2000). External factors, called exogenous pyrogens, like specific surface components of microbes (e.g., lipopolysaccharides), probably induce fever by prompting the production of pyrogenic cytokines that affect the hypothalamus, similar to interleukin-1β (Nock *et al*., 2022). These signals prompt the release of other agents, with prostaglandin E2 (PGE2) being a significant contributor in the pre-optic area of the hypothalamus (POAH) (Sultana *et al*., 2015; Nock *et al*., 2022). PGE2, among various prostaglandins, has been identified as a pivotal player in the fever response. Neurons in the pre-optic region have E-prostanoid receptors on their membranes, which, upon activation by PGE2, modify their activity, leading to an increase in the fever set point (McKay *et al*., 1996; Mora *et al*., 2013). PGE2 has been confirmed as the downstream mediator in the fever response among other prostaglandins (McKay *et al*., 1996; Mora *et al*., 2013).

The plant *Ficus asperifolia* is found in savannah regions, especially along river banks and marshy areas (Ojo and Akintayo, 2014). It is widely distributed across Africa. Its presence has been reported in Senegal, Cameron, Sudan, Nigeria, Central and East Africa (Nkafamiya *et al*. 2010; Omoniwa and Luka, 2012). The common names of the plant include; **English** *Sandpaper tree,* **Nupe/Hausa** *Kawusa,* **Yoruba** *Ipin* and **Igbo** *Anmerenwa* or *Asesa* (Burkill, 1997). Traditionally, people have used the plant latex, leaf, bark, and root to treat various health issues such as headaches, menstrual pain, gum inflammation, malaria, diabetes, high blood pressure, dysentery, liver ailments, urinary and respiratory infections, and even tumors (Burkill, 1997; Arbonnier, 2004; Watcho *et al*. 2009).

The water-based fruit extract from the plant has been found to exhibit uterotonic effects, while research by Nkafamiya *et al*. (2010) highlighted that the crude fiber from *Ficus asperifolia* leaves contains higher levels of protein and minerals compared to certain Nigerian vegetables. The plant has also been reported to possess gastroprotective and hypoglycemic properties (Raji *et al*., 2011; Omoniwa and Luka, 2012). Analysis of *Ficus asperifolia* leaves identified phytochemical constituents like saponins, tannins, cardiac glycosides, terpenes, steroids, and flavonoids (Omoniwa *et al*., 2013). Various studies have established the antibacterial activity of the aqueous bark extract of the plant (Nwanko and Ukaegbu-obi, 2014) and antioxidant potential of the aqueous leaf extract (Ojo and Akintayo, 2014), and antimicrobial properties of the essential oils extracted from the leaves of the plant (Lawal *et al*., 2016). Recent investigations have demonstrated the significant anti-inflammatory and analgesic activities of the methanol leaf extract of *Ficus asperifolia* (Abdullahi *et al*., 2020).

Materials and methods

Preparation of plant material

Fresh leaves of *Ficus asperifolia* Miq were collected from Toro district, Toro Local Government Area of Bauchi State, Nigeria. The plants were identified and authenticated by Baha'uddeen Said Adam of the herbarium unit of the Department of Biological Sciences, Bayero University, Kano, Nigeria. A voucher specimen number BUKHAN 0106 was collected for future reference.

Experimental Animals

Wistar rats and Swiss albino mice of both sexes, weighing between 100-150g and 20-25g respectively, were sourced from the

Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. These animals had unlimited access to standard feed and water. They were housed in clean cages with sawdust, changed every three days. The study strictly adhered to ethical guidelines for the use and care of laboratory animals, aligning with Ahmadu Bello University's Research Policy (Revised 2010). The ethical committee at Bayero University approved the utilization of animals in the experimental procedures (BUK/CHS/REC/VII/54).

Drugs and Chemicals

The drugs and chemicals used for the studies include: Naloxone hydrochloride (Martindale Pharma, Building A2 Glory Park, Glory Park Avenue, Wooburn Green, HP100DF, United Kingdom); Morphine sulphate (Martindale pharma, Essex, England); Piroxicam (Pfizer); Acetic acid (Sigma-Aldrich, Co., 3050 Spruce Street, St Louis MO 63103, USA); Ethyl acetate (MERCK Eurolab); N-Butanol (KESHI, USA); Chloroform (Sigma Aldrich, St. Louis Mo, USA); Carrageenan (Sigma Aldrich, USA); Hydrochloric acid, Sulphuric acid (May and Baker, UK), ferric chloride anhydrous (Avishkar, India), ammonia (Loba chemie, India).

Equipment

Thermostat Oven (DHG-9101, USA), Water Bath (HH-4 ENGLAND Lab science), Electric Weighing Balance (FA2104A, Gulfed Medical and Scientific England), Electronic Hot Plate Digital (DB-1A, PEC MEDICAL USA), Digital Vernier callipers, Animal weighing balance (SF-400), Animal cages, pestle and mortar, syringes (1 ml, 2 ml, 5 ml and 10 ml), What man Filter Paper No. 1, crucibles, separating funnel, conical flask, beakers and retort stand.

Extraction

Fresh leaves of *Ficus asperifolia* were collected from the plant, rinsed with clean water and shade-dried. The plant materials were then pulverized into fine powder using porcelain mortar and pestle and sieved. Powdered plant material weighing 2 kg was macerated with 7 L of 70%v/v 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 What man filter paper (1mm mesh size) and then concentrated on water bath maintained at 45 ^{o}C until greenish black residues were obtained and stored in a desiccator.

Phytochemical Screening

Standard phytochemical tests as described by Trease and Evans, (2009) were employed in screening the crude extract of *Ficus asperifolia* leaf. The extract was screened for the presence or absence of secondary metabolites including alkaloids, flavonoids, saponins, cardiac glycosides, tannins, triterpenes and anthraquinones.

Acute Toxicity Studies (LD_{50})

Oral acute toxicity study of methanol leaf extract of *Ficus asperifolia* was investigated in mice and rats using the method described by Lorke (1983). This was conducted in two phases. In the first phase, mice were divided into 3 groups of three mice each and treated at doses of 10, 100 and 1000 mg/kg, respectively with the FME extract per oral (*p.o*). The treated animals were then observed for 24 h for signs of toxicity and death. In the second phase, mice were divided into 3

groups of one mouse each based on the result of the first phase and were treated with specific doses of 1600, 2900 and 5000 mg/kg. The oral LD_{50} was calculated from the results of the second phase as the geometric mean of the lowest lethal dose and the highest non-lethal dose.

Antipyretic Studies

Brewer's yeast induced pyrexia model

The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats as described by (Kumar *et al*., 2004). Fever was induced by administering 20 ml/kg of 20 % aqueous suspension of Brewer's yeast in normal saline subcutaneously 17 h before treatment. Thirty (30) wistar rats of both sexes were divided into five groups. Groups 1 and 2 served as negative control (distilled water 10 ml/kg) and positive control (Aspirin 300 mg/kg), while groups 3, 4, and 5 received 125 mg/kg, 250 mg/kg, and 500 mg/kg of the extract. All administrations were per oral. Rectal temperatures were taken by the use of digital thermometer (Mediklin, China) before yeast injection, 19 h after the injection, and at 1, 2, 3, and 4 hours after drug administration.

Analgesic studies

Hot plate method

The method described by Eddy and Leimback (1953) 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 125, 250 and 500 mg/kg of methanol leaf extract of *Ficus asperifolia* (*p.o*), while the fifth group (positive control) received 10 mg/kg morphine (*p.o*). 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. A maximum cut off time of 20 seconds of exposure to thermally-induced pain was allowed to observe response in the experiment.

Acetic acid induced writhing in mice

The method described by Koster *et al*., (1959) 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 which served as negative control. Groups 2, 3 and 4 received 125, 250 and 500 mg/kg of *Ficus asperifolia* methanol leaf extract (*p.o*) respectively 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 and the percentage inhibition of writhes was calculated using the relationship below:

$$\text{Percentage (\%) inhibition} = \frac{\text{Average number of writhes in control} - \text{Average number of writhes in test}}{\text{Average number of writhes in control}} \times 100$$

Investigation of possible mechanisms of analgesic activities

Interaction of naloxone with FME as assessed by hot plate test

Six groups of six (6) mice each were used as described by Woode *et al*., (2009). The first group served as control and received 10 ml/kg distilled water. Group 2, received 2 mg/kg

naloxone alone, group 3, was given 500 mg/kg of FME alone and group 4 was also given 4 mg/ml morphine solution alone. Groups 5 and 6 were pretreated with naloxone 2 mg/kg then 15 minutes later 500 mg/kg of FME was given to animals in group 5 and 4 mg/kg of morphine solution to those in group 6. All treatments were administered orally. Sixty minutes after treatment, each mouse was placed on a digital electronic hot plate which was set and maintained at 55 ± 1^0C. The index of pain response latency was assessed at 30-, 60-, 90- and 120-minutes post treatments, using hot plate method (Table 1).

Interaction of glibenclamide with FME as assessed by acetic acid induced writhing test

The participation of pain pathways in the analgesic activity of FME was investigated using mouse model of acetic acid induced writhing as previously described by Koster *et al*., (1959). The pathways investigated involved the administration of glibenclamide (a K^+ ATP channel blocker, 5 mg/kg) together with FME extract to exploit the potassium ATP channel pathway (Table 1) while activity was observed (Figure 2).

Interaction of L-arginine with FME as assessed by acetic acid induced writhing test

The participation of pain pathways in the analgesic activity of FME was investigated using mouse model of acetic acid induced writhing as previously described by Koster *et al*. (1959). To verify the possible involvement of nitric oxide (NO) pathways in the antinociceptive activity of FME, mice were pre-treated with N-L-nitro- arginine methyl ester (L-NNA or L-NAME, a NO synthase inhibitor) and the nociceptive responses to acetic acid induced writhing test were evaluated 60 minutes after administration of FME. Same was carried out with distilled water and L- arginine (NO synthase precursor) pre-treated mice and as shown in (Table 1) while activity was observed (Figure 3).

Table 1: Grouping for mechanistic study

Groups	**Interactive pathways**		
	Opiodergic	**K^*ATP channel**	**Nitric oxide**
I	DW (10 ml/kg)	DW (10 ml/kg)	DW (10 ml/kg)
II	FME(500 mg/kg)	FME(500 mg/kg)	FME (500 mg/kg)
III	Morphine (10 mg/kg)	Piroxicam(10 mg/kg)	Piroxicam (10 mg/kg)
IV	Naloxone (2 mg/kg)	Glibenclamide (5 mg/kg)	L-arginine (50 mg/kg)
V	Naloxone + FME	Glibenclamide + FME	L-arginine + FME
VI	Naloxone + Morphine	Glibenclamide + Piroxicam	L-arginine + Piroxicam
VII	-	-	L-NAME (50 mg/kg)
VIII	-	-	L-arginine + L-NAME

Statistical Analysis

Results were expressed as mean ± standard error of mean and presented as charts and tables. Data obtained for Brewer's yeast and hot plate tests were analyzed using repeated measure ANOVA followed by Bonferroni's post hoc test while data for acetic acid induced writhing test were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Results were considered significant at $p< 0.05$ when compared with the distilled water (DW) control group.

Results

Preliminary phytochemical constituents

The results of the preliminary phytochemical screening of FME revealed the presence of various phytochemicals such as cardiac glycosides, tannins, flavonoids, alkaloids, anthraquinone, saponins, steroids and triterpenes.

Acute toxicity study

The estimated oral median lethal dose of the methanol leaf extract of *Ficus asperifolia* was found to be greater than 5,000 mg/kg in both rats and mice.

Effect of FME on Brewer's yeast-induced pyrexia

The rats in all treated groups exhibited an increase in their rectal temperature 18 hours after receiving Brewer's yeast. The initial measurement at zero hour served as the baseline for all treatment groups and was compared across subsequent hourly intervals at 1, 2, 3, 4, and 5 hours post-administration. The extract showed a dose-depended reduction in the temperature at the second hour which was significant ($p<0.05$) at the highest dose (500 mg/kg). The FME at all the doses showed significant ($p<0.05$) decrease in the rectal temperature at the third, fourth and fifth hour in a dose-dependent (Table 2).

Effect of methanol leaf extract of *Ficus asperifolia* on hot plate test in mice

The FME produced significant ($p<0.05$) increase in pain latency when compared with the negative control. The extract demonstrated activity at both the early and later stage of pain modulation (Tables 3).

Effect of methanol leaf extract of *Ficus asperifolia* on acetic acid-induced writhing test in mice

The FME elicited significant ($p<0.05$) and dose-dependent analgesic activities compared to the negative control. FME (125, 250 and 500 mg/kg) produced a reduction in the number of writhes with percentage inhibition of 85.39, 90.98 and 92.23% respectively (Table 4). However, piroxicam which was used as a standard control produced

significant (p<0.05) analgesic activity with percentage inhibition of 85.85%.

Table 2: Effect of the Methanol Leaf Extract of *Ficus asperifolia* (FME) on Brewer's yeast induced pyrexia in Rats

Treatment	Rectal temperature ± SEM (^{0}C)					
(mg/kg)	0 h	1 h	2 h	3 h	4 h	5 h
DW 1ml/kg	36.61±0.13	36.59±0.33	36.57±0.34	36.58±0.26	37.58±0.29	37.57±0.21
By 20ml/ kg	36.58±0.16	37.48±0.11	38.59±0.04	38.41±0.24	38.40±0.19	38.57±0.01
Asp (100)	36.60±0.09	37.12±0.30	36.87±0.14	36.58±0.36	36.28±0.27*	36.07±0.20*
FME (125)	36.59±0.11	37.29±0.32	36.67±0.08	36.46±0.16*	36.23±0.22*	36.07±0.19*
FME (250)	36.68±0.04	37.15±0.03	36.49±0.44	36.44±0.26*	36.18±0.18*	36.04±0.31*
FME (500)	36.61±0.12	36.54±0.30	36.37±0.32*	36.34±0.19*	36.13±0.17*	36.01±0.11

Values are Mean ± SEM; Data were analyzed using repeated measures ANOVA followed by Bonferroni's post hoc test, *= p<0.05 significant statistical decrease in mean rectal temperature compared to; FME = Methanol leaf extract of *Ficus asperifolia* ASP = Aspirin; DW = Distilled water; n= 6.

Table 3: Effect of the Methanol Leaf Extract of *Ficus asperifolia* (FME) on Hot Plate Test in Mice

Treatment (mg/kg)	Reaction time± SEM (Sec) 0 min	30 min	60 min	90 min	120 min
DW (10ml/kg)	1.02±0.01	1.29±0.03	1.55±0.02	1.63±0.02	1.78±0.01
FME (125)	0.98±0.01	1.88±0.03*	2.00±0.01*	2.97±0.02*	2.95±0.03*
FME (250)	1.10±0.04	1.98±0.01*	2.44±0.01*	3.02±0.02*	3.39±0.03*
FME (500)	0.98±0.04	2.37±0.04*	2.75±0.09*	2.86±0.08*	2.99±0.08*
MP (4)	1.09±0.02	2.31±0.06*	3.28±0.03*	3.07±0.02*	2.85±0.05*

Values are presented as Mean ± SEM; Data were analyzed using repeated measures ANOVA followed by Bonferroni post hoc test, *= $p<0.05$ significant statistical increase in mean reaction time compared to; FME = Methanol leaf extract of *Ficus asperifolia* MP = Morphine; DW = Distilled water; n= 6.

Table 4: Effect of FME on Acetic Acid-induced Writhes in Mice

Treatment (mg/kg)	Mean Number of Writhes	%Inhibition
DW (10ml/kg)	24.71± 1.03	-
FME (125)	3.61±0.09*	85.39
FME (250)	2.23 ±0.11*	90.98
FME (500)	1.92 ±0.01*	92.23
PRC (10)	3.99±0.33*	83.85

Values are presented as Mean ± SEM; Data were analyzed using one way ANOVA followed by Dunnett's post hoc test, *= $p<0.05$ statistically significant decrease in mean number of writhes compared to; FME= Methanol leaf extract of *Ficus asperifolia*; DW = Distilled water; PRC= Piroxicam; n=6.

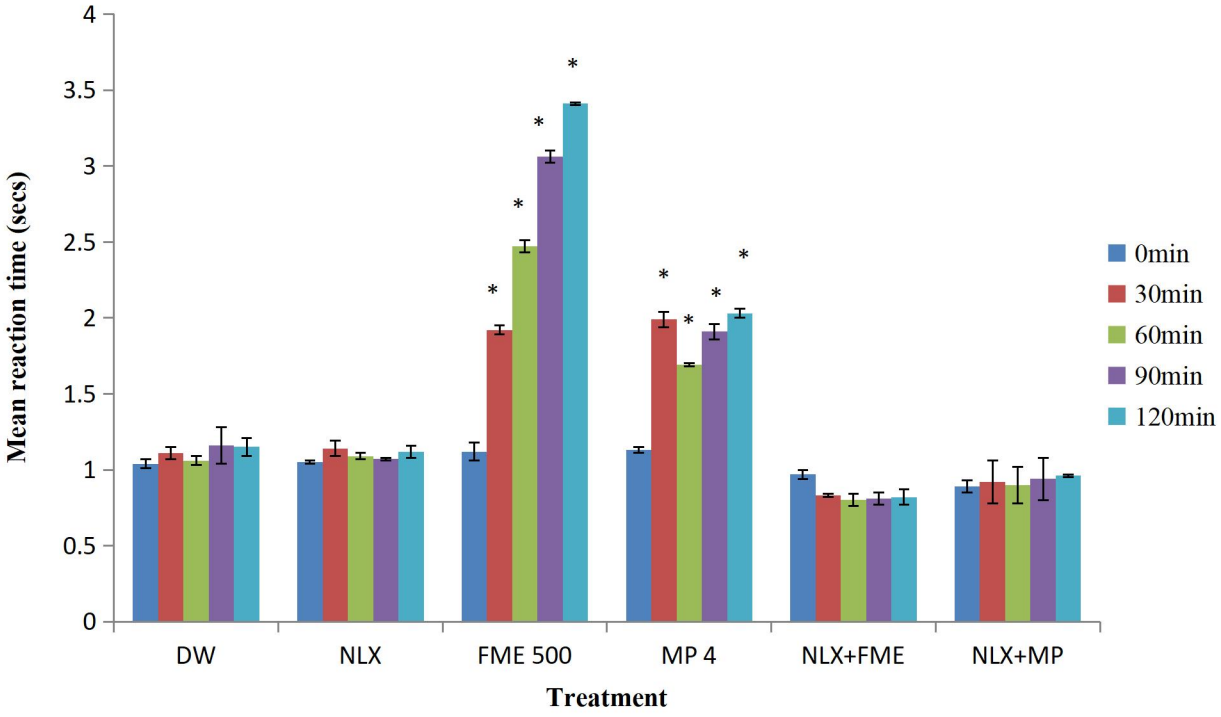

Figure 1: Effect of the Interactive Study between FME and Naloxone. Values are presented as Mean ± SEM; Data were analyzed using repeated measures ANOVA followed by Bonferroni post hoc test, *= $p<0.05$ significant statistical increase in mean reaction time compared to; FME=Methanol extract of *F. asperifolia*, MP = Morphine; NLX = Naloxone; DW = Distilled water; n= 6

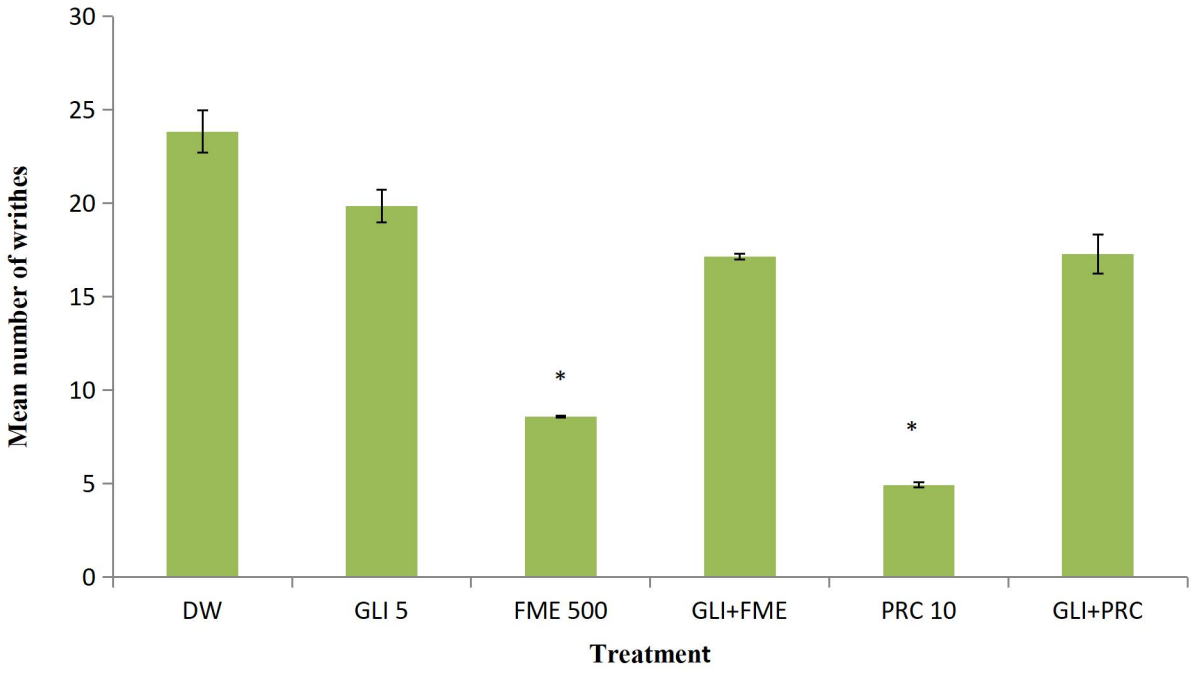

Figure 2: Effect of Interaction between Glibenclamide and Methanol Leaf Extract (FME) of *Ficus asperifolia* on Acetic acid Induced Writhing test. Values presented as Mean ± SEM; Data were analysed using one way ANOVA followed by Dunnett's post hoc test, *=$p<0.05$ significant statistical difference compared to DW group. DW=Distilled water, FME = Methanol leaf extract of *F. asperifolia*, GLI = Glibenclamide, PRC=Piroxicam, n=6.

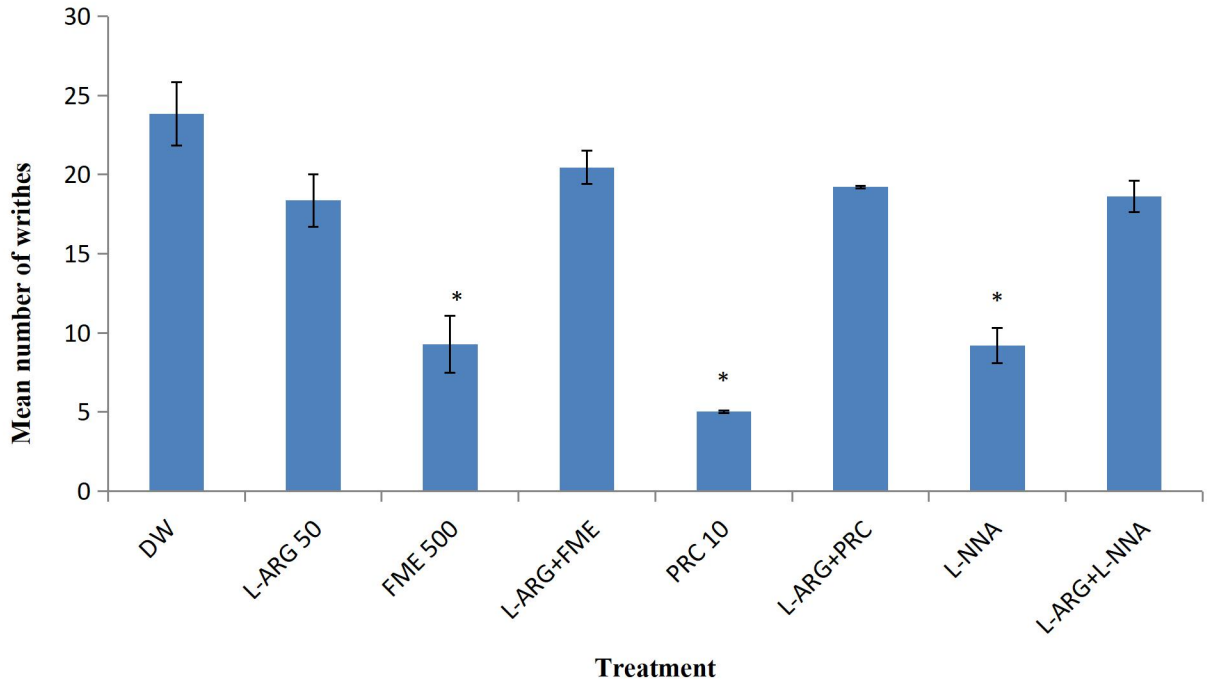

Figure 3: Effect of Interaction between L-NNA and Methanol Leaf Extract (FME) of *Ficus asperifolia* on Acetic acid Induced Writhing test. Values presented as Mean ± SEM; Data were analyzed using one way ANOVA followed by Dunnett's post hoc test, *=$p<0.05$ significant statistical difference compared to DW group. DW=Distilled water, FME = Methanol leaf extract of *F. asperifolia*, L-ARG=L-arginine, L-NNA= N-L-nitro- arginine methyl ester, PRC=Piroxicam, n=6.

Discussion

The leaves of *Ficus asperifolia* have been used traditionally to treat various conditions including pain, fever and inflammatory conditions. The present study attempted to investigate the anti-nociceptive and antipyretic activities of the methanol leaf extract of *Ficus asperifolia* in mice and rats.

Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, anthraquinones, cardiac glycosides, tannins, saponins, steroids and triterpenes which agrees with reported work of Omoniwa *et al*., (2013) and Abdullahi *et al*., (2020). These phytochemicals obtained from plants have shown anti-inflammatory and analgesic activities using animal models (Adebayo *et al*., 2015).

Oral administration of *Ficus asperifolia* leaf extract did not produce any sign of toxicity or death in both the first and second phase of the study. The examination of acute systemic toxicity involves studying the harmful impacts that arise when organisms are exposed to a single or multiple doses of a test substance within 24 hours through a known pathway (Subramanian *et al*., 2018). Evaluating the acute toxic potential of substances is crucial to identify potential adverse effects resulting from unintentional or intentional short-term exposure (Clemedson *et al*., 2000). Moreover, findings from acute toxicity tests help in determining appropriate

dosages for long-term toxicity investigations and other research involving animal use (Colerangle *et al*., 2017). Based on the estimated oral LD_{50}, the methanol leaf extract of *Ficus asperifolia* is considered practically safe in mice and rats (Loomis, 1996).

Administration of Brewer's yeast in mice through the subcutaneous route triggers a fever by increasing prostaglandin synthesis. This makes it a useful model for testing the effects of natural or synthetic drugs on reducing fever (Devi *et al*., 2003; Olorukooba *et al*., 2018). This type of fever, induced by yeast, is known as pathogenic fever, likely caused by prostaglandin production. Hence, the potential antipyretic action of drugs could involve inhibiting prostaglandin synthesis, achievable by blocking the cyclooxygenase enzyme (Hullati and Sharada, 2007). The decrease in temperature observed in the present study suggests that the methanol leaf extract of *Ficus asperifolia* reduces fever in a dose-dependent manner, similar to the standard drug. The mechanism behind its temperature-lowering effect might involve inhibiting prostaglandins through the blockade of cyclooxygenase enzyme activity.

Fever is controlled by the body's immune response, which triggers the release of natural substances that lower body temperature (Jordan *et al*., 1999). Two theories explain how antipyretics work: one involving the cyclooxygenase enzyme and the other unrelated to it (Xu *et al*., 2013). In the cyclooxygenase-dependent theory, antipyretics like aspirin inhibit prostaglandin production by blocking COX (Xu *et al*., 2013). Aspirin and other NSAIDs inhibit COX-2 by disrupting its transcription through a regulator called nuclear factor-kB (NFkB) (Xu *et al*., 2013). The non-cyclooxygenase theory suggests that antipyretics might reduce fever by suppressing tissue inflammation, decreasing interactions between white blood cells and blood vessel walls (Pierce *et al*., 1996), lowering the production of fever-causing cytokines (Ahrens, 1996), increasing anti-inflammatory molecules (Ahrens, 1996), or enhancing the function of natural fever-reducing messengers (Wilkinson and Kasting, 1990). The study revealed that the methanol leaf extract of *Ficus asperifolia* possesses antipyretic activity which is comparable to that of the standard drug aspirin.

The study investigated analgesic activity using the hot plate and the acetic acid induced mouse writhing tests. The hot plate model serves as a standard approach for assessing centrally mediated pain relief (Eddy and Leimbach, 1953; Malami *et al*., 2009). Narcotic substances like morphine, pentazocine, and codeine exert their pain-relieving effects through this central mechanism (Leonard *et al*., 2006). The observed pain relief from the methanol leaf extract (FME) might stem from its activity via a central mechanism similar to narcotic painkillers. These centrally acting analgesics work by elevating the pain threshold and changing the body's response to pain. Reactions like paw-licking, sudden movements, and jumping observed in mice during the test are integrated above the spinal level (Gupta and Bhatia, 2008). The centrally mediated activity produced by both FME and morphine was nullified when pretreated with naloxone. Naloxone, a broad-spectrum opioid receptor antagonist, can block mu (μ), kappa (κ), and delta (δ) receptors (Tripathi, 2013). This suggests that the extract might have interacted with these receptors to produce its central pain-relieving activity. Pre-administration of naloxone, a non-specific opioid antagonist, also negated the pain-relieving effects of FME, indicating the

potential involvement of opioid receptors and/or the body's natural opioids in the pain relief caused by the extract (Bovill, 1997; Kiran and Sinha, 2015).

The abdominal constriction response triggered by acetic acid serves as a sensitive method to assess both peripherally acting pain relievers like NSAIDs (Gene *et al*., 1998) and also centrally acting painkillers like morphine (Donkor *et al*., 2013). NSAIDs such as piroxicam diminish the writhing caused by acetic acid by preventing the release and/or creation of inflammatory pain signals (Donkor *et al*., 2013). FME demonstrated analgesic effects by effectively reducing the pain induced by acetic acid. This ability of FME to alleviate acetic acid-induced pain suggests that it might generate its pain-relieving effect by inhibiting COX in peripheral tissues, potentially by obstructing the release and/or production of inflammatory mediators. Additionally, there could be a central action of FME in reducing the sensory impact of acetic acid. The extract's analgesic effectiveness using this pain model could be attributed to its action on peritoneal receptors, inhibiting the production of arachidonic acid derivatives, or by hindering the transmission of pain signals to the central nervous system (Sini *et al*., 2010). NSAIDs function by impeding the conversion of arachidonic acid into the intermediary cyclic endoperoxide, PGH2, which serves as a common substrate for various synthases generating numerous lipid mediators, including major prostaglandins (PGE2, PGD2, and PGF2α), thromboxane A2 (TXA2), and prostacyclin (PGI2) (Su *et al*., 2011). The decrease in the number of writhes induced by the extract suggests that the analgesic effect may be mediated peripherally through the inhibition of prostaglandin and other endogenous substance synthesis and release (Hassan *et al*., 2015).

Potassium ATP (KATP?) channels, present in both the central and peripheral nervous systems (Yamada and Inagaki, 2005), play diverse roles in neuronal functions like neuroprotection, regulation of neurotransmitter release, and control of membrane excitability (Yamada and Inagaki, 2005; Miki and Seino, 2005; Soundarapandian *et al*., 2007). Notably, the activation of KATP channels and subsequent cellular hyperpolarization are implicated in the pain-relieving effects of various drugs with different mechanisms, such as aspirin, ketorolac (Lazaro-Ibanez *et al.,* 2001), baclofen, and a2-AR agonists (Ocana and Baeyens, 1993).

In our research, GLIB notably reduced the pain-relieving effect of FME, confirming the significant role of KATP channels in pain relief produced by a2-AR agonists. Pinpointing the exact location of KATP channel blockade is challenging, but potential sites include the spinal, brain, and peripheral levels (Ocana, 2004). The absence of KATP current potentially contributes to neuropathic pain through various mechanisms like increased membrane excitability, amplification of neurotransmitter release, and modulation of neuronal excitability (Yamada and Inagaki, 2005; Miki and Seino, 2005; Nichols, 2006; Soundarapandian *et al*., 2007). This therefore suggests that FME might have caused decreased membrane excitability, decreased in neurotransmitter release and decrease neuronal excitability to abolish pain sensations at peripheral levels.

Nitric oxide (NO) is a constantly synthesized soluble gas from L-arginine amino acid in endothelial cells by the nitric oxide synthase (NOS) enzyme (Mayer and Hemmensy, 1997).

Nitric oxide is known to play a complex part in the transmission of nociceptive signals peripherally and centrally (Cury *et al*. 2011; Galdino *et al*. 2015). The pre-treatment of mice with L-NNA a nitric oxide inhibitor largely reversed the analgesia caused by FME and piroxicam as well the analgesia caused by l-arginine a substrate of nitric oxide synthase, when assessed in the acetic acid-induced writhing test in mice. The marked reversal of FME anti-nociceptive activity by NOS antagonist, L-NNA, is an indication of the possible involvement of NO cGMP pathway in the extract's mode of anti-nociceptive activity. Furthermore, pre-treatment with naloxone, a non-selective opioid antagonist also blocked the anti-nociceptive effects of FME which might suggest the involvement of opioid receptors and/ or endogenous opioids in the anti-nociceptive effects of the extract (Bovill 1997; Kiran and Sinha 2015).

In conclusion, it was observed that pre-treatment of mice with naloxone (a non-selective opioid receptor antagonist) and glibenclamide (a potassium channel blocker) each significantly reversed the analgesic action of the extract. Also, pre-treatment of animals with L-NNA (a nitric oxide synthase inhibitor), significantly reversed the analgesic action of the extract thereby suggesting the involvement of nitric oxide pathways in the analgesic activity of methanol leaf extract of *Ficus asperifolia*.

The methanol extract of *Ficus asperifolia* leaf has demonstrated significant antipyretic and analgesic activities. The observed analgesic activity of methanol leaf extract of *Ficus asperifolia* is likely mediated via the involvement of opioidergic, potassium ion channels and the nitric oxide pathways. These findings confirm its traditional use in the treatment of pain and management of malaria symptoms.

Acknowledgement

The authors acknowledged the technical support of Malam Aliyu Ahmad of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical sciences, Bayero University, Kano, Nigeria.

Conflict of Interest

The Authors have declared that there is no any conflict of interest with regards to this work or its publication.

References

Abdullahi ID, Yaro AH and Nazifi AB (2020): Anti-nociceptive and anti-inflammatory activities of solvent fractions of methanol leaf Extract of *Ficus asperifolia* miq. (Moraceae) in murine models. Nig. J. Pharm. sci. Res. Vol. 19 No.2, 25-37.

Adebayo SA, Dzoyem JP, Shai LJ, Eloff JN (2015): The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. BMC Compl Alt Med. Res. 15:1-10.

Arbonnier M (2004): Trees, Shrubs and Lianas of West African Dry Zones. 1st Edn., CIRAD, Margraf Publishers GMBH MNHN,USA., Pp:574.

Ahrens FA (1996): Pharmacology. 1st, Wiley, Iowa ed: Wiley. P: 328.

Bovill J (1997): Mechanisms of actions of opioids and non-steroidal anti-inflammatory drugs. Eur J Anaesthesiol. 14:9–15.

Burkill HM (1997): The Useful Plants of West Tropical Africa. Vol. 4 2nd edition. Royal Botanic Gardens, Kew, Richmond, Survey TW 8 3AE Pp 293.

Clemedson C, Barile FA, Chesne C, Cottin M, Curren R, Eckwall B, Ferro M, Gomez-Lechon MJ, Imai K, Janus J, Kemp RB, Kerszman G, Kjellstrand P, Lavrijsen K, Logemann P, McFarlane-Abdulla E, Roguet R, Segner H, Thuvander A, Walum E, Ekwall B (2000): Evaluation of acute systemic toxicity. Part VII. Prediction of human toxicity by results from testing of the first 30 reference chemicals with 27 further in vitro assays. ATLA. Res. 28:159-200.

Colerangle JB (2017): Preclinical development of nononcogenic drugs (Small and large molecules). (2nd ed). In: Faqi AS (Ed). A comprehensive guide to toxicology in nonclinical drug development. U.K.: Academic Press. 659-683 p.

Croft A, Duffield T, Menzies P, Leslie K, Bagg R, Dick P (2000): The effect of tilmicosin administered to ewes prior to lambing on incidence of clinical mastitis and subsequent lamb performance. Can. Vet. J. 41:306.

Cury Y, Picolo G, Gutierrez VP, Ferreira SH (2011): Pain and analgesia: the dual effect of nitric oxide in the nociceptive system. Nitric Oxide. 25:243–254.

Devi BP, Boominathan R and Mandal SC (2003): Evaluation of antipyretic potential of Cleome viscosa Linn. (Capparidaceae) extract in rats. Journal of Ethnopharmacology; 87(1):11–13.

Donkor K, Stephen A, Jerry A, Nutifafa T, Nii OM and Laud KO (2013): Analgesic and Anti-inflammatory Activities of *Asena*, A Herbal Preparation for Treatment of Arthritis, using Rodent Models. Medicinal and Aromatic Plant Research Journal. 1(2): 20-29.

Eddy NB and Leimbach, D.J. (1953): Synthetic Analgesic II-Dithienbutyl and Dithienyl butylamine. J. Pharmacology and Experimental Therapeutics, 107(3): 385 – 393.

Galdino G, Duarte I, Perez A (2015): Central release of nitric oxide mediates antinociception induced by aerobic exercise. Braz J Med Biol Res. 48:790–797.

Gene RM, Segura L, Adzet T, Marin E and Igelsias J (1998): *Heterotheca inuloides*: Inflammatory and Analgesic effects. J. Ethnopharmacol. 60, 157-162.

Gupta P and Bhatia V (2008): Corticosteroid Physiology and Principles of Therapy. Indian J. of Pediatrics. 75: 1039-1044.

Hassan FI, Zezi AU, Yaro AH and Danmalam UH (2015): Analgesic, Anti-inflammatory and Antipyretic activities of the Methanol Leaf Extract of *Dalbergia saxatilis* in Rats and Mice. J. Ethnopharmacology. 166(1) 74-78.

Hullati KK and Sharada MS (2007): Comparative antipyretic activity of path: An Ayurvedic drug. Pharmacognosy Magazine; 3:173-176.

Jordan F, Forrester C, Hodge A, Reeve-Johnson L (1999): The comparison of an aqueous preparation of tilmicosin with tylosin in the treatment of Mycoplasma gallisepticum

infection of turkey poults. Avian Disease. pp. 521-525.

Kiran A and Sinha AN (2015): Elucidation of analgesic effects of butorphanol compared with morphine: A prospective cohort study. J Biol Life Sci. 6:130–140.

Koster, R., Anderson, M. and De-Beer, E. J. (1959). Acetic acid for analgesics screening. Federal proceedings, 18: 412-417.

Kumar R, Clermont G and Vodovotz Y (2004): The Dynamics of Acute Inflammation: J. of Theoretical Biology. 230: 145–155. 53

Lawal OA, Adebayo MA, Sikiru AA and Ogunwande IA (2016): Chemical composition and antimicrobial activity of essential oils of *Ficus asperifolia* Miq. and *Ficus capensis* Thunb From Nigeria. J. Essential Oil Bearing Plants. 19: 1693- 1700.

Lazaro-Ibanez GG, Torres-Lopez JE, Granados-Soto V (2001): Participation of the nitric oxide-cyclic GMP-ATP-sensitive K(+) channel pathway in the antinociceptive action of ketorolac. Eur. J. Pharmacol. 426 39–44.

Leonard JP, Daniel SB. and Yonoli XMS (2006): Increasing Death from OpioidAnalgesics in the United States. J. Pharmacoepidemiology andDrug safety. 19(3):54-67.

Loomis TA, Hayes AW. Loomis's essentials of toxicology. (4th ed), California: Academic press; 1996. 17-32 p.

Lorke D (1983): A New Approach to Practical Acute Toxicity Testing. Archives of Toxicology, springer –Verlag, 54: 275-287.

Magaji MG, Yaro AH, Adamu A, Yau J, Malami S, Abubakar Y and Hussaini IM (2009): Some neuropharmacological studies on hydroalcoholic extract of *Maerua angolensis* DC (Capparidaceae) in mice and chicks. Int. J. Pure and Applied Sciences. 3**:** 14 - 21. 34

Malami S, Kwanashie HO and Hussaini IM (2009): Anticonvulsant effects of methanol extract of *Ficus vallis* - *choudae* (moraceae) stem bark. Nig. J. Pharmaceutical Sciences; Vol. 9 No. 1, P. 44 – 51.

Mayer B, Hemmens B (1997): Biosynthesis and action of nitric oxide in mam malian cells. Trends Biochem Sci. 22:477–481.

McKay S, Morck D, Merrill J, Olson M, Chan S, Pap K (1996): Use of tilmicosin for treatment of pasteurellosis in rabbits. American Journal of Vetenary Research; 57:1180-1184.

Miki T and Seino S (2005): Roles of KATP channels as metabolic sensors in acute metabolic changes. J. Mol. Cell. Cardiol. 38 917–925. 36

Mora N, Uribe-Querol E, Rosales C (2013): Molecular Aspects of Inflammation. In: Pérez-Martínez L, Pedraza-Alva G, Osorio EF, editors. Research signpost. Kerala, India: Research Signpost. pp.15-41.

Nichols CG (2006): KATP channels as molecular sensors of cellular metabolism. Nature. 440 470–476.

Nkafamiya II, Osemeahon SA, Modibbo UU and Aminu A (2010): Nutritional Status of Non-conventional Leafy Vegetables,*Ficus*

asperifolia and *Ficus sycomorus.* African J. Food Science 4(3): 104-108.

Nwanko IU and Ukaegbu-Obi KM (2014): Preliminary Phytochemical Screening and Antibacterial Activity of two Nigerian Medicinal Plants (*Ficus asperifolia* and *Terminalis catappa*). J. Medicinal Plant and Herbal Therapy Research, 2: 1-5.

Nock DD, Abraham IG and Ahmad MH (2022): Potential Pharmacological Properties of Methanol Leaves Extract of *Culcasia angolensis* (Araceae): Antinociceptive, Antiinflammatory and Antipyretic Activities in Laboratory Animals. J. Basic and Applied Zoology; 83:7 https://doi.org/10.1186/s41936-022-00269-8

Ocana M and Baeyens JM (1993): Differential effects of K+ channel blockers on antinociception induced by alpha 2-adrenoceptor, GABAB and kappa-opioid receptor agonists. Br. J. Pharmacol. 110 1049–1054.

Ocana M, Cendan CM, Cobos EJ, Entrena JM, Baeyens JM (2004): Potassium channels and pain: present realities and future opportunities. Eur. J. Pharmacol. 500 203–219.

Ojo AO and Akintayo CO (2014): Assessment of Antioxidant Activity of *Ficus asperifolia* Miq aqueous extract –Invitro studies. J. Phytopharmacology. 3(1):16-21

Olorukooba AB, Ejiofor JI and Anafi SB (2018): Evaluation of anti-nociceptive, anti-inflammatory and antipyretic activities of the n-butanol Fraction of *Uapaca togoensis* pax in Mice and Rats

Omoniwa BP and Luka CD (2012): Antidiabetic and toxicity evaluation of aqueous stem extract of *Ficus asperifolia* in normal and alloxan-induced diabetic albino rats. Asian J. Experimental Biological sciences; 3:726-732.

Omoniwa BP, Luka CD and Soji-Omoniwa O (2013): Effect of aqueous leaf extract of *Ficus asperifolia* on cardiac enzymes and lipid profile in male albino rats. J. Medical Sciences, 13: 373-378.

Pierce JW, Read MA, Ding H, Luscinskas FW, Collins T (1996): Salicylates inhibit I kappa B-alpha phosphorylation, endothelialleukocyte adhesion molecule expression, and neutrophil transmigration. J. Immunol. 156:3961-3969.

Pierre W, Esther N, Pepin AN, Telesphore BN and Albert K (2009): Evaluation of In-vitro uterotonic activities of fruit extracts of *Ficus asperifolia* in rats. Evidence-Based Complementary and Alternative Medicine, 2(11): 7.

Raji Y, Oyeyemi WA, Shittu ST and Bolarinwa AF (2011): Gastro-Protective Effect of methanol stem bark extract of *Ficus asperifolia* on Indomethacin induced gastric ulcer in rats. Nigerian J. Physiological Sciences; 26: 43-48.

Sini JM, Yaro AH, Ayanwuyi LO, Aiyelero OM, Mallum SM and Gamaniel KS (2010): Antinociceptive and anti-inflammatory activities of the aqueous extract of the root of *Combretum sericeum* in rodents. African J. Biotechnology, 9 (51): 8872-8876.

Soundarapandian MM, Zhong X, Peng L, Wu D and Lu Y (2007): Role of K(ATP) channels in protection against neuronal excitatory insults. J. Neurochem. 103 1721–1729.

Su F, Nguyen ND, Wang Z, Cai Y, Rogiers P and Vincent JL (2011): Fever control in septic shock: beneficial or harmful? Plos Medicine, 23 (6): 516–520.

Subramanian K, Sankaramourthy D and Gunasekaran M (2018): Toxicity studies related to medicinal plants. In: Mandal SC, Mandal V, Konishi T (Eds). Natural Products and Drug Discovery: An Integrated Approach. U.K.: Elsevier. 491-505 p.

Sultana S, Asif HM, Akhtar N and Ahmad K (2015): Asian Pacific journal of tropical disease medicinal plants with potential antipyretic activity : A review. Asian Pacific J. Tropical Disease, 5(Suppl 1), S202 – S208. https:// doi. org/ 10. 1016/ S2222- 1808(15) 60890-8

Trease GE and Evans WC (2009): Pharmacognosy 16th Ed. Bailliere Tindall Ltd., London, pp: 60-75.

Tripathi KD (2013): Essential of Medical Pharmacology. Jaypee Brothers Medical Publisher. 7th Edition. Pp 219-220.

Watcho P, Ngadjui E, Nkeng-Efouet PA, Nguelefack TB and Kamanyi A (2009): Evaluation of in-vitro uterotonic activities of fruit extracts of *Ficus asperifolia* in rats. Evidence Based Complimentary Alternative Medicine; 783413: 1-7.

Wilkinson MF, Kasting NW (1990): Central vasopressin V1-blockade prevents salicylate but not acetaminophen antipyresis. J. Appl. Physiol. 68:1793-1798.

Woode E, Boakye-Gyasi E, AK, Ainooson GK, Ansah C and Duwiejus M (2012): Anti-nociceptive effects and the mechanism of *Palisota hirsuta* K. schum leaf in murine models International journal of Pharmacology. 5(2): 101-103.

Xu Z, Zhou J, Cai J, Zhu Z, Sun X and Jiang C (2013): Anti-inflammation effects of hydrogen saline in LPS activated macrophages and carrageenan induced paw oedema. J. Information Sciences. 9: 2.

Yamada K. and Inagaki N (2005): Neuroprotection by KATP channels. J. Molecular Cell. Cardiolog. Res.38, 945–949.