Original Research Article

Anti-Inflammatory Activty of Ethanol Leaf Extract of *ArthropterisOrientalis* in Wistar Albino Rat.

ABSTRACT

The study investigated the anti-inflammatory activity of ethanol leaf extract of Arthropterisorientalis in wistar albino rat. The plant extract was obtained with the procedure as described by Khalifa et al., 2017 and both acute and chronic anti-inflammatory properties was assessed. Five groups of five animals each were utilized for acute and chronic inflammation respectively. Anti-inflammatory effect of the extract was evaluated on egg albumin induced paw edema in albino rats and edema induced by formalin in the wistar albino rats' paw for acute and chronic inflammation respectively. The extract had 26.665% tannin while flavonoid, saponin, phenol, and glycoside had 0.784%, 0.631%, 0.183% and 0.057% respectively. The result revealed that the extract at 500 mg/kg exhibited significant inhibition ($p<0.05$) of systemic acute paw edema at the 4th hour of the second phase of edema in comparison with the standard drug. The results reveal that intraperitoneal administration of ethanol leaf extract of Arthropterisorientalis on formalin-Induced paw edema in wistar albino rats at (100 and 500mg/kg) and (100mg/kg) exhibited significant inhibition ($p<0.05$) in mean paw volumes on day 1 and 2 respectively. The extract did not produce any significant hematological change. These results show that Arthropterisorientalis has potential anti-inflammatory properties.

Keywords ?

Comment [MB1]: Name of species should be italic.

Introduction

Herbal healing is the most ancient form of healing known to mankind that is as old as the human society. The importance of traditional medicine as a source of primary health care was first officially recognized by World Health Organization (WHO), in the Primary Health care Declaration of Alma Ata and has been globally addressed since 1976 by the Traditional Medicine Program of WHO. According to WHO, about 80% of the world inhabitants rely mainly on traditional medicine for primary health-care (Winiger 2022). The ability of plants to act as a drug, herb, ethno-medicine, essential oil or even cosmetics is derived from the secondary product

of plant metabolism which produces alkaloids, terpenoids and flavonoids. These substances actually evolved as a response of the plant to stress, predation and competition leading to the enormous chemistry library of biological systems (Jan 2021).

Ferns are members of a large and diverse group of plants commonly referred to as the lower plants. It has been estimated that only 5% of all bryophytes have been studied with regard to any phytochemical properties, ferns, including *Arthropterisorientalis*, show a lot of diversity in their practical uses. They are normally used for food, medicinal, economic, decorative and environmental purposes in many countries such as United States, Europe, New Zealand, Japan, Africa and the Philippines and Nigeria (Addo-Fordjour*et al.*, 2007, Petkov*et al*., 2021). This study attempted to investigate the anti-inflammatory activities of ethanol leaf extract of *Arthropterisorientalis* in wistar rat.

Inflammation is a biochemical response of the body against an assertive agent such as pathogens, damaged cells, or irritants. It acts as a protective response involving immune cells, blood vessels, and molecular mediators. Its function is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair (Chen *et al*., 2018). The five cardinal signs of inflammation include heat, pain, redness, swelling and loss of function (Ferrero-Miliani*et al*., 2007: Chen *et al.*, 2018). Non-steroidal anti-inflammatory drugs (NSAID) have been used globally for the treatment of inflammation, pain and fever, as well as for cardiovascular protection. However, it causes severe side-effects, which include gastric ulcer, renal damage, bronchospasm and cardiac abnormalities, thus restricting their use.. Examples of these drugs are aspirin, ibuprofen, naproxen, fenoprofen, indomethacin, diclofenac, fenamates, piroxicam, ketorolac, nimesulide, rafecoxib, paracetamol etc. These side effects necessitated the need to find a better drug to treat the ailment. Research has proven the significance of drugs of natural origin as an important source for the treatment of many diseases worldwide (Sriutth*aet al*., 2018).

Medicinal plants, which is very rich in Africa flora, have played exceptional and indispensable roles in early times in alternative traditional medicine and the research of plants employed as pain-relievers and anti-inflammatory agents in traditional medicine is one of the productive and logical strategies in the search for new drugs,with the prevalence of bacteria and fungi infections in our society that leads to various diseases like inflammation, rheumatism, venereal and skin

diseases, the search for new, safer and affordable drugs especially from plants is of utmost importance (Okaiyeto and Oguntibeju 2021)..

Materials and Methods

Drugs and Chemicals

Standard drug Diclofenac was obtained from a community pharmacy in Enugu Nigeria. Sigma Aldrich produced Tween 80, formalin and egg albumin. All the chemicals, reagents and solvents used, were of analytical grade.

Plant Collection, Identification and Extract Preparation

Fresh leaves of *Arthropterisorientalis*were collected from a wide growing habitat in Awka South Local Government Area, Anambra State, Nigeria. The plant was identified by Prof. A.O. Nwadinigwe in the Department of Life Sciences and Biotechnology (Botany), University of Nigeria, Nsukka. It was deposited in the herbarium of the Department and Voucher number UNH No 259 was assigned to it. Prior to Extraction the leaves were washed, air dried and mancerated into powdered form. Thereafter Ajirioghene*et al.*,plant crude extraction methods were modified (2022). 70 percent ethanol was used to extract the mixed powder, which was the filtered using Whatman No. 2 filter paper.Rotary evaporator was then used to vacuum concentrate the solvent to dryness. The extract was then dried at 40°C in an incubator before being kept at 0-4°C. The yield of the extract was 13%. Finally, the dried extract was diluted in normal saline to get the needed dose and was kept in the refrigerator until use

Comment [MB2]: What is the particle size of the powder ?

Source of Experimental Animals

Fifty mature Wistar albino rats 120-200 g were purchased from the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka central animal house. The animals were kept in regular laboratory conditions as per the University ethical guidelines which adhere to the "Principle of Laboratory Animal Care" (NIH Publication No. 85-23).The rats were given free access to the regular rat meal (Topfeeds) and water for one week (12–12 h light–dark cycle, 28 2°C).

Comment [MB3]: Whether all the rats male, or female or mixed.

Experimental Design

The animals were divided into five groups of five (5) rats each for both acute and chronic inflammation. The animals received treatment as follows;

Group 1- Negative control, received 1.0 ml of Tween 80

Group 2- Standard drug, received 1.0 ml of diclofenac

Group3- Received 0.5ml of Ethanol extract of Ao, (EEAO) (100mg/kg)

Group 4- Received 0.5ml of Ethanol extract of Ao, (EEAO) (200mg/kg)

Group 5- Received 0.5ml of Ethanol extract of Ao, (EEAO) (500mg/kg)

Why there are different doses bethween group 1 &2 recieved 1.0 ml, group 3,4 &5 ecieved 0,5 ml ? Usually all of group recieved the same doses 1.0 ml or 0.5 ml.

Phytochemical Analysis

The phytochemical analysis was done using standard methods.

Qualitative phytochemical screening of ethanol leaf extract of ArthropterisOrientalis (ELEAO)

Comment [MB4]: Name of species should be italic.

Phytochemical analysis of ELEAO was done to evaluate the bioactive constituents, using standard procedures as described by Khalifa*et al*., 2017: Ajirioghene*et al*., 2018. Glycosides was determined using Keller Killiani testsaponinusing foam test, tanninusing gelatine test, flavonoidusing Shinoda test, phenolusing ferric chloride test, alkaloidusing Mayer's test, carbohydrate using iodine test, **amino acid**, **aromatic acid**, steroidusing Salkowski's test and terpenoidusing chloroform test.

Quantitative phytochemical screening of **ArthropterisOrientalis (ELEAO)**

Quantitative analysis was determined for Phenol, Flavonoid, Saponin, Tannin and Glycosideof **ELEAO** was determined using standard procedures. Total phenols and tannins content were determined using the method described by Prabhavathi*et al*., (2016); Total flavonoids,saponins content and glycosidewerewere determined by the method of Madhu*et al*., (2016).

Determination of the Acute Toxicity of Ethanol Leaf Extract of ArthropterisOrientalis (ELEAO)

The acute toxicity was carried out using methods outlined by Lorke(1983):Ajirioghene*et al*., (2018). It was done to determine the dose used for the experiment. This was carried out in two stages with a total of eighteen Wister albino rats were used for this study.

Comment [MB5]: How old the age of the wistar albino rats, all of them female or male or mix ?

In the first stage in determining the toxic range of the plant extract, the Wister albino rats were divided into tree (3) groups of 3 animals each. The Wister albino rats were fasted overnight, the groups received a dose (10, 100, or 1000mg/kg body weight) of plant extract suspended in 3% v/v Tween 80 and it was administered orally. All the Wister albino rats were allowed free access to food and water. Then they were observed for clinical signs of acute toxicity and mortality for 24hrs.

The stage two was carried out since no death was recorded in stage one. Wister albino rats were divided into 3 groups of 3 Wister albino rats each, and given one of three different higher doses 1600, 2900, and 5000mg/kg body weight of the plant extract were administered orally. The Wister albino rats were observed for number of deaths for 24hr.

Anti-inflammatory Study (**Effect of extract on acute inflammation**)

The method described by Shabbir*et al.*, (2018) was used. A total of Twenty five adultWistar albino rats(120-200g) were divided into five groups of five rats per group. They were placed ino cages according to their groups. The animals were deprived of feed for 12 hours prior to the experiment but were allowed access to pure drinking water. They were not allowed access to both feed and pure drinking water during the experiment.

The crude ethanol leaf extract of *Arthropterisorientalis* and Diclofenec was separately administered intraperitoneally. Group 1 was used as negative control thus received 0.9% Tween 80 in normal saline 2ml/kg, group II -positive control received10 mg/kg of Diclofenac, Group III, IV and V received 100, 200 and 500 mg/kg of the plant extract.

The animals were left for 30minutes after which 0.1ml of fresh egg albumin was injected into the sub- plantar of the right hind paw of each of the rat. Using a vernier caliper, the diameter of the right hind paw was measured at 1, 2, 3 and 4 hours respectively.Percentage inflammation and inhibition of inflammation were calculated using the formular below:

Inhibition (%) = [1- (Xt / Xc) x 100]

Where, Xt and Xc are the mean paw diameter of the treated and control groups, respectively, at 1, 2, 3, and 4 h.

Anti-inflammatory Study (Effect of extract on chronic inflammation)

Comment [MB6]: How old the age of the wistar albino rats, all of them female or male or mix ?

Comment [MB7]: The title should not be separated from the content, move to the next page.

Assessment of Anti Inflammatory Activity was done using methods of Oyeleke*et al*., (2018)Wistar albino rats were used, twenty-five (25) weighing 120-200g were divided into five groups of five rats per group. Group I negative control received 0.9% Tween 80 in normal saline 2ml/kg, Group II received 10mg/kg of Diclofenac. Group III, IV and V received 100, 200 and 500mg/kg of the plant extract respectively through intraperitoneal injection.

One hour later, chronic inflammation was induced by a single sub-plantar injection of 0.lml freshly prepared 2% formalin in the right hind paw of all the wistar albino rats in each group. The administration of the extracts 100, 200 and 500 mg/kg, Tween 80 in normal saline and Diclofenac was continued once daily for seven consecutive days. The rat paw thickness was measured usingvernier caliper daily and the level of inhibition was calculated using the formular below:

Inhibition (%) = [1- (Xt / Xc) x 100]

Where Xt = means increase in paw diameter of treated Wistar albino rats (group) and Xc = means increase in paw diameter of control Wistar albino rats (group).

The difference in the paw diameter before and after induction of inflammation was expressed as a percentage of inhibition of the paw.

Preparation of Blood Samples for Hematological Analysis

At the end of the treatment duration, the rats were subjected to deep ether anaesthesia before euthanasia. Retro-orbital puncture was used for blood collection; thewhole blood samples were placed in EDTA(ethylenediaminetetraacetic acid) bottles and used for determination of Erythrocyte sedimentation rate (ESR), Hemoglobin concentration, Total White Blood Cell count and Pack cell volume (PCV).

Comment [MB8]: Give spases bethween the phrases

Determination of Hematological Parameters

Hematology parameters using blood samples in EDTA such as **erythrocyte sedimentation rate (ESR), packed cell volume (PCV), white blood cell count and hemoglobin levels (HB).**

They were analyzed haematology analyzer (Mindray Auto Hematology Analyzer, BC-5200, USA) following the manufacturer's instructions.

Statistical Analysis

Data analysis was carried out using Statistical Package for Social Sciences (SPSS- version-20). Significance of any differences between control and treatment groups was determined at $p < 0.05$. Results were presented as tables and graphs with Mean ± Standard Error of Mean (SEM) where applicable. The means of the control and treated groups were compared using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc tests*. $P < 0.05$ was considered statistically significant.

Comment [MB9]: The title should not be separated from the content, move to the next page.

Results

Phytochemical Screening of the Ethanol Leaf Extract of *AthropterisOrentalis.*

The qualitative analysis of the ethanol leaf extract of *Athropterisorentalis*was done to determine its various phytochemical content.

Table 1: Qualitative and Quantitative analysis of ethanol leaf extract of *Athropterisorientalis*

Comment [MB10]: We recommend that the letters in the Table must be smaller than the text

TEST(constituents)	Qualitative	Quantitative %
Glycoside	++	0.06
Saponnin	++	0.63
Tannin	++	26.67
Flavonoid	++	0.78
Phenol	++	0.18
Alkaloid	-	
Carbohydrate	++	
Amino acids	+	
Aromatic amino acid	++	
Steroid	+	
Terpenoid	-	

Keys: ++ (Moderately present), + (present in trace amount) – (absent)

The table above shows the results of the Qualitative and Quantitative phytochemical screening of the ethanol leaf extract of *Arthropterisorientalis*. The qualitative analysis revealed the presents of

glycoside, saponin, tannin, flavonoid, phenol, carbohydrate, aromatic amino acid, Amino Acids, and steroid while alkaloid, reducing sugar, starch, proteins and terpenoid were absent.

The Quantitative analysis shows that tannins had the highest percentage of 26.665% followed by flavonoid 0.784%, saponin 0.631%, phenol 0.183%, and least of all glycoside with 0.057%.

Acute Toxicity Study

There were no signs of toxicity or death following treatment with various doses of the ethanol leaf extract of *Arthropterisorientalis*. (Up to 5000 mg/kg) for 48-hours after treatment of rats.

Effect of Ethanol Leaf Extract of *Arthropterisorientalis* on Acute Inflammation

Table 2: Effect of Ethanol Leaf Extract of *ArthropterisOrientalis*Leaf Oon Egg Albumin-Induce

Hind Paw Edema in Wistar Albino Rats

Comment [MB11]: We recommend that the letters in the Table must be smaller than the text

Gps	Treatment	Doses (mg/kg)	Paw edema (cm) and % inhibition of paw edema				
			30minutes	1st hour	2nd hour	3rd hour	4th hour
I	Control	-	1.08 ± 0.04	0.92±0.04	0.84±0.04	0.78±0.02^{b}	0.64± 0.04^{b}
II	Diclofenac (Standard drug)	10	1.20 ± 0.10 (-17%)	0.84± 0.09 (25%)	0.76±0.11 (31.28%)	0.70± 0.08^{ab} (38.46%)	0.64 ±0.07^{b} (16.67%)
III	EEAO	100	1.20 ± 0.08 (-28.57%)	0.74± 0.16 (-10%)	0.80±0.06 (0)	0.68 ±0.08^{ab} (23.08%)	0.52±0.04^{ab} (66.67%)
IV	EEAO	200	1.20 ± 0.06 (-156.14%)	1.00± 0.12 (-169%)	0.72±0.10 (8.75%)	0.56± 0.11^{ab} (7.69%)	0.40 ±0.08^{ab} (33.3%)
V	EEAO	500	1.08 ± 0.04 (-28.57%)	0.72± 0.04 (10%)	0.62±0.02 (18.7%)	0.40 ± 0.06^{a} (84.62%)	0.36 ±0.04^{a} (100%)
	p-values		(0.570)NS	(0.336)NS	(0.347)NS	(0.029)*	(0.010)*

Results are expressed in mean ± SEM; n = 5, P-value =NS, *Significant P<0.05; Mean values with the same super script alphabet do not differ significantly among the groups while those with the different alphabet superscript differ significantly using the Turkey Post Hoc.

The result revealed that ethanol leaf extract of *Arthropterisorientalis* exhibited non-significant inhibition at the first phase of edema for all the tested doses, but 500 mg/kg showed significant inhibition of systemic acute paw edema within the second phase of edema when compared with the control group. However, the extracts at 500 mg/kg exhibited significant inhibition of systemic acute paw edema only at the fourth hour of the second phase of edema in comparison with the standard drug.

Effect of Ethanol Leaf Extract of *Arthropterisorientalis*on Chronic Inflammation

Table 3: Effect of ethanol leaf extract of *Arthropterisorientalis* on formalin Induced paw edeman

Comment [MB12]: We recommend that the letters in the Table must be smaller than the text

Grp	Treatment	Paw edema (cm) and % inhibition of paw edema						
		Day1 1hr	Day2	Day3	Day4	Day5	Day6	Day7
I	Control	0.61±0.01^{b}	0.52±0.02^{a}	0.69±0.01^{ab}	0.58±0.02^{ab}	0.58 ±0.01	0.56 ± 0.01	0.57 ±0.01
II	Diclofenac 10mg/kg	0.56±0.01^{ab}	0.63±0.03^{ab}	0.64±0.02^{ab}	0.61±0.01^{ab}	0.56 ± 0.04	0.57 ± 0.02	0.57 ±0.02
	(Standard drug)	(11.76)	(-91.67)	(17.24)	(-66.67)	(11.11)	(37.50)	(0)
III	EEAO 100mg/kg	0.52 ±0.02^{a}	0.76 ±0.05^{b}	0.72±0.04^{b}	0.68±0.03^{b}	0.65 ± 0.03	0.62 ± 0.02	0.58 ±0.01
		(29.41)	(-200)	(-10.34)	(-55.56)	(-38.89)	(-37.5)	(-5.88)
IV	EEAO 200mg/kg	0.55±0.02^{ab}	0.58 ±0.02^{a}	0.60±0.01^{a}	0.57±0.02^{a}	0.54 ±0.01	0.55 ± 0.02	0.52 ±0.01
		(11.96)	(-50)	(31.03)	(5.56)	(22.22)	(6.25)	(29.41)
V	EEAO 500mg/kg	0.53 ±0.02^{a}	0.58±0.04^{a}	0.59±0.03^{a}	0.56±0.02^{a}	0.58 ± 0.03	0.57 ± 0.03	0.55 ±0.03
		(23.5)	(-50)	(34.48)	(11.11)	0	(-6.25)	(11.76)
	P-value	(0.025)*	(0.002)*	(0.035)*	(0.020)*	(0.148)NS	(0.381)NS	(0.386)NS

Results are expressed in mean ± SEM; n = 5, P-value =NS, *Significant P<0.05; Mean values with the same super script alphabet do not differ significantly among the groups while those with the different alphabet superscript differ significantly using the Tukey Post Hoc.

The results in Table 3 above reveal that intraperitoneal administration of ethanol leaf extract of *Arthropterisorientalis* on formalin-Induced paw edema in Wistar albino rats at (100 and 500mg/kg) and (100mg/kg) exhibited significant inhibition in mean paw volumes on day 1 and 2

respectively, when compared with the control group while all the doses exhibited non-significant inhibition when compared the standard drugs. However, within days 3, 4 and 7 the extract showed non-significant inhibition of the edema at 200 and 500mg/kg, while on days 1, 5 and 6 only 200mg/kg showed non-significant inhibition of the edema in comparison with the control group.

Hematological Analysis

Table 4: Mean Values and Comparison of Hemoglobin (g/dL), Pack Cell Volume (%) and Erythrocytes Sedimentation Rate (mm/h)

Comment [MB13]: We recommend that the letters in the Table must be smaller than the text

Groups	Treatment	Hemoglobin (g/dL)	Packed Cell Volume (%) (PCV)	Erythrocytes Sedimentation Rate (mm/hr)
I	Control	8.50 ± 0.26	26.26 ± 0.26	4.00 ± 0.00
II	Diclofenac 10mg/kg	8.36 ±0.41	25.66 ± 1.20	3.00 ± 0.57
III	EEAO 100mg/kg	9.33 ± 0.67	28.33 ±2.18	4.00 ± 0.00
IV	EEAO 200mg/kg	9.86 ± 0.33	30.33 ± 0.66	3.33 ± 0.66
V	EEAO 500mg/kg	8.73 ± 0.38	27.00 ± 0.57	2.83 ± 0.16
	P-value	(0.163)NS	(0.114)NS	(0.194)NS

Results are expressed in mean ± SEM; n = 5, P-value =NS, *Significant P<0.05; No significant difference between the groups.

The resultsin Table 4 above revealed that intraperitoneal administration of ethanol leaf extract of *Arthropterisorientalis*at all the tested doses of 100, 200 and 500mg/kg exhibited a non-concentration dependent and non-significant increase in both Hemogblobin level and pack cell volume, when compared with Standard and Negative control groups that received standard drug (diclofenac) and Tween 80 respectively, while 200mg/kg dose exhibiting the highest non-significant increase when compared with the other doses.

While at the tested doses of 100 and 200 mg/kg exhibited a non-concentration dependent and non-significant increase in erythrocyte sedimentation rate and 500 mg/kg that exhibited non-

significant decrease when compared with standard group that received Diclofenac. When compared with control group that received Tween 80 at the tested doses of 100 and 200mg/kg exhibited a non-concentration dependent and non-significant decrease in erythrocytes sedimentation rate and 100mg/kg had the same erythrocytes sedimentation rate with the control group.

Table 5: Mean Values and comparison of Total White Blood Cell Count (mm3) and Neutrophils (%).

Comment [MB14]: We recommend that the letters in the Table must be smaller than the text

Groups	Treatment	Total White Blood Cell Count (WBCT) (mm3)	Neutrophils (%)
I	Control	8700.00 ± 173.20	29.93 ± 0.06
II	Diclofenac 10mg/kg	8566.66 ± 1933.33	39.67 ± 2.96
III	EEAO 100mg/kg	8333.33 ± 1576.21	35.33 ± 4.48
IV	EEAO 200mg/kg	5966.66 ± 1266.66	29.33 ± 4.66
V	EEAO 500mg/kg	7500.00 ± 1563.11	33.00 ± 1.52
	P-value	(0.657)NS	(0.228)NS

Results are expressed in mean ± SEM; n = 5, P-value =NS, *Significant P<0.05; No significant difference between the groups.

The results reveal that intraperitoneal administration of ethanol leaf extract of *Arthropterisorientalis* at all the tested doses of 100, 200 and 500mg/kg exhibited a non-concentration dependent and non-significant decrease in Total White Blood Cell Count (WBC^T), when compared with standard drug (Diclofenac) and Negative control groups (Tween 80).

While all the tested doses 100, 200 and 500mg/kg exhibited a non-concentration dependent and non-significant decrease in percentage Neutrophils count when compared with standard group and exhibited a non-concentration dependent and non-significant increase in percentage Neutrophils count when compared with control groups, except at 200mg/kg that showed non-significant decrease in comparison with the negative control.

Table 6: Mean Values and comparison of Leukocytes (%) and monoccytes (%)

Comment [MB15]: We recommend that the letters in the Table must be smaller than the textand preferably Table in one page.

Groups	Treatment	Lymphocytes (%)	Monocytes (%)
I	Control	69.33 ± 0.33	0.767 ± 1.3279
II	Diclofenac 10mg/kg	59.33 ± 3.17	0.867 ± 1.4154
III	EEAO 100mg/kg	64.00 ± 5.13	0.833 ± 1.4434
IV	EEAO 200mg/kg	70.33 ± 4.84	0.33 ± 0.5774
V	EEAO 500mg/kg	66.33 ± 1.33	0.33 ± 0.5774
	P-value	(0.252)NS	(0.950)NS

Results are expressed in mean ± SEM; n = 5, P-value =NS, *Significant P<0.05; No significant difference between the groups.

The results reveal that intraperitoneal administration of ethanol leaf extract of *Arthropterisorientalis*at the tested doses of 100, 200 and 500mg/kg exhibited a non-concentration dependent and non-significant increase in percentage lymphocytes when compared with standard group and exhibited a non-concentration dependent and non-significant decrease in percentage lymphocytes when compared with control group with the exemption of 200mg/kg that exhibited non-significant increase in percentage leukocytes.

While the results reveal that intraperitoneal administration of ethanol extract of *Arthropterisorientalis* leaf at the tested doses of 200 and 500mg/kg exhibited a non-significant decrease in percentage Monocytes and 100mg/kg exhibited a non-significant increase in percentage Monocytes when compared with control group that received tween 80. When compared with the standard drug, all the tested doses of 100, 200 and 500mg/kg exhibited a concentration dependent and a non-significant decrease in percentage Monocytes.

Discussion

Inflammation, a response triggered by damage to living tissues, has a purpose to localize and eliminate injurious agents and to remove damaged tissues so that the body can begin to heal. This action is usually beneficial but when it stays for a longer duration, chronic inflammation sets in. The study observed that the plant extract contained glycoside, saponnin, tannin,

flavonoid, phenol, carbohydrate, amino acids, aromatic amino acid and steroid while alkaloid, reducing sugar, starch, protein and terpenoid were absent. Glycosides as well as flavonoids, saponin, and tannin show potent anti-inflammatory action on proliferative phases of inflammation based on the research conducted by Owolabi*et al*., 2018. This is also in agreement Hamalainen*et al*., 2007, which asserted that flavonoids inhibits inflammation by inhibiting signal transducer and activator of transcription 1 (STAT-1) and nuclear factor kappa beta (NF-kβ) activations. Also, the anti-inflammatory properties of the plant extract could also be due to the presence of a high percentage of tannin which has been used as an anti-inflammatory agent (Ambreem andMirza, 2020).

The search for a suitable anti-inflammatory drug with very minimal side effects has been on the increase. Most anti-inflammatory drug is associated with deleterious side effects such as gastric ulcer, renal damage, bronchospasm and cardiac abnormalities, thus restricting their use. (Kehinde*et al*., 2023). The acute toxicity testing carried out on the plant extract showed that, at a very high dose of about 5000mg/kg, there was no sign of toxicity or death indicating that the plant extract is relatively safe. There is a need for further molecular studies to ascertain the active compound that elicited the anti-inflammatory property.

The Egg albumin-induced hind paw edema is a major test used for screening anti-inflammatory properties of new agent. It measures the ability of the compound to reduce local edema which was induced in the right paw by inoculating an irritant agent. Our result shows the effect of the ethanol extract with regards to the mean hind paw diameter,it showed a statistical significant decrease in the induced edema as the time interval increased. The percentage inhibition was also seen to increase steadily therefore establishing that the anti-inflammatory effect of the extract was expressed with time. This finding agrees with the study of anti-inflammatory and diuretic effects of ethanol leaf extract of *Piper guineense* on wistar albino rats (Omodamiro andJimoh, 2014 and Barung*et al*., 2021). The anti-inflammatory effect of the extract observed may be due to its phytochemical constituents such as tannins and it is supported by research that tannins possess anti-inflammatory effect (Duke, 1992). Our result also observed that the plant extract showed statistical significantly inflammatory reduction when compared with the standard drug and gives a higher % inhibition (84.62%) than the standard drug. This could mean that there is a possibility of getting a better drug for the treatment of inflammation from the plant extract.

The study also evaluated the effect of the plant extract on chronic inflammation and used the formalin Induced paw edema. Formalin test is a biphasic response where first phase is the direct effect of formalin, which involves neurogenic pain. The second phase is involved in the inflammatory reactions mediated by prostaglandin, serotonin, histamine, bradikinin and cytokines, such as interleukin-1 beta, interleukin-6 tumor necrosis factor-alpha eicosanoids, and Nitric Oxide (Fu *et al*., 2001:Tong*et al*., 2021). The results showed statistical significant anti-inflammatory effect of *Arthropterisorientalis*in a dose dependent manner at doses 100 and 500mg/kg produced significant ($p<0.05$) reduction in paw edema and inhibition when compared with the control group. However, there was no significant difference in paw edema when compared to the standard drug. This effect can be attributed to the presence of polyphenolic compounds in *Arthropterisorientalis,* also present in several medicinal plantswhich are responsible for anti-inflammatory capacity as shown in previous studies(Wu *et al*., 2006, Sarkar *et al*., 2021).

The study showed that there was no statistical significant difference in hematological parameters especially white blood cells and neutrophils which are involved in inflammation,this could be attributed to the extract's anti-inflammatory properties(Betancourt-Alonso *et al*., 2011, Nana*et al*., 2023)

Conclusion

It is concluded that ethanol extract of *Arthropterisorientalis*has anti-inflammatory properties and could be used to treat both acute and chronic inflammation. This justifies its use traditionally for the purpose.

Declarations

Data Availability: The data are available on request

Human and Animal Rights

The University of Nigeria Animal Research Ethics Committee, which agreed with the "Guide to the Care and Use of Laboratory Animals in Research and Teaching" as prescribed in NIH publications volume 25 No.28 revised in 1996, approved the use of animals for this study with approval number UNN-AREC/056.

References

Comment [MB16]: There are 7 from 30 references not up to date (more than 10 years)

Addo-Fordjour, P., Yeboah-Gyan, K., Lawson, B. W. L., &Akanwariwiak, W. G. (2007). Diversity and distribution of ferns on the campus of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. *Journal of the University of Science and Technology*, *27*(1). https://doi.org/10.4314/just.v27i1.33022

Ajirioghene AE, Ani CO, Ajebor DN, ElavienisoAF,Okolo KO. (2022) Methanolic leaf extract of *Dryopterisdilatata*reverses kidney injury on streptozotocin-induced diabetic male Wistar rats. Niger J ExpClinBiosci 2022;10:65-73.

Ajirioghene. E. Akpotu,, Ani, C, Nworgu, C., Pamela, O., Igwe, U., Uzoigwe, J., Adeyemo, M., Nwaeme, O. and Onwujekwe, O. (2018), Antidiabetic and Anti- hyperlipidemic effects of ethanolic extract of *Dryopterisdilatata* leaves, *Journal of Diabetes and Endocrinology*, Vol. 9(3), pp. 20-27.

Ambreen, M., and Mirza, S. A. (2020). Evaluation of anti-inflammatory and wound healing potential of tannins isolated from leaf callus cultures of Achyranthesaspera and Ocimumbasilicum. *Pakistan Journal of Pharmaceutical Sciences*, *33*(1(Supplementary)), 361–369.

Asakawa, Y. (2001). Recent advances in p h y t o c h e m i s t r y o f bryophytesacetogenins, terpenoids and bis(bibenzyl)s from selected Japanese, Taiwanese, New Zealand, Argentinean and European liverworts. *Phytochemistry*, *56*, 297–312.

BarungElisabeth Natalia , Jovie Mien Dumanauw, MeilanyFeronikaDuri,and Donald Emilio Kalonio (2021), Egg white-induced inflammation models: A study of edema profile and histological change of rat's paw. J Adv Pharm Technol, doi: 10.4103

Betancourt-Alonso, M. A., Orihuela, A., Aguirre, V., Vázquez, R., & Flores-Pérez, F. I. (2011). Changes in behavioural and physiological parameters associated with Taeniapisiformisinfection in rabbits (Oryctolaguscuniculus) that may improve early detection of sick rabbits. *World Rabbit Science*, *19*(1). https://doi.org/10.4995/wrs.2011.801

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., and Deng, J. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget. Impact Journals, LLC*, *9*(6), 7204–7218. https://doi.org/10.18632/oncotarget.23208.PMC5805548

Duke, J. A. (1992). *Handbook of Biologically active phytochemicals and their activities.* CRC Press.

Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S., &Girardin, S. E. (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. *Clinical and Experimental Immunology*, *147*(2), 227–235. doi.org/10.1111/j.1365-2249.2006.03261.x

Fu, K.-Y., Light, A. R., and Maixner, W. (2001). Long-lasting inflammation and long-term hyperalgesia after subcutaneous formalin injection into the rat hindpaw. *The Journal of Pain: Official Journal of the American Pain Society*, *2*(1), 2–11. doi.org/10.1054/jpai.2001.9804

Hamalainen, M., Nieminen, R., Vuorela, P., Heinonen, M., &Moilanen, E. (2007). Anti-Inflammatory Effects of Flavonoids: Genistein, Kaempferol, Quercetin, and Daidzein Inhibit STAT-1 and NF-κBActivations,Whereas Flavone, Isorhamnetin, Naringenin, and Pelargonidin Inhibit only NF-κB Activation along with Their Inhibitory Effect on iNOS Expression and NO Production in Activated Macrophages. *MediatInflamm*, 1–10.

Jan Rahmatullah, SajjadAsaf, Muhammad Numan, Lubna and Kyung-Min Kim, (2021), Plant Secondary Metabolite Biosynthesis and Transcriptional Regulation in Response to Biotic and Abiotic Stress Conditions *Agronomy*, doi.org/10.3390/agronomy11050968

Kehinde P. Olamilosoye, Rufus O. Akomolafe , Olumide S. Akinsomisoye [a], Modinat A. Adefisayo, Quadri K. Alabi (2023), The aqueous extract of *Ocimumgratissimum* leaves ameliorates acetic acid-induced colitis via improving antioxidant status and hematological parameters in male Wistar rats. Egyptian Journal of Basic and Applied Sciencesdoi.org/10.1016/.

Khalifa, N. M., Al-Omar, M. A., & Ali, O. M. (2017). Synthesis and reactions of some new (E)-2-cyano-N′-[1-(pyren-3-yl)ethylidene]acetohydrazide derivatives. *Russian Journal of General Chemistry*, *87*(9), 2083–2086. doi.org/10.1134/s1070363217090250.

Lorke D. (1983) A new approach to practical acute toxicity. Archive Toxicol 53: 275-289.

Madhu, M., Sailaja, V., Satyadev, T., Satyanarayana, M. V. (2016). Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. *Journal of Pharmacognosy and Phytochemistry*, 5(2): 25-29.

Nana William Yousseu, Justin RodrigueBillongMimb,AlbertDonatienAtsamo,EricGonzalTsafack,Stephanie Flore DjuichouNguemnang,Zenab Linda FagniNjoya,VanessaMbaMatahMarthe,YacineKarelleMadjoKouam,MariusMbiantcha,and Gilbert Ateufack (2023), *In Vitro* and *In Vivo* Anti-Inflammatory Properties of the Hydroethanolic Extract of Roots of Vennoniaguineensis (Asteraceae). International Journal of Inflammation, doi.org/10.1155/2023/7915367

OkaiyetoKunle, Oluwafemi O. Oguntibeju (2021),African Herbal Medicines: Adverse Effects and Cytotoxic Potentials with Different Therapeutic Applications, *Int. J. Environ. Res. Public Health*, doi.org/10.3390/ijerph18115988

Omodamiro, O., and Jimoh, M. (2014). A Evaluation of Anti-inflammatory and Diuretic Effects of Ethanol Leaf Extract of Piper guineense on Wistar Albino Rats. *American Journal of Ethnomedicine*, *1*(4), 250–259.

Owolabi, O. O., James, D. B., Sani, I., Andongma, B. T., Fasanya, O. O., & Kure, B. (2018). Phytochemical analysis, antioxidant and anti-inflammatory potential of FERETIA

APODANTHERA root bark extracts. *BMC Complementary and Alternative Medicine, 18*(1). doi.org/10.1186/s12906-017-2070-z

Oyeleke, S. A., Ajayi, A. M., Umukoro, S., Aderibigbe, A., and Ademowo, O. G. (2018). Anti-inflammatory activity of Theobroma cacao L. stem bark ethanol extract and its fractions in experimental models. *J. Ethnopharmacol.* 222, 239–248. doi:10.1016/j.jep.2018.04.050

PetkovVenelin, Iva Slavova, DesislavaTeneva, TzvetelinaMladenova, PlamenStoyanov, and Mariana Argirova (2021) Phytochemical Study and Biological Activity of Three Fern Species of the Asplenium Genus Growing in Bulgaria. The Natural Products Journal, DOI:10.2174/2210315511666210512024716

Prabhavathi, R. M., Prasad, M. P., and Jayaramu. (2016). Studies on qualitative and quantitative phytochemical analysis of Cissusquadrangularis Adv. *Adv. Appl. Sci. Res*, *7*, 11–17.

Sarkar Chandan, MilonMondal, BilkisKhanom, Md. Monir Hossain, Md. Solayman Hossain, AntoniSureda, Muhammad Torequl Islam, MiquelMartorell, Manoj Kumar, JavadSharifi-Rad, Ahmed Al-Harrasi, and Ahmed Al-Rawahi (2021), *Heliotropiumindicum* L.: From Farm to a Source of Bioactive Compounds with Therapeutic ActivityEvid Based Complement Alternat Med.doi: 10.1155/2021/9965481

Shabbir, A., Batool, S. A., Basheer, M. I., Shahzad, M., Sultana, K., Tareen, R. B., et al. (2018). Ziziphoraclinopodioides ameliorated rheumatoid arthritis and inflammatory paw edema in different models of acute and chronic inflammation. *Biomed. Pharmacother.* 97, 1710–1721. doi:10.1016/j.biopha.2017.11.118

Sriuttha P, Sirichanchuen B, Permsuwan U. (2018) Hepatotoxicity of Nonsteroidal Anti-Inflammatory Drugs: A Systematic Review of Randomized Controlled Trials. Int J Hepatol. ;2018:5253623.

Tong Zhenkai, Wenfeng He, Xiao Fan, and AiweiGuo (2021), Biological Function of Plant Tannin and Its Application in Animal Health. Front Vet Sci. doi: 10.3389/fvets.2021.803657

WinigerFabian (2022) **The Spirituality of Others and the WHO Discourse on Traditional Medicine.** doi.org/10.1093/oso/9780192865502.003.0005

Wu, Y., Zhou, C., Li, X., Song, L., Wu, X., Lin, W., Chen, H., Bai, H., Zhao, J., Zhang, R., Sun, H., & Zhao, Y. (2006). Evaluation of antiinflammatory activity of the total flavonoids ofLaggerapterodonta on acute and chronic inflammation models. *Phytotherapy Research: PTR*, *20*(7), 585–590. doi.org/10.1002/ptr.1918