Niger. J. Physiol. Sci. 36 (2021): 221 – 226
www.njps.physiologicalsociety.com
https://doi.org/10.54548/njps.v36i2.11

Niger. J. Physiol. Sci.

Full-Length Research Article

Ficus vogelii Methanol Leaf Extract Possess Anti-Arthritis, Anti-Inflammatory and Membrane Stability Potentials in C57BL/6J Mice

***Nwaehujor C.O.[1], Adika O.A. [2], Uwagie-Ero E.A.[3], Igile, G.O.[1]**

[1]*Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar. Calabar, Nigeria*
[2]*Department of Animal Production and Health, Faculty of Agriculture, Federal University Oye-Ekiti. Ekiti State, Nigeria.*
[3]*Department of Surgery, Faculty of Veterinary Medicine, University of Benin, Benin City, Nigeria.*

Summary: The study evaluated the membrane-stabilizing potentials in red blood cells and anti-inflammatory properties in C57BL/6J mice of the methanol leaf extract of *Ficus vogelii*. Animals were treated orally with different doses of the extract (50, 100, 200 mg/kg) for 30 days and their blood was measured for membrane stability at different saline concentrations. Diclofenac (12.5 mg/kg) or Indomethacin (10 mg/kg) was used as standard in the anti-inflammatory studies. The mean corpuscular fragility (MCF) values and their corresponding percentage stabilization increased significantly ($p \leq 0.05$) in the treatment groups compared to the negative control. Treatment of mice with 50, 100 and 200 mg/kg of the extract significantly ($p \leq 0.05$) inhibited carrageenan-induced paw oedema in mice. The highest dose (200 mg/kg) showed lower anti-inflammatory activity compared to Diclofenac (12.5 mg/kg). Daily administration of the extract significantly ($p \leq 0.05$) suppressed adjuvant-induced paw arthritis by day 15 and 30 post arthritis induction. *Ficus vogelii* extract inhibited granuloma formation significantly. The anti-inflammatory effects of methanol leaf extract of *Ficus vogelii* on granuloma formation were comparable to that of Indomethacin (10 mg/kg). In summary, this study showed that the methanol leaf extract of *Ficus vogelii* possessed membrane-stabilizing potentials and anti-inflammatory properties, therefore, providing further proof that the leaves contain an active compound with potent anti-inflammatory activity.

Keywords*: Ficus vogelii*, membrane stability, arthritis, anti-inflammatory, oedema, C57BL/6J mice

**Address for correspondence: E-mail: chinaka_n@yahoo.com; Tel: +2348035450300*

Manuscript received- November 2020; Accepted- October 2021

INTRODUCTION

The cell membrane that surrounds a cell is selectively permeable, allowing only certain molecules to enter and exit the cell. It is well known that mammalian cells are equipped with extensive repair mechanisms that help to constantly maintain the integrity of the cell membrane, thus highlighting the importance of the cell membrane to the survival of cells (Dias and Nylandsted, 2021). In events that lead to the compromise of the health of the cell membrane, the cell becomes susceptible to acute or chronic injuries or completes disruption that may lead to cell death (Cooper and McNeil, 2015; Boye and Nylandsted, 2016). Known cell membrane repair mechanisms includes; membrane fusion and replacement strategies (via exocytosis-mediated repair), removal of damaged membranes (by endocytosis-mediated repair or shedding), and protein-driven membrane remodelling and wound closure (Dias and Nylandsted, 2021). However, these repair mechanisms do not occur individually but co-operate in unison for maximum repair to be effective (Moe *et al*., 2015; Jimenez and Perez, 2017; Dias and Nylandsted, 2021). In all, a rise in the intracellular calcium level, which the cell interprets as danger, is the key stimulus that initiates the cell membrane repair mechanism, intended to rapidly reseal the wound (Draeger *et al*., 2011; Dias and Nylandsted, 2021).

Understanding the process of membrane repair had enabled the identification of therapeutic strategies and boosting of membrane repairs in diseases related to poor membrane integrity (Boye and Nylandsted, 2016). Different therapeutic strategies which are mainly the use of membrane stabilising agents and exogenous expression of several recombinant proteins are currently in use. Membrane stabilization strengthens or reinforces the cell membrane to become stronger and able to withstand influences from the outside environment (Anosike *et al.*, 2012). The use of membrane stabilizers in the management of inflammation and consequently pain, both acute and chronic is gaining wide acceptance (Boniface *et al.*, 2014). They have been used for many years to treat painful conditions such as trigeminal neuralgia, complex regional pain syndrome, and headaches (Honoro *et al.*, 2018). Poloxamer 188, a membrane-stabilising agent is a multi-block copolymer surfactant which seals cell membrane defects in various cell types caused by different types of injuries (Murphy *et al*., 2010; Gu *et al*., 2013; Inyang, *et al*., 2020). Most anti-inflammatory drugs have been reported to have membrane stabilising effects on cell membranes (Ogbomade *et al.*, 2019). The anti-inflammatory actions of steroids such as Vamorolone have been used as a membrane stabilising agent (Sreetama *et al*., 2018; Dias and Nylandsted, 2021). Furthermore, proteins such as the MG53, dysferlin, GRAFI and the annexins have shown enhancement of cell

membrane repairs (Benevolensky *et al*., 2000; Matteo and Moravec, 2000; Cai *et al*., 2009; Lenhart *et al*., 2015).

Inflammatory response of tissues is a defensive and protective mechanism aimed at removing the harmful stimuli causing injuries and subsequently initiates healing processes to the injured tissue (Maldini *et al*., 2009). In the treatment of the consequences of inflammatory conditions, steroids and non-steroidal anti-inflammatory drugs are commonly used. An effective anti-inflammatory drug should potentially inhibit the development of inflammation with fewer side effects on the integrity of the tissues or system (*Fakhrudin et al., 2014*). However, the unpleasant side effects of these agents such as gastrointestinal ulcers (Goldsby *et al*., 2003) have necessitated the search for newer, safer and more effective conventional and herbal-derived anti-inflammatory agents.

Studies have shown that several herbal derived drugs have the properties to be classified as anti-inflammatory agents (Chi *et al*., 2001; Mohini *et al*., 2012; Lui *et al*., 2014; Sarkar *et al*., 2016; Ren *et al*., 2017). Furthermore, some herbal extracts have both anti-inflamatory and membrane stabilising activities (Oyedapo *et al*., 2004, 2020; Arawwawala *et al*., 2010; Debnath *et al*., 2013). The aim of this research work was to evaluate the membrane stabilising effects and anti-inflammatory activities of the extract of the leaf of *F. vogelii* in order to relate its medicinal activities to natural and bioactive constituents. The leaf of *Ficus vogelii*, a commonly used green-leafy vegetable in various dishes in Northern Cross River State of Nigeria is traditionally believed to guarantee good health and well-being. Its ethanol extract is used by adults for well-being, while its aqueous extract is used for weaning children and for treatment of pediatric anemia (Igile *et al.*, 2018). Relevant literature discovered that traditionally, there are claims that the bark and root are used in treating urinary tract infection, asthma, diabetes, malaria, cardiovascular diseases, kidney diseases and cough (Igile *et al.*, 2018).

MATERIALS AND METHODS

Extraction of Plant materials: *F. vogelii* leaves were obtained from Akamkpa Local Government Area in Cross River state, Nigeria were identified at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. The leaves were dried at room temperature (25 °C) without direct sunlight and grinded. The ground leaves (1.5 kg) were extracted by cold maceration with 80 % methanol (Sigma Aldrich, Germany) at room temperature for 48 hours then filtered with a Whatman No. 1 filter paper. The filtrate was concentrated with a rotary evaporator (Büchi, Switzerland) at 40 °C and stored in a refrigerator at 4 °C.

Animal and experimental design: All experiments with animals were approved by the Ethical Committee of the University of Nigeria, Nsukka (Approval Number: UNNRA019/V0411). C57BL/6J mice of both sexes, aged 6 weeks, purchased from the Department of Veterinary Animal Laboratory, University of Nigeria, Nsukka. The mice were acclimatized for 10 days in polyethylene cages with free access to water and a 15 % fat diet in a controlled environment and relative humidity, with a 12 h light-dark cycle. All animals were humanely handled and their welfare respected throughout this study as stipulated in the 1964 Helsinki Declaration, as amended (World Medical Association, 2013)

Acute toxicity test: Thirty (30) mature albino mice of both sexes (27.15±2.31 g) were weighed and randomly separated into 5 groups (A–E) of 6 rats per group. Groups A–D were dosed orally with varying doses (100, 500, 1000, and 2000 mg/kg) of the methanol leaf extract *of F. vogelii* respectively while group E (5th group) was given an equivalent volume of distilled water (10 ml/kg). The mice were allowed access to feed and water *ad libitum* for 72 h and observed for signs of toxicity and death**.**

Osmotic fragility of Red Blood cells in C57BL/6J mice: Twelve (12) mature mice of both sexes (27.15±2.31 g) were weighed and randomly separated into 4 groups (A–D) of 3 rats per group. Group A-C were treated orally with 50, 100 and 200 mg/kg of methanol leaf extract of *Ficus vogelii* respectively. Group D was given distilled water and served as a negative control. Blood was collected from mice in each group into heparinized tubes after a 30-days treatment. For each blood sample, test tubes were numbered from 1 to 17. Five ml of buffered NaCl ranging from 0.1 to 0.85% was placed in tubes 1 through 16. Five ml of distilled water was placed in tube 17. 0.1 ml of blood was added to each tube and mixed gently and then incubated at 37 °C for 1 h. the tubes were centrifuged at 2000 rpm for 10 min to sediment any intact red cells. The haemoglobin content of the supernatant was measured at 540 nm with a spectrophotometer using the 0.85%-saline tube as a blank and the distilled water tube as the 100%-haemolysis as standard. The highest value of optical density which corresponded to an incubation concentration of 0.1% NaCl was taken as 100% haemolysis. Percentage lysis was calculated using the formula:

$$\frac{\text{Abs of test}}{\text{Abs of standard}} \times 100$$

% haemolysis was plotted against % NaCl concentrations. The mean corpuscular fragility (MCF), which is the concentration of NaCl producing 50 % lysis was extrapolated from the graphs and recorded. Also, % stabilization was calculated.

Anti-Inflammatory Studies

Carrageenan-induced paw edema: Thirty (30) mice of both sexes were placed in groups of 6 mice per group and treated orally with either methanol leaf extract of *F. vogelii* at 50, 100, 200 mg/kg, Diclofenac (12.5 mg/kg) or distilled water, 60 min prior to an injection of 1 % carrageenan (Ramchandran *et al*., 2002) into the plantar tissue of the right hind paw. The contra-lateral hind paws were injected with 0.1 ml of normal saline as control. Paw edema volume was measured using an Orchid digital plethysmometer at 0, 1, 2 and 3 h after carrageenan injection.

Adjuvant-induced polyarthritis: The arthritic syndrome was induced in mice by an injection of 0.1 ml of Freund's complete adjuvant into the sub-plantar region of the right hind paw according to methods of Ramchandran *et al.* (2002) and Kasture *et al*. (2001). Twenty-five mice of both sexes were grouped into 5 groups of 5 animals each. The

groups were orally treated with either methanol leaf extract of *F. vogelii* at 50, 100, 200 mg/kg, Diclofenac (12.5 mg/kg) or distilled water (0.03 ml/10 g) for 30 days. Plethysmographic determination of paw volume was performed on both injected and contra-lateral foot. Paw volume after 18 h of adjuvant injection was taken as sub-acute phase of inflammation and that of the 15th and 30th days were observed as an index of chronic inflammation.

Cotton pellet-induced granuloma test: Cotton pellet induced granuloma test was performed as described by Niemegeers *et al.* (1975). Formation of granuloma was induced by subcutaneous implantation of 50 mg of sterile cotton pellets into the left and right axillae of each mouse under pentobarbitone (35 mg/kg) anaesthesia. Twenty-five mice of both sexes were grouped into 5 groups of 5 animals each. Post cotton pellet implantation, each group was treated once daily with either the extract (50, 100 and 200 mg/kg) respectively, or indomethacin (10 mg/kg) or distilled water intraperitoneally for a period of 7 days. On day 8, the animals were euthanized using chloroform and the pellets surrounded by granuloma tissues were carefully dissected out. The moist pellets were weighed and dried at 60 °C for 24 h. The dry pellets were then weighed to obtain their dry weights. The weights of the granuloma formed were calculated as the difference between the wet and dry weights (Udegbunam *et al.*, 2011).

Data Analysis

Median Corpuscular Fragility (MCF) values and % stabilization was calculated by the one-way analysis of variance (ANOVA) using unpaired student's t test. The student t-test was applied at 5 % confidence level. The mean oedema size and granuloma weights of the treated groups and those of the control were compared using one-way analysis of variance. All analyses were done in the SPSS version 16.0. Duncan multiple range test was used to separate variant mean at $P < 0.01$ or 0.05.

RESULTS

Yield and Acute toxicity test: The total yield of the extract was 72 g. Acute toxicity studies revealed no extract-induced mortality or overt serious clinical manifestation even at the highest test dose of 2000 mg/kg.

Median Corpuscular Fragility (MCF) values and % stabilization in C57BL/6J mice treated with methanol leaf extract of *F. vogelii*: The methanol leaf extracts of *F. vogelii* significantly and dose dependently reduced the corpuscular fragility and increased cell membrane stabilization in the test animals. The percentage reductions in corpuscular fragility were 0.277±0.023, 0.317±0.028, 0.387±0.042 for the 50, 100 and 200mg/kg of the extract while the percentage stabilization were 25.34, 43.44 and 75.11 respectively (Table 1).

Effect of methanol leaf extract of *F. vogelii* on carrageenan-induced paw edema in C57BL/6J mice: The effect of the doses (50,100,200mg/kg) of the methanol leaf extracts of *F. vogelii* were comparable to those of the reference drug (diclofenac) and was more pronounced at 3h after the injection of the carrageenan (Table 2).

Table 1:
Median Corpuscular Fragility (MCF) values and % stabilization in C57BL/6J mice treated with methanol leaf extract of *F. vogelii*

	Dose (mg/kg)	MCF (% [NaCl])	stabilization
Ficus vogelii	50	0.277±0.023	25.34[a]
	100	0.317±0.028	43.44[a]
	200	0.387±0.042	75.11[b]
Control	-	0.221±0.015	

The MCF values and their corresponding % stabilization were increased in the treatment groups as compared to the negative control. The highest dose (200 mg/kg) gave the highest % stabilization.

Table 2:
Effect of methanol leaf extract of *F. vogelii* on carrageenan-induced paw oedema in C57BL/6J mice

Treatment	Dose (mg/kg)	Paw volume (ml)			
		0 h	1 h	2 h	3 h
Ficus vogelii	50	0.35 ±0.01	0.35 ±0.02	0.44 ±0.01*	0.48 ±0.03*
	100	0.34 ±0.03	0.33 ±0.01*	0.50 ±0.02	0.51 ±0.03
	200	0.35 ±0.02	0.34 ±0.01*	0.45 ±0.02*	0.44 ±0.02*
Diclofenac	12.5	0.36 ±0.03	0.44 ±0.02	0.31 ±0.02*	0.31 ±0.02*
Distilled water	0.03ml/10g	0.33 ±0.01	0.53 ±0.03	0.59 ±0.03	0.63 ±0.04

*Values are expressed as mean±S.E.M.; n = 6; *P <0.01 compared with negative control*

Effects of methanol leaf extract of *F. vogelii* on Adjuvant-induced paw arthritis in C57BL/6J mice: The result shows a dose dependent effect of leaf extracts of *F. vogelii* on paw inflammation when compared against the positive control. The volume of the paw oedema decreased as the phases progressed from the 0h to Day 30. The doses significantly decreased the paw size at day 30 when compared against the previous phases (Table 3).

Table 3:
Effects of methanol leaf extract of *F. vogelii* on Adjuvant-induced paw arthritis in C57BL/6J mice

Treatment	Dose (mg/kg)	Paw volume (ml)			
		0 h	18 h	Day 15	Day 30
Ficus vogelii	50	0.34 ±0.02	0.58 ±0.03*	0.51 ±0.32	0.45 ±0.02*
	100	0.33 ±0.02	0.56 ±0.02*	0.51 ±0.03	0.41 ±0.03**
	200	0.33 ±0.03	0.59 ±0.03	0.47 ±0.02*	0.42 ±0.02**
Diclofenac	12.5	0.34 ±0.01	0.48 ±0.02**	0.44 ±0.02**	0.35 ±0.01**
Distilled water	0.03ml/10g	0.34 ±0.02	0.77 ±0.04	0.80 ±0.04	0.77 ±0.02

*Values are expressed as mean±S.E.M.; n = 5. *P<0.05; **P<0.01 compared with negative control*

Effect of methanol leaf extract of *F. vogelii* on Granuloma weight in C57BL/6J mice: The methanol leaf extracts of *F. vogelii* significantly and also dose-

dependently reduced the granuloma weight and increased percentage inhibition in mice. The granuloma weights were 0.24±0.03, 0.16±0.02 and 0.11±0.01 for the 50, 100 and 200mg/kg of the extract while the percentage inhibition were 11.11, 40.74 and 59.26 respectively (Table 4).

Table 4:
Effect of methanol leaf extract of *F. vogelii* on Granuloma weight in in C57BL/6J mice

Treatment	Dose	Granuloma wt (g).	% inhibition
Distilled water	1 ml/kg	0.27 ± 0.03 _	
Indomethacin	10 mg/kg	0.09 ± 0.02*	66.67*
Extract	50 mg/kg	0.24 ± 0.03	11.11
Extract	100 mg/kg	0.16 ± 0.02*	40.74*
Extract	200 mg/kg	0.11 ± 0.01*	59.26*

Values are expressed as mean±S.E.M. or %;
*n = 5. *P<0.05; **P<0.01 compared with negative control.*

DISCUSSION

The present study showed that oral administration of 50 mg/kg, 100 mg/kg and 200 mg/kg of the methanol leaf extract of *F. vogelii* significantly reduced the percentage hemolysis of the erythrocytes in comparison to the control. The inhibition of cell membrane lysis in this study could be attributed to the anti-inflammatory activities of the leaf extract as reported in some other studies with extracts (Mounnissamy *et al*., 2008; Debnath *et al*., 2013; Oyedapo *et al*., 2020) Furthermore, the highest dose of 200 mg/kg showed most significant percentage of stabilization and membrane protective capacity with a relatively low flagiligram. This suggests that the methanol leaf extract of *F. vogelii* confers significant level of cell membrane protection to the cells when compared to the control. Similar result was obtained from works on *Pilostigma thonningii*, a plant used in the treatment of various diseases characterized with anaemia, jaundice and fatigue (Fongang *et al*., 2014). Furthermore, osmotic fragility tests are used clinically as a diagnostic and management tool in hereditary spherocytosis, elliptocytosis and other red cell membrane disorder as a measure of cell viability (Brown *et al*., 1983; Da Costa *et al*., 2013). However, they may also be useful in the interpretation of inflammatory and prolonged malfunctions due to high blood pressure and other oedema-associated diseases in poor countries where automated blood-cell counters are readily available. Thus the efficacy of any membrane stabilizing agent is dependent on its ability to either reduce the harmful effects or restore the physiology of the cell membrane that has been disrupted by the toxicant. This is so since the free radicals (superoxide, hydroxyl radicals, hydrogen peroxide, nitric oxide radical) produced as a result of oedema during inflammation sometimes all act to disintegrate cell membranes.

Upon inducement of paw oedema by carrageenan test, the results showed marked reduction with the extract of *F. vogelii* when compared with group treated with the diclofenac and distilled water which served as positive and negative controls respectively. This suggests that the extract may have reduced the oedema, pain and inflammation that is normally associated with tissue injury. Pain and inflammation accompanies tissue damage or injury. Most analgesics act on the peripheral or central nervous system where they either block the generation of impulses at the chemoreceptor sites of pain or raise the threshold of pain. Analgesics also alter the physiological responses to pain, suppress anxiety and apprehension which are essential to the repair process (Chandana *et al.,* 2011). The pathway of the analgesic abilities of the *F. vogelii* which were in phases in this work may not be known presently, however, the analgesic properties have been highlighted in this study. Similar result was reported on a related plant, *Ficus glomerata*, where the early phase of analgesia was attributed to the release of histamine and serotonin and the later phases sustained by the leukotriene and prostaglandins (Menezes *et al.,* 2011). Flavonoids and tannins have been reported to inhibit PG synthesis as identified in work done on *Ficus religiosa* (Yaso *et al.,* 2018) as most NSAIDs have well balance anti-inflammatory and ulcerogenic activities which can be attributed to its PG synthase inhibitor activities.

The extract of *F. vogelii* when used in the treatment of the inflammatory conditions of synovitis showed promising results with pronounced effects. These effects (decrease in paw volume) increased in subsequent days when compared to the group administered diclofenac and the distilled water serving as controls. Even though the group administered diclofenac showed more effects in the Adjuvant-induced paw arthritis test, the *F. Vogelii l*eaf extract showed significant reduction in the induced inflammation and synovitis, thus implying that the extract was able to suppress inflammation and synovitis. Rat adjuvant induced arthritis is a commonly used animal model for the studies of NSAIDs and disease modifying anti-rheumatic drugs (Srinivasa *et al.,* 2019). Development of arthritis is divided into phases. It starts with the induction phase without any evidence of synovitis, followed by early synovitis and finally late synovitis with progressive joint destruction. An effective anti-rheumatic drug should be able to block one or more of these phases. *F. vogelli* at the dose of 200 mg/kg showed very effective prevention of systemic inflammation as seen in the reduced destruction of joints. Similar findings were seen in works where extracts of *Ficus benghalensis* roots where used to alleviate arthritis in rats (Bhardwaj *et al*., 2016). This activity can be attributed to its tannins and flavonoid contents.

The extracts of the *F. vogelii* showed marked decrease in granuloma weight with increased administered dose when compared to the group administered indomethacin which served as the positive control group. Inflammation is known to be an integral part of the body's defence mechanism. Furthermore, inflammation and granuloma normally develop within several days with the proliferative phase of inflammation involved with the production of macrophages, fibroblasts and neutrophils which subsequently leads to formation of granuloma. The marked level of inhibition with increased doses and subsequent decrease in weight of the granuloma indicates that the proliferative phase was effectively suppressed by the extract. This is in tandem with work by Arul *et al* (2005) on *Morus indica.* This ability of the methanol extract of *F. vogelii* to suppress the proliferative phase of inflammation may be because of inhibition of the mediators of inflammation such as histamine, serotonin as well as prostaglandin.

In conclusion, the efficacy of *F. vogelii* as an effective therapeutic agent for treatment of cases of inflammation and

stabilization of anaemic patient have been shown in this work and hence, authenticates its folkloric use in treatment of pediatric anaemia. It also shows that methanol leaf extract of *F vogelii* possess a modulatory potentials on changes that occur within the erythrocyte and vascular system in cases of acute inflammation by creating changes to the mediators of inflammatory reaction. Further studies are required to elucidate the mechanism of its anti-inflammatory properties and also possible isolation of active constituent responsible for the stated properties.

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