JOURNAL OF SCIENTIFIC INNOVATIONS AND CREATIVE RESEARCH
eISSN: xxxxxxxxxxxxxxxx
DOI: xxxxxxxxxxxxxxxxxx
Journal Homepage: https://jsicr.org

(RESEARCH ARTICLE)

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Anti-Inflammatory Activity of Methanolic Extract of *Vitex doniana* Fruits in Wistar Rats

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Journal of Scientific Innovations and Creative Research, 2025, 1(1), 019–030

Publication history: Received on 15th June, 2025; revised on 29th June, 2025; accepted on 24th July, 2025

Article DOI: xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

Abstract

Vitex doniana is used globally by different communities to treat various ailments including inflammation though without any scientific proof. Although some proven pharmacological properties are available for the leaves, roots, stems and barks; literatures reviewed depicts that there is yet to be an up-to-date information on the fruits. Thus, the aim is to validate this knowledge gap. Extraction was done using cold maceration method. Acute toxicity (LD50) test of methanolic extract of *Vitex doniana* fruits was determined by the method of Lorke while the phytochemical analyses was done using the method of Trease and Evans. Anti-inflammatory investigation was done using the method of egg albumen and formalin induced paw oedema models. Group I (positive control) received 10 mg/Kg of diclofenac sodium, Group II & III received 200 and 400 mg/Kg body weight of *V. doniana* fruits' extract while Group IV (negative control) received 10 mL/Kg of sterile water. Oedema was induced in rats by injecting 0.2 mL of the edematogenic agents into the right hind paw 40 minutes after treatment. Paw oedema size was measured before and after induction of inflammation at 60, 120, 180, and 240 minutes for egg albumen model while at a fixed time for 7 consecutive days duration for the formalin model. The anti-inflammatory effect was calculated as percentage (%) inhibition of oedema in the animals treated groups in comparison to the control groups respectively. Statistical analysis was done using one-way ANOVA followed by a Dunnett's post hoc analysis. Acute toxicity (LD50) test for *Vitex doniana* fruits' extract revealed the absence of toxic symptoms or mortality at dose of 5000 mg/Kg. Phytochemical screening of methanolic extract of *Vitex doniana* fruits reveals the presence of flavonoids, steroids, carbohydrates, and reducing sugars. In both egg albumen and formalin models, the extract was significant in reducing paw oedema at $P<0.05$ when compared to the controls. This study has therefore provided some vital scientific information about the anti-inflammatory effect of *Vitex doniana* fruits that may possibly contribute to its use as a form of herbal therapy in the treatment and management of inflammatory conditions.

Keywords: *Vitex doniana* fruit; Egg albumen model; Formalin model; Acute toxicity; Anti-inflammatory activity.

1. Introduction

Nature has provided safe and effective medicine for human ailments over many centuries, of which most were harvested from higher plants. Many plants in reaction to stress, infections, danger, or environmental changes yield diverse secondary metabolites that offer lots of therapeutic potentials for several human illnesses and thus; owe their medicinal values to the secondary metabolites e.g. alkaloids, glycosides, flavonoids, terpenoids, saponins, etc. [1,2,3,4]. Since secondary metabolites have been elaborated within living systems, they are often perceived as showing more drug-likeness and biological friendliness than totally synthetic molecules [2]. The large proportion of drugs discovered stem up from the diverse structures and intricate carbon skeletons of natural products; thus, secondary metabolites from higher plants are generally discerned to afford small organic molecules of outstanding chemical diversity that are highly relevant in elucidating a new mechanism of interaction with a biological target for a disease

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condition under investigation and consequently enhancing contemporary drug discovery process [2,3]. Report by Onwudiwe *et al*. [5] showed that extracts of many plants used in Africa have anti- inflammatory activity.

Vitex doniana (family Verbenaceae), is a sweet fruity plant that serves as a source of food and income for mankind with over 270 known species of trees and shrubs. It is popularly called black plum in English while its local names vary according to location e.g. in Tanzania (Mfuru, Mgwobe), Benin Republic (Fontin, Fontchi), and Uganda (Munyamazi, Muhomozi) whereas in several Nigeria dialects like Hausa is Dinya, Igbo is Ucha koro, and Yoruba is Oori nla [6,7,8]. This large genus (Vitex) is widely distributed in savannah regions, the tropics, and subtropics of West Africa and native to Togo, Kenya, Nigeria, Ghana, Angola, Botswana, Sudan, Tanzania, Uganda, etc. [6,8,9,10]. Phytochemistry of the extracts of *Vitex doniana* (leaves, stem & root barks) have been found to contain alkaloids, anthraquinones, flavonoids, saponins, tannins, glycosides, steroids, and terpenoids [10,11].

Ethnobotanical uses of black plum include treatment of ulcers, sterility, cough, seizures, eye problems, dermatosis, cutaneous wounds, burns, inflammatory and gastrointestinal disorders, rheumatic and menstrual pains, etc. [9,12]. *Vitex doniana* (i.e. leaves, stem and root barks) have been shown to possess pharmacological activities like hepatoprotective effects by Bolanle *et al*., [13] whereas the leaves extract was significantly documented to inhibit paw oedema induced by agar in rats at $P<0.05$ and increases reaction latency to thermal pain in a dose-dependent manner by Sifuma [10]. Leaves' extract of *Vitex doniana* significantly reduced blood glucose level at $P<0.05$ and also exerts hypolipidemic effect by Njoku *et al*., [14]. The stem bark possesses antiepileptic and analgesic activities with a marked dose-related hypotensive effect in both normotensive & hypertensive rats by Sifuma, [10] and excellent wound healing effect by Amegbor *et al*., [7] while the leaves according to Adelodun *et al*., [6]; Ezekwesili *et al*., [12] to be semen restorative and antidiabetic respectively. Although some proven pharmacological properties are available for the leaves, roots, stems, and barks; literatures reviewed depicts that there is yet to be an up-to-date collection of scientifically proven information on *Vitex doniana* (especially the fruits) for its folklore claims in medicine; which is important for the frontier of research and drug development. Hence, the need to investigate the claimed anti-inflammatory effect of the extract of *Vitex doniana* fruits is unequivocally essential [15]. Thus, the objective here is to validate this existing knowledge gap.

Inflammation is a complex process involving the interplay of several reactions including activation of receptors and enzymes, release of chemical mediators, fluid leakage, and cell migration leading to tissue damage. All illnesses are inflammatory in nature and may serves as the basis from where many diseases progress to more serious degenerative conditions that ultimately get diagnosed as a disease i.e., arthritis, allergies, hay fever, endometriosis, eczema and major disorders like cancers, dementia, diabetes, & systemic lupus erythematous [2,16]. Although inflammatory processes depend on the precise nature of the initial stimulus and its location in the body, they all share a common mechanism summarized as follows: cell surface pattern receptors recognizes detrimental stimuli, the inflammatory pathways are then activated to ensure release of inflammatory markers, and consequently; ensuing the inflammatory response [17].

Inflammatory reactions may be triggered by a variety of noxious stimuli such as biologic agents (bacteria, viruses, fungi, parasites, and their microbial toxins), tissue necrosis (nutritional derangement e.g. ischemia, irradiation, physical trauma, mechanical and thermal injuries (e.g. burns or frostbite), chemical agents (foreign bodies e.g. splinters, allergens e.g. peas, drugs e.g. penicillin G, endogenous substances e.g. urate crystals, and both organic & inorganic poisons), immunological reactions (autoimmunity e.g. rheumatoid arthritis, immunodeficiency e.g. asthma, hypersensitivity e.g. dermatitis, etc.), lifestyle factors (poor diet, lack of exercise, and exposure to environmental & household toxins), and genetic or metabolic disorders like Alzheimer disease, gout, cancer, etc. [18,19,20]. The five cardinal signs of inflammation include redness, swelling, heat, pain, and loss of function [21,22].

Inflammation is classified crudely based on the defense capacity of the host, duration of the response, and histologic appearances into acute and chronic inflammation. Acute inflammation is a rapid host response (hours to few days) that serves to deliver plasma proteins and leukocytes to the site of the injurious agent(s). It is characterized by the exudation of fluids and plasma proteins with the emigration of predominantly neutrophils to the injury site [20,22]. Whereas, chronic inflammation is a prolonged inflammatory process (weeks to months or years), which develops from an active acute inflammation and tissue destruction or persistent stimuli that induces chronic inflammation from the onset. Chemical mediators also regarded as permeability factors or endogenous mediators; are soluble diffusible molecules that act systemically and locally at the site of injury or infection once released by the plasma, cells, or the damaged tissue itself. They are either cell-derived mediators e.g. prostaglandins, histamine etc. or plasma-derived mediators e.g. Hageman factor, C3b opsonin etc. [23,24,25]. Chemical mediators initiate, regulate, propagate, and

intensify the inflammatory reaction responses leading to organ dysfunction and major problem in many clinical conditions like sepsis, severe burns, acute pancreatitis, hemorrhagic shock, etc. [2,26].

The use of anti-inflammatory drugs are also considered as the mainstay of analgesic therapy [27]. Most anti-inflammatory agents mediate their effects through blockades of prostaglandins synthesis and likewise, competitively inhibiting histamine receptors [2]. Example, non-steroidal anti-inflammatory drugs (NSAIDs) block cyclooxygenase (COX) enzymes through inhibition of prostaglandins synthesis whereas corticosteroids (steroidal anti-inflammatory agents); prevent the formation of both prostaglandins and leukotriene through the release of lipocortin that then inhibits the phospholipase A2 and consequently blocking arachidonic acid synthesis [28,29]. However, the best way to correct and eliminate inflammation is to improve comprehensive lifestyle and augment dietary changes rather than just relying on pharmaceutical drugs alone that can cause unintended harm in the form of side effect. Several dietary factors like fibre-rich foods, whole grains, fruits (e.g. berries), omega-3 fatty acids, antioxidant vitamins (e.g. Vitamin C & E), and trace minerals (e.g., zinc), have been documented to reduce blood concentrations of inflammatory inducers [2].

2. Material and methods

2.1 Materials

2.1.1 Collection and Identification of Plant Materials

The fruits of *Vitex doniana* were obtained from a local market in Eke Awka, Awka South Local Government Area of Anambra State; Nigeria. In the herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Agulu Campus, Anambra State, Nigeria, the fruits were taxonomically identified, confirmed and assigned voucher specimen number: PCG/474/A/069.

2.1.2 Chemicals, Consumables and Equipment

These includes fresh fruits of *Vitex doniana*, clean water, 80% methanol (Sigma Aldrich, St Louis, USA), formalin (BDH Analar®, UK), egg albumen, diclofenac sodium (50 mg tablet), rodent feed (Guinea feed Nig. Ltd.). Whatman filter paper No.1 (Whatman Ltd., England), muslin-bag, beakers, syringes and needles, water bath (Techmel & Techmel, Texas, USA), rope and ruler, electronic weighing scale (Metler H30, Switzerland), and gastric cannula. All other reagents and chemicals used were of analytical grade sorted from local commercial sources.

2.1.3 Animals, Treatment and Design

Healthy and matured Wistar rats of both sexes weighing (131-338 g) were obtained from the colony breed of the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam Campus along with the ethical permit numbered PHACOOU/AREC/2021/003 from Faculty of Pharmacy COOU Animal Research Ethics Committee; which is in line with National Institute of Health (NIH) guidelines for the care and use of laboratory animals. Animals were fed with normal rodent feed and had unrestricted access to clean drinking water as well as kept at a temperature of 25 ±2°C with 12 hours of light and dark cycles. Before the experiment, animals were adapted to the laboratory condition for 2 weeks and also, deprived of food overnight prior to the experiment day. Two sets of twenty (20) experimental rats (for egg albumen and formalin models), were divided into 4 groups of 5 animals per group following the approach described by Dako *et al*., [30] as shown below:

- Group I (gold standard/positive control) received 10 mg/Kg of diclofenac sodium
- Group II (crude extract) received 200 mg/Kg body weight of *V. doniana* fruit extract
- Group III (crude extract) received 400 mg/Kg body weight of *V. doniana* fruit extract
- Group IV (negative control) received 10 mL/Kg of sterile water

2.2 Methods

2.2.1 Sample Preparation and Extraction

This was done using cold maceration method described by Shah and Seth [31]. Fresh fruits were adequately washed with clean water to remove any contaminants. The waterproof-like bark of the fruits was peeled off and pulverized before initiating cold maceration in 80% methanol for 72 hours with intermittent stirring. Subsequently, the mixture was first filtered using muslin-bag and then, twice filtered with Whatman filter paper No.1 to remove all extraneous matters that were insoluble in the extraction solvent. Thereafter, the filtrate was concentrated in a water bath at 45°C to obtain the crude extract. Afterwards, an 80 g weight of the concentrated crude extract was stored in an enclosed container in a refrigerator at 4°C till when needed.

2.2.2 Acute Toxicity Test of the Extract

Acute toxicity (i.e. LD_{50}) of the methanolic extract of *Vitex doniana* fruits was done in rats orally using the Lorke method described in 1983 (one of the conventional methods of LD_{50} determination) as reviewed by Erhirhie *et al.*, [32]. Animals were monitored at 1 hour, 3 hours, and 24 hours for behavioral changes (like food or water refusal, diarrhea, weakness, etc.) and mortality.

2.2.3 Phytochemical Analyses of the Fruits' Extract of *Vitex doniana*

The phytochemical qualitative analyses of the methanolic extract of *Vitex doniana* fruits was carried out in accordance to the methods described by Aziz [33], Evans [34] and Shah and Seth [31] in order to determine chemical constituents that are characteristics of the pharmacological responses.

2.2.4 Pharmacological Studies (Anti-inflammatory Investigation)

Various pharmacological methods have been developed for testing acute and chronic inflammation, proliferative phase of granuloma formation, and testing of immunological factors; since for a fact, the mechanism of inflammation involves a series of cascaded events regulated by an array of chemical mediators as a salutatory response to the injurious stimuli that are usually accompanied by the clinical signs of erythema, oedema, pain, or hyperalgesia [2].

2.2.4.1 *Egg Albumen Induced Paw Oedema Model (Acute Inflammation Test)*

The egg albumen model was done with slight adjustment and combinations of the methods described by both Singh *et al.*, [35] and Uka *et al.*, [36]. Here, diclofenac sodium (standard drug dose at 10 mg/Kg), test sample (methanolic extract of *V. doniana* fruits at doses of 200 and 400 mg/Kg respectively), and sterile water (negative control dose at 10 mL/Kg); were all administered orally using gastric cannula. About 40 minutes later, a 0.2 mL of fresh egg albumen was injected into the sub plantar region of the rats' right hind paw. The paw thickness was taken before injecting fresh egg albumen and also at 60, 120, 180, and 240 minutes accordingly with the aid of a rope and ruler. The anti-inflammatory effect was deduced as a percentage (%) inhibition of oedema in the treated groups (i.e., test extract and standard drug) in comparison to the control group using the formula below:

$$\%\,\text{inhibition} = \frac{\left(T_o - T_t\right)}{T_o} \times 100$$

Where; T_t = thickness of paw of rats given test extract and standard drug at corresponding time

T_o = thickness of paw of the control group rats at the same corresponding time

2.2.4.2 *Formalin Induced Paw Oedema Model (Chronic Inflammation Test)*

In formalin model, the method described by Singh et al., [35] was used for induction of oedema in experimental animals. Here, 0.2 mL of the edematogenic agent (2% v/v of freshly prepared formalin solution in distilled water) was administered accordingly to all the groups in likewise similar fashion as in egg albumen model above. The paw thickness was measured before injecting the formalin and after injecting the formalin every day at a fixed time for 7 consecutive days using a rope and ruler. The anti-inflammatory effect was deduced as a percentage (%) inhibition of oedema in the treated groups (i.e., test extract and standard drug) in comparison to the egg albumen control group; just as shown in the above formula.

2.3 Statistical Analysis

Results were presented as mean ± standard deviation. The statistical data comparison between extract treated groups and control was made using one-way Analysis of Variance (ANOVA) and then followed by a Dunnett's test (post hoc analysis). *P<0.05 was considered to be statistically significant while ^{ns}P>0.05 was considered to be statistically non-significant. Data analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 23, IBM software.

3. Results

3.1 Acute Toxicity (LD_{50})

The Lorke's method of LD_{50} assay reveals no lethality or any behavioural changes observed at all phases of the acute toxicity determination. Animals were healthy and active during and after the period of LD_{50} evaluation. Thus, the LD_{50} of the methanolic extract of *Vitex doniana* fruits was estimated to be >5000 mg/Kg in rats with the Lorke formula. Summary is shown in table 1 below.

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where; D_0 = 5000 mg/Kg and D_{100} = > 5000 mg/Kg

Table 1 displays the fruits' extract acute toxicity done using Lorke conventional method of LD_{50} assay

ACUTE TOXICITY (LD_{50}) OF METHANOLIC EXTRACT OF *Vitex doniana* FRUITS

Phases	*Groups*	*Dose (mg/Kg)*	*No. of Animals*	*No. of Death*
	I	10	3	0/3
1	***II***	100	3	0/3
	III	1000	3	0/3
	I	1600	1	0/1
2	***II***	2900	1	0/1
	III	5000	1	0/1

3.2 Phytochemical Analysis

Qualitative phytochemical screening of methanolic extract of *Vitex doniana* fruits reveals the presence of flavonoids, steroids, carbohydrates and reducing sugars while saponins, terpenoids, and alkaloids were not detected. Summary of the findings is shown in the table 2 below. They are responsible for different colours, flavours, and smell [37]. Phytochemicals are produced via secondary metabolism in relatively small amounts and have been employed for reducing the risk of major chronic diseases [38]. Several types of flavanols stimulate human peripheral blood leucocyte proliferation and possesses anti-bacterial, anti-allergic, anti-viral, anti-inflammatory, and anti-neoplastic activities [39].

Table 2 depicts the identified phytochemicals of the methanolic extract of *Vitex doniana* fruits

PHYTOCHEMISTRY OF METHANOLIC EXTRACT OF *Vitex doniana* FRUITS

S/N	**Phytochemicals**	**Test Type/Name**	**Inferences**
1	Carbohydrates	Molisch's test	**+**
2	Reducing sugars	Fehling's test	**+**
3	Flavonoids	Alkali test	**+**
4	Steroids	Liebermann's burchourd test	**+**
5	Saponins	Frothing test	**-**
		Emulsion test	**-**
6	Terpenoids	Salkowski's test	**-**

7	Alkaloids	Dragendorff's test	-
		Mayer's test	-
		Wagner's test	-
		Picric acid test	-

Where (+) means presence of specific phytochemicals while (-) means absence of specific phytochemicals

3.3 Anti-inflammatory Effect

3.3.1 *Egg Albumen Paw Oedema Model (Acute Inflammation)*

As depicted in table 3 and figure 1 below, there was time dependent inhibition of oedema in both the diclofenac (standard drug) and extract (test sample) treated groups as compared to the egg albumen control group at various hours for the egg albumen induced paw oedema model. At 60 minutes duration; there was no significant reduction in paw oedema in all the groups. At 120 minutes duration, there was significant reduction in paw oedema for the diclofenac treated group (standard drug dose at 10 mg/Kg with a percent inhibition of 32.56%) while the extract treated groups (test extract given at doses of 200 and 400 mg/Kg orally with a percent inhibition of 13.95% and 16.28% respectively; were not significantly different from the control group even though there was reduction in paw oedema). At 180 and 240 minutes duration respectively, both the diclofenac (with a percent inhibition of 41.46% and 63.16%) and extract treated groups (with percent inhibition of 26.83% and 50.00% for 200 mg/Kg dose while 31.71% and 52.63% inhibition for 400 mg/Kg dose accordingly); were all significant in reducing paw oedema as compared to the control group.

Table 3 shows the anti-inflammatory effects of the drug and methanolic extract sample of *Vitex doniana* fruits on egg albumen induced paw oedema model

	PAW OEDEMA SIZE (in cm)			
Groups	***60 minutes***	***120 minutes***	***180 minutes***	***240 minutes***
I Positive Control (Diclofenac, 10mg/Kg)	0.72 ±0.08^{ns}	0.58 ±0.04*****	0.48 ±0.04*****	0.28 ±0.04*****
II Test Extract (200mg/Kg Dose)	0.88 ±0.11^{ns}	0.74 ±0.15^{ns}	0.60 ±0.12*****	0.38 ±0.04*****
III Test Extract (400mg/Kg Dose)	0.84 ±0.21^{ns}	0.72 ±0.24^{ns}	0.56 ±0.19*****	0.36 ±0.15*****
IV Negative Control (Water, 10mL/Kg)	0.78 ±0.15	0.86 ±0.11	0.82 ±0.13	0.76 ±0.11

Values are presented as mean ± SD, Dunnett: n=5, *P<0.05 is significantly different from control while ^{ns}P>0.05 is not significantly different from control

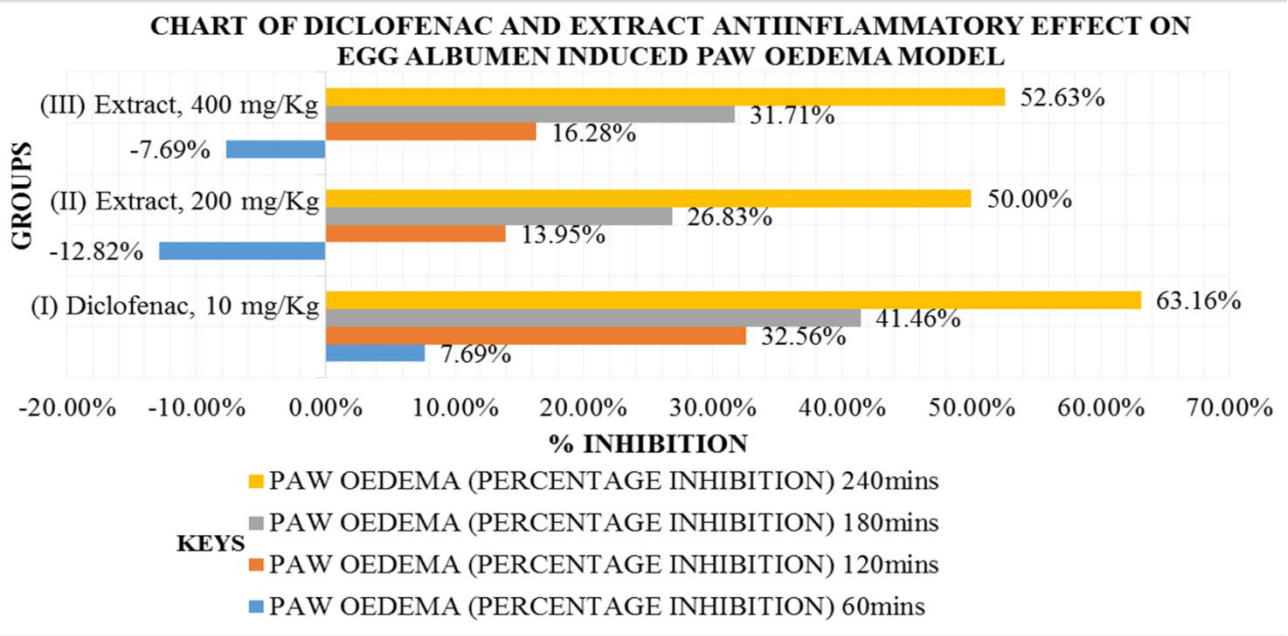

Figure 1 is an egg albumen induced paw oedema model that compares the relative anti-inflammatory effect of the crude extract with the standard drug (diclofenac sodium) at various time duration

3.3.2 Formalin Paw Oedema Model (Chronic Inflammation)

Table 4 and figure 2 below, depict a continuous significant day wise reduction of oedema in both groups of the methanolic extract of *Vitex doniana* fruits and the diclofenac sodium (standard drug) as compared to the formalin control group in the formalin induced paw oedema model. Mean significant decrease in oedema was attained on day three (3) with percent inhibition of 51.79% for diclofenac sodium dose at 10 mg/Kg and 55.36% and 50% for the extract dose at 200 mg/Kg and 400 mg/Kg respectively. At day six (6) and seven (7) respectively, both the 200 mg/Kg and 400 mg/Kg doses of the extract completely attained maximum decrease in oedema with 100% percent inhibition of oedema respectively while the standard drug (diclofenac sodium dose at 10 mg/Kg) inhibits oedema by 85.71% on day six (6) and attained maximum decrease in oedema on day seven (7) with 100% inhibition of oedema.

Table 4 correlates the anti-inflammatory effect of the standard drug and methanolic extract of *Vitex doniana* fruits against the control group for the formalin induced paw oedema model

	PAW OEDEMA SIZE (in cm)						
Groups	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
(I) Diclofenac 10 mg/Kg	0.76 ±0.05*	0.66 ±0.05*	0.54 ±0.05*	0.38 ±0.04*	0.24 ±0.05*	0.10 ±0.00*	0.00 ±0.00*
(II) Extract 200 mg/Kg	0.78 ±0.08*	0.64 ±0.11*	0.50 ±0.07*	0.36 ±0.05*	0.16 ±0.05*	0.00 ±0.00*	0.00 ±0.00*
(III) Extract 400 mg/Kg	0.88 ±0.08*	0.74 ±0.09*	0.56 ±0.05*	0.34 ±0.11*	0.12 ±0.04*	0.00 ±0.00*	0.00 ±0.00*
(IV) Sterile H_2O 10 mL/Kg	1.26 ±0.05	1.24 ±0.05	1.12 ±0.04	1.02 ±0.13	0.86 ±0.09	0.70 ±0.10	0.56 ±0.05

Values are presented as mean ± SD, Dunnett test: n=5, *P<0.05 is significantly different from control

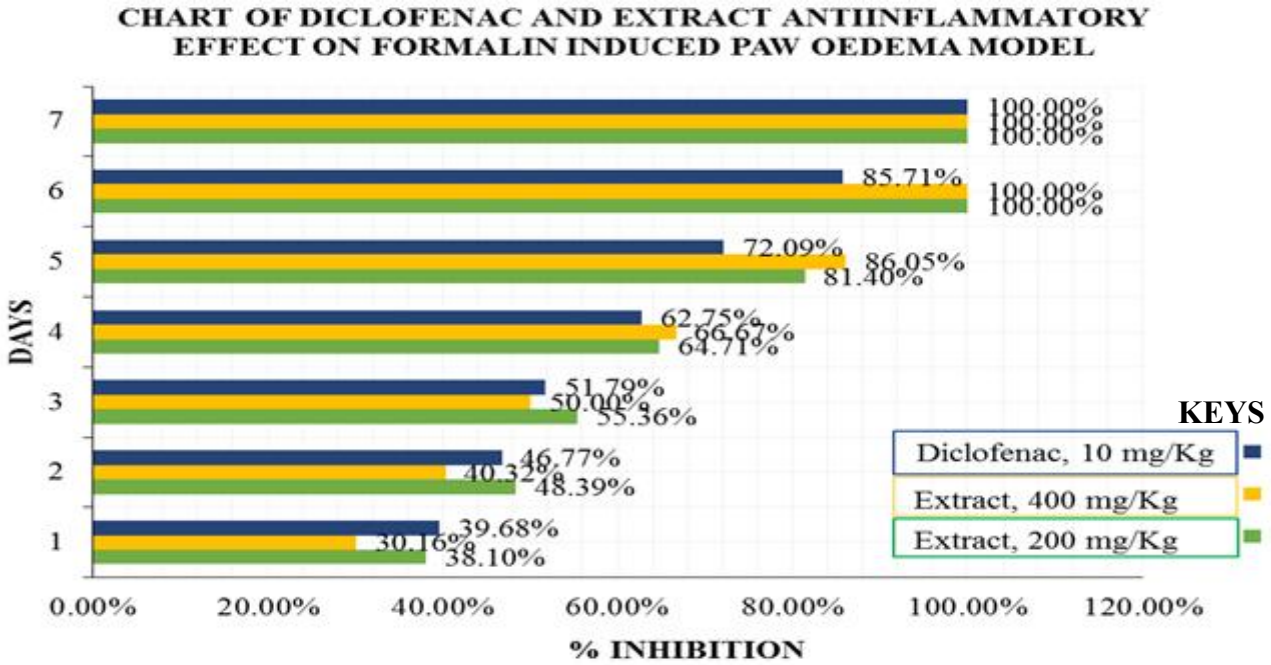

Figure 2 is a formalin induced paw oedema model chart comparing the relative anti-inflammatory activity of the methanolic extract of *Vitex doniana* fruits to the standard drug (diclofenac sodium) at different days

4. Discussion

During the evaluation of candidate plant materials for pharmacological activities, the characterization of their chemical nature is essential in the field of drug discovery and development; since this preliminary screening facilitates the recognition of bioactive compounds [33]. These natural products have been reported to be safe, efficacious, biocompatible, and cost-effective alternatives to treat inflammatory diseases in folklore medicine as compared with the non-steroidal anti-inflammatory drugs (NSAIDs) and also; that several substances of plants' origin such as flavonoids, saponins, steroids, and tannins offer potential therapeutic agents ensuing the inhibition of inflammatory activities [36, 40, 41]. Thus, it could be inferred that the anti-inflammatory effect of the fruits' extract of *Vitex doniana* are due to the presence of these identified bioactive constituents (mainly flavonoids and steroids). Inflammatory disorders constitute a major global health challenge. Although synthetic drugs for the alleviation of these disorders are dominating the market, their adverse effects raise a lot of concerns, hence; safety and efficacy governs scientific exploration of natural product as alternative anti-inflammatory agents [42]. The result of the acute toxicity study is as displayed in table 1. It showed that the LD_{50} of methanolic fruits' extract of *Vitex doniana* was greater than 5000 mg/Kg as no animal died or showed sign of toxicity during both phases of the study. This is in consonant to the studies done by Adjei *et al*., [43]; Bolanle *et al*., [13]; Sifuma [10]; which also recorded no toxicity at doses of 3000 mg/Kg, 5000 mg/Kg and 4000 mg/Kg body weight respectively. Signs of acute toxicity, which includes decreased locomotors activity, decreased feed intake, tremor, change of hair color, prostration and death as reported by Barbosa-ferreira *et al*., [44] was not noticed in the experimental rats administered with the methanolic extract of *Vitex doniana* fruits; thus, suggesting that the fruits' extract of *Vitex doniana* is practically nontoxic in the treatment of acute and chronic inflammatory conditions.

Egg albumen paw oedema model, has been a well-established testing procedure for the evaluation of anti-inflammatory agents in acute condition [36]. Pain associated with the early phase of inflammation is attributed to injection trauma or direct effects of inflammatory inducers on nociceptors [33]. Egg albumen paw oedema model is known to facilitate its inflammatory actions by initiating the release of histamine and serotonin concomitantly thereby increasing activation of the vascular permeability; which further amplifies blood flow and vasodilation, hence, ensuing the inflammatory process [45]. As seen from the results above in table 3 and figure 1, the early phase of the inflammatory process can be attributed to histamine, substance P, injection trauma, and serotonin; which reflects the

non-significant inhibition of paw oedema in all the groups i.e. diclofenac sodium (7.69%), 200 mg/Kg dose extract (-12.82%), and 400 mg/Kg dose extract (-7.69%) for the 60 minutes duration whereas at 120 minutes duration, both the 200 mg/Kg (13.95%) and 400 mg/Kg (16.28%) doses extract still depicts no significant inhibition of paw oedema except for the diclofenac group (32.56%) with significant inhibition of paw oedema in a time-dependent manner. During the late phase of inflammation in the egg albumen paw oedema model; there was significant inhibition of paw oedema at both 180 and 240 minutes duration for all the groups i.e. diclofenac sodium (41.46% and 63.16%), 200 mg/Kg dose extract (26.83% and 50.00%), and 400 mg/Kg dose extract (31.71% and 52.63%) accordingly in a dose-dependent manner. The anti-inflammatory effect of the fruits' extract of *Vitex doniana* is comparable to diclofenac; even though diclofenac sodium inhibits paw oedema far more than the extract groups.

Diclofenac sodium is a non-steroidal anti-inflammatory drug that acts by blocking the enzyme cyclooxygenase which leads to the inhibition of prostaglandin synthesis responsible for inflammation [45]. A reviewed study conducted by Takeuchi [46] disclosed that damage to the gastric mucosa of a normal stomach can only occur when expression of both COX-1 and COX-2 are inhibited. This also suggest that the possible mechanism of anti-inflammatory activity is not directly associated with COX-1 inhibition but COX-2 [42]. Thus, the anti-inflammatory activities of the fruits' extract of *Vitex doniana* could be credited to the inhibition of cyclooxygenase an enzyme that is responsible for prostaglandin production. As earlier noted by Singh et al., [35] and reported by Aziz [33] respectively; formalin induced paw oedema model is one of the most suitable test procedure used for evaluation of chronic anti-inflammatory effects, since it closely resembles human arthritis whereas pain during the late phase of inflammation reflects pain in the induced inflammatory models due to release of chemical mediators of inflammation (such as serotonin, histamine, prostaglandins, and bradykinin). Hence, as dictated by the results in table 4 and figure 2 above, it could be seen that the methanolic extract of *Vitex doniana* fruits significantly prevented formalin induced paw oedema in a dose-dependent manner. The mean significant inhibition of oedema was attained on day three (3) and by day six (6), the maximum inhibition of paw oedema (100%) was met by both the 200 and 400 mg/Kg doses extract groups and remained the same on day seven (7) while that of diclofenac sodium (standard drug) was attained on day seven.

5. Conclusion

The methanolic fruits' extract of *Vitex doniana* possesses anti-inflammatory properties which could be as a result of its phytochemical constituents mainly steroids and flavonoids. These findings support the use of the extract in traditional system of medicine for the management of inflammatory conditions.

Compliance with ethical standards

Acknowledgments

The authors acknowledge the Management of Faculty of Pharmacy COOU Animal Research Ethics Committee for granting the ethical approval, without which this study would not have been possible.

The authors also acknowledge Mrs Onwunyili Amaka and Mr Akwuba Mbazuluike Joseph; both of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Agulu for their help in the identification and authentication of plant sample respectively.

Disclosure of conflict of interest

The authors declare no conflict of interest

Contributions of the Authors

Ngozi U. Madubogwu, Joseph O. Efenure and Theophine C. Akunne conceptualized and executed the study.

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