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Anti-Inflammatory And Membrane Stabilization Activities Of Methanol Extract of *Cissus aralioides* leaves.

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ABSTRACT

This study aimed at investigating the anti-inflammatory and membrane stabilization activities of methanol extract of Cissus aralioides leaves. Fresh leaves of the plant were air dried, macerated in methanol, filtered and concentrated at reduced pressure with a rotary evaporator. Adult wistar albino rats (120-150g) of both sexes were used for the animal experiments while fresh whole blood (3ml) collected from healthy human volunteers was used for the membrane stabilization studies Anti-inflammatory activities were determined using egg albumin-induced rat paw oedema, acetic acid-induced vascular permeability and agar-induced leukocyte migration in rats, and membrane stabilization effect was by heat and hypotonic solution-induced haemolysis of human red blood cell membrane. The extract doses (100-400 mg/kg) significantly ($p< 0.05$) inhibited egg albumin-induced rat paw oedema and also significantly ($p< 0.05$) reduced exudate volume and vascular permeability in rats induced by acetic acid. The varied extract doses (100-800 µg/ml) stabilized human erythrocyte membrane against heat and hypotonic-induced lysis, as was evidenced by the high percentage inhibition of haemolysis of the red cell membrane. From the results in this study, it can thus be concluded that methanol extract of Cissus aralioides has potent anti-inflammatory and membrane stabilization properties.

Keywords: anti-inflammatory**,** Cissus aralioides, erythrocyte membrane, leukocyte migration, vascular permeability

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INTRODUCTION

Inflammation is the response of tissue to injury (infection, trauma and hypersensitivity), characterized in the acute phase (microscopically) by increased blood flow (vasodilation) and vascular permeability along with the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines. Macroscopically, it is characterized by redness; swelling, heat, pain and loss of function and these cause considerable pain and discomfort [1]. At the onset of an inflammation, cells undergo activation and release inflammatory mediators which include histamine, serotonin, slow reacting substances of anaphylaxis (SRS-A), prostaglandins and some plasma enzyme systems such as the complement system, the clotting system, the fibrinolytic system and the kinin system [2]. These mediator molecules work collectively to cause increased vasodilation and permeability of blood vessels, thus, leading to increased blood flow, exudation of plasma proteins and fluids, and migration of leukocytes, mainly neutrophils, outside the blood vessels into the injured tissues. Immigration of these cells into peripheral tissues is one of the principal purposes of inflammation, bringing to a site of injury the immune cells which combat infection and clean up damaged tissue [3]. Although inflammation is a defence mechanism, the complex events and mediators involved in the inflammatory reactions can induce, maintain or aggravate many diseases.

Currently available anti-inflammatory agents such as steroids and non-steroidal anti-inflammatory drugs have several drawbacks or limitations to their use. Increased incidence of stroke, atherosclerosis, cancer, gastric disorder and coronary heart related diseases has been attributed to prolonged use of the non-steroidal anti-inflammatory drugs such as indomethacin, largely due to the implication of prostanoids in these pathological conditions [4].

Cissus aralioides is a lofty climber, woody at the base with stout green succulent stems constricted at the nodes and sometimes sub-succulent leaves (fig 1). The whole plant is covered with irritating hairs, and leaves contain an acid and slightly acrid red sap. They are commonly found in tropical Africa especially Cameroon and Nigeria (Igbo name- eririagwo) [5]. In Nigeria folkloric medicine, the leaves are used for treatment of cuts, wounds, internal and external microbial infections and swellings. It is also used for the treatment of arthritis, rheumatism, dropsy, gout swelling, oedema, analgesic, pulmonary troubles [5].

Several medicinal plants and natural products are used in the treatment of disorders arising from inappropriate deployment of inflammatory mediators and microbial infections. *Cissus aralioides* has been reported to have antidiabetic, antimicrobial as well as anti-inflammatory properties [6]. This study was therefore aimed at investigating the anti-inflammatory and membrane stabilization activities of methanol extract of *Cissus aralioides* leaves.

Fig 1: *Cissus aralioides* leaves

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MATERIALS AND METHODS

Materials

Plant Material

Cissus aralioides leaves were collected from the environs of University of Nigeria, Nsukka campus in September, 2014. The plant was identified by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme, Aku road, Nsukka, Enugu State, Nigeria and Mr. Francis, Chijioke of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka campus. A voucher specimen was deposited in the Department of Plant Science and Biotechnology herbarium, University of Nigeria, Nsukka for reference purposes.

Experimental Animals

Swiss albino mice (22-30g) and adult Wistar albino rats (120-150g) of both sexes obtained from the Zoological Garden, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, were used for the experiments. These were housed in metal steel cages and acclimatized in the laboratory for two weeks before the experiments. They were maintained in standard environmental conditions of temperature (28±2^{o}C) and fed with standard rat chow and tap water *ad libitum*. The animal study was conducted in accordance with the ethical rules and recommendation of University of Nigeria, Nsukka Committee on the Care and Use of Laboratory Animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85-23, revised, 1985).

Extraction

The fresh leaves plucked from the plant stalk were dried at room temperature for three weeks and crushed. Extract of the plant material was obtained by macerating the leaves in methanol for 24hrs and evaporating the solvent using a rotary evaporator. The methanol extract of *Cissus aralioides leaves* obtained was stored in the freezer (-4^{o}C) until needed.

Acute Toxicity Study

Acute toxicity study of methanol extract of *Cissus aralioides* was carried out using the method of Lorke [7] to define the range of lethal dose and safe dose for the extract. Eighteen (18) Swiss albino mice starved of food for 18 hours but allowed access to water were used for this study. They were divided into six groups of three (3) mice each and treated orally with varying doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight. The animals were observed for 24 hours for nervousness, dullness, in-coordination and/or mortality.

Anti-Inflammatory Studies

Anti-inflammatory activities of the methanol extract of *Cissus aralioides* were studied using egg albumin-induced rat paw oedema, acetic acid-induced vascular permeability, and agar-induced leukocyte migration in rats.

Egg Albumin-Induced Rat Paw Oedema

The rat paw oedema method of Winter *et al*. [8] was used. Twenty-five Wistar albino rats were fasted overnight but had free access to water prior to the day of the experiment. The rats were divided into five groups of five animals each.

Group 1 (normal control) received normal saline
Group 2 received 100 mg/kg b.w of the extract
Group 3 received 200 mg/kg b.w of the extract
Group 4 received 400 mg/kg b.w of the extract
Group 5 (standard control) received indomethacin (50mg/kg) intraperitoneally (i.p).

Vehicle, standard drug and test compounds were administered 30 minutes prior to egg albumin (philogistic agent) injection. After 30minutes, 0.1 ml of fresh undiluted egg albumin was injected subcutaneously into the sub-plantar region of right hind paw, and the oedema was assessed in terms of volume of distilled water displaced by the paw before, and at 0.5, 1, 2, 3, 4 and 5 hours after induction of inflammation [9].

The percentage inhibition of oedema was calculated using the relation.

$$\% \text{ Inhibition} = 100(1-(a-x/b-y))$$

Where a= mean paw volume of treated animals after egg albumin injection
x= mean paw volume of treated animals before egg albumin injection
b= mean paw volume of control animals after egg albumin injection
y= mean paw volume of control animals before egg albumin injection

Acetic Acid-Induced Vascular Permeability in Rats

The effect of the extract on acetic acid induced vascular permeability was examined using the modified method of Whittles [10]. Twenty-five Wistar albino rats of either sex were divided into five groups of five rats each.

The animals were starved for 10 hours prior to the experiment and then were administered varied doses of the extract and drugs as above.

After 3 hours, each animal was given 0.5ml intravenous injection of 1% Evans blue solution.

Vascular permeability was induced 30 minutes afterwards by (i.p) injection of 1ml of 0.6% glacial acetic acid. The animals were sacrificed 20 minutes later and their peritoneum was washed with 10 ml of normal saline. The recovered peritoneal fluid was centrifuged at 3000 rpm for 10 minutes and the absorbance was measured at 610nm using spectrophotometer. The percentage inhibition of vascular permeability was calculated thus:

$$\% \text{ inhibition of vascular permeability} = 1- (T/C) \text{ X } 100$$

Where T and C is the mean absorbance of treated and control groups.

Agar Induced Leukocyte Migration in Rats

The effect of methanol extract of *Cissus aralioides* on *in vivo* leukocyte mobilization induced by an inflammatory stimulus in albino rats was evaluated using the method of Rebeiro *et al*., [11].

Twenty-five (25) Wistar albino rats of either sex divided into five groups of five rats each were used for the test. The animals were fasted for 10 hours prior to the experiment and were then administered with varied doses of the extract and drug as stated above.

Each animal in the respective groups received intraperitoneal injection (i.p) of 0.5 ml of a 3% w/v agar suspension in normal saline. After 4 hours, the animals were sacrificed and the peritoneal cavities washed with 5ml of a 5% solution of EDTA in phosphate buffer saline (PBS). The peritoneal fluid was recovered. Total and differential counts (TLC and DLC) on the perfusates were performed after staining with Wright's stain.

The percentage inhibition of leukocyte migration was calculated using the formula below:

$$\% \text{ Leukocyte inhibition (\% LI)} = 1- (T/C) \text{ X } 100$$

Where T and C represent the leukocyte count of the treated and control groups respectively.

Total Leukocyte Count

The total leukocyte count was determined following the standard technique as described by Ramnik [12]. The sample was diluted 1:20 with Turk's solution, which is 2 % glacial acetic acid. The diluted sample was loaded into a Neubaer counting chamber with the aid of Pasteur pipette. The total leukocyte was calculated by counting the required number of squares on the counting chamber under a microscope.

Differential Leukocyte Count

Differential leukocyte count was carried out using standard techniques as described by Ramnik [12]. The sample was centrifuged at 2000g for 5 minutes, at the end of which the solution was poured out. The pellet was re-suspended and a drop made on a clean grease-free slide. The film was covered with Wright's stain for 3 minutes and was diluted twice the volume with distilled water and left for 7 minutes. The stain was drained off and allowed to air dry. The cells were counted with oil immersion using X100 objective lens on a microscope.

Membrane Stabilization Studies

Heat and water-induced haemolysis of human red blood cell (HRBC) was used to assess membrane stabilization activity of the extract using the method of Shinde *et al*. [13].

Preparation of Erythrocyte Suspension

Fresh whole blood (3ml) collected from healthy human volunteers were put in heparinised tubes and centrifuged at 3000 rpm for 10 minutes. A volume of saline equivalent to that of the supernatant was used to dissolve the red blood pellets. The volume of the dissolved red blood pellets was measured and reconstituted as a 40% v/v suspension with isotonic buffer solution (10 mM Sodium Phosphate buffer, pH 7.4). The buffer solution contained 0.2 g of NaH_2PO_4, 1.15 g of Na_2HPO_4 and 9 g of NaCl in 1litre of distilled water. The reconstituted red blood cell (re-suspended supernatant) was used as such. All experiments were performed in keeping with the guidelines published in the Helsinki Declaration for the Use of Human Subjects for research. Ethical clearance approval with the approval number UNN/FBS/EC/1022 was obtained from the Faculty of Biological Sciences, University of Nigeria, Nsukka Committee on Ethics and Biosafety.

Heat-Induced Haemolysis of Red Blood Cell

Samples of the extract were dissolved in isotonic phosphate buffer solution. A set of five centrifuge tubes containing respectively, 5ml graded doses of the extracts (100, 200, 400, 600, 800µg/ml) was arranged in quadruplicates (4 sets per dose). Two (2) sets of control tubes contained 5ml of the vehicle and 5ml of 200µg/ml of indomethacin respectively. Human red blood cell suspension (0.1 ml) was added to each of the tubes and mixed gently. A pair of the tubes was incubated at 54°C for 20 minutes in a regulated water bath. The other pair was maintained at −10°C in a freezer for 20 minutes. Afterwards, the tubes were centrifuged at 1300g for 3 minutes and the haemoglobin content of the supernatant was estimated using a spectrophotometer at 540 nm.

The percent of inhibition of haemolysis of the extract was calculated thus

$$\% \text{ Inhibition of haemolysis} = 1 - \frac{(OD_2 - OD_1)}{(OD_3 - OD_1)} \times 100$$

Where OD_1 = Absorbance of test sample unheated
OD_2 = Absorbance of test sample heated
OD_3 = Absorbance of control sample heated

Hypotonic-Induced Haemolysis of Red Blood Cell

Samples of the extract were dissolved in distilled water (hypotonic solution). The hypotonic solution (5 ml) contained graded doses of the extracts (100, 200, 400, 600 and 800µg/ml) put into duplicate pairs (per dose) of the centrifuge tubes. Isotonic solution (5 ml) containing graded doses of the extract (100-800 µg/ml)

were also put into duplicate pairs (per dose) of the centrifuge tube. Control tubes contained 5 ml of the vehicle (distilled water) and 5ml of 200µg/ml of indomethacin respectively. Erythrocyte suspension (0.1 ml) was added to each of the tubes and mixed gently. The mixture was incubated for 1 hour at room temperature (25°C) and centrifuged afterwards for 3 minutes at 1300 g.

Absorbance (OD) of the haemoglobin content of the supernatant was estimated at 540nm using a spectrophotometer. Percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%.

The percent inhibition of haemolysis of the extract was calculated thus

$$\% \text{ Inhibition of haemolysis} = 1 - \frac{(OD_2 - OD_1)}{(OD_3 - OD_1)} \times 100$$

Where OD_1 = Absorbance of test sample in isotonic solution
OD_2 = Absorbance of test sample in hypotonic solution
OD_3 = Absorbance of control sample in hypotonic solution

Statistical Analysis

Data obtained from the study were analyzed using statistical product and service solutions (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA). All values were expressed as mean ± standard deviation and analysed by one way analysis of variance (ANOVA). Differences between means was assessed by Duncan's new multiple range. Values with $p<0.05$ was considered statistically significant.

RESULTS

Acute Toxicity Study

No mortality or significant change in the behaviour of the animals was recorded at the administration of methanol extract of *Cissus aralioides* up to the dose of 5000mg/kg per body weight.

Table 1: Effect of Methanol Extract of *Cissus aralioides* on Egg Albumin-Induced Rat Paw Oedema

Treatment	Dose (mg/kg)	Mean Paw volume ± S.D (ml)					
		30mins	60mins	120mins	180mins	240mins	300mins
Control	Saline	0.53 ± 0.21 (-)	0.48 ± 0.10 (-)	0.55 ± 0.13 (-)	0.63 ± 0.05 (-)	0.65 ± 0.06 (-)	0.70 ± 0.08 (-)
Extract	100	0.47 ± 0.14 (11)	0.42 ± 0.11 (12)	0.30 ± 0.12* (45)	0.24 ± 0.11* (62)	0.16 ± 0.11* (75)	0.08 ± 0.10* (88)
	200	0.47 ± 0.09 (11)	0.42 ± 0.04 (12)	0.41 ± 0.02* (25)	0.38 ± 0.10* (40)	0.10 ± 0.00* (85)	0.07 ± 0.15* (90)
	400	0.47 ± 0.16 (11)	0.33 ± 0.05 (31)	0.26 ± 0.17* (53)	0.20 ± 0.00* (68)	0.10 ± 0.08* (85)	0.04 ± 0.05* (94)
Indomethacin	50	0.42 ± 0.01 (21)	0.30 ± 0.08 (37)	0.23 ± 0.21* (58)	0.15 ± 0.10* (76)	0.10 ± 0.14* (85)	0.05 ± 0.08* (93)

n= 5 Level of significance * = P<0.05. Values in parenthesis are percentage inhibition of paw oedema calculated relative to the control.

Egg albumin-induced rat paw oedema

Egg albumin induced oedema in the experimental animals which was sustained over 5 hrs. The inflammatory oedema induced by egg albumin was significantly inhibited ($p<0.05$) by the methanol extract of *Cissus aralioides* from the 2nd to 5th hour. The effect of the extract was dose dependent at various time intervals; the mean paw volume was reduced from 0.47 ± 0.14 to 0.08 ± 0.10, 0.47 ± 0.09 to 0.07 ± 0.15 and

0.47 ± 0.16 to 0.04 ± 0.05 for the doses of 100, 200 and 400mg/kg body weight respectively at the 5th hour interval (Table 1). The highest inhibition of rat oedema of 94% was observed in the group administered with 400mg/kg body weight of the extract at the 5th hour. The inhibition by the extract was comparable with that of indomethacin at a single dose of 50 mg/kg body weight.

Acetic acid-induced vascular permeability in rats

Result in Table 2 showed that *Cissus aralioides* extract and indomethacin significantly ($p<0.05$) inhibited acetic acid-induced vascular permeability in rats. *Cissus aralioides* extract at doses of 100, 200 and 400 mg/kg body weight showed percentage inhibitions of vascular permeability of 19, 40 and 64 respectively, while indomethacin (50mg/kg) showed inhibition of 86% when results were calculated relative to the control (normal saline).

Table 2: Effect of Methanol Extract of *C. aralioides* on Acetic Acid-Induced Vascular Permeability in Rats

Treatment	Dose (mg/kg)	Absorbance	% Inhibition of Vascular Permeability
Control	-	0.70±0.08	
Extract	100	0.57±0.07*	19
	200	0.42±0.12**	40
	400	0.25±0.05***	64
Indomethacin	50	0.10±0.03***	86

n= 5 Level of significance * = p< 0.05, ** = p< 0.01, *** = p<0.001. Percentage inhibition of vascular permeability was calculated relative to the control.

Agar-induced leukocyte migration in rats

Result from Table 3 showed that the extract exerted a marked inhibition of leukocyte migration into the peritoneal cavity. There were significant ($p<0.05$) differences between the total leukocyte count of group 1 (control group) and that of groups treated with the extract as indicated by their percentage inhibition of 21, 31 and 47 for the doses of 100, 200 and 400mg/kg body weight of the extract respectively. The effect of the extract was comparable to that of the reference anti-inflammatory drug (indomethacin) which produced an inhibition of 58%.

Table 3: Effect of Methanol Extract of *C. aralioides* on Agar-Induced Leukocyte Migration in Rats

Treatment	Conc. (mg/kg)	TLC (mm^{-3})	% Inhibition	Differential Leukocyte Count (%)				
				N	L	M	B	E
Control	-	14240 ± 5517.52		60.40	35.20	2.06	1.16	1.14
Extract	100	11300 ± 3628.36	21	60.56	36.00	1.40	0.73	1.24
	200	9800 ± 3158.32	31	57.00	39.50	1.40	0.92	0.89
	400	7600 ± 3184.34*	47	54.00	43.00	0.68	0.68	0.97
Indomethacin	50	6000 ±3140.06**	58	60.00	37.00	1.15	0.62	1.20

n= 5 Level of significance * = p< 0.05, ** = p<0.01. Percentage inhibition of leukocyte migration was calculated relative to the control.
Key: N= Neutrophils, L= Lymphocytes, M= Monocytes, E= Eosinophils, B= Basophiles

Membrane stabilization of human erythrocyte cell membrane

Effect of Methanol Extract of *C. aralioides* on Heat-Induced Haemolysis of Human Red Blood Cell

Result in table 4 showed that the extract at varied doses (100-800µg/ml) significantly ($p<0.05$) protected the human erythrocyte membrane against lysis induced by heat as shown by the high percentage inhibitions of haemolysis exerted by the extract in a dose-dependent manner. The result also showed that indomethacin (200µg/ml) produced the highest percentage inhibition of haemolysis with a value of 99%.

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Table no 4: Effect of Methanol Extract of *Cissus aralioides* on Heat-Induced Haemolysis of Human Red Blood Cells (HRBCs)

Treatment	Dose (µg/ml)	Mean absorbance ± S.D		% Inhibition of haemolysis
		Heated solution	Unheated solution	
Control	-	0.78±0.18	0.07±0.01	
Extract	100	0.43±0.01***	0.05±0.01	48
	200	0.46±0.03***	0.18±0.03	53
	400	0.59±0.04***	0.44±0.01	56
	600	0.64±0.04***	0.55±0.01	61
	800	0.69±0.01***	0.67±0.04	82
Indomethacin	200	0.08±0.01***	0.07±0.01	99

Level of significance * = P<0.05, ** = P<0.01, *** = P<0.001. Percentage inhibition was calculated relative to the control. n= 2

Effect of Methanol Extract of *C. aralioides* on Hypotonicity-Induced Haemolysis of Human Red Blood Cell

Result in table 5 showed that *Cissus aralioides* extract significantly (p<0.05) inhibited lysis induced by water (hypotonic solution). This is evidenced by the high percentage inhibition (19, 55 and 78 %) of haemolysis obtained at doses of 400, 600 and 800µg/ml respectively. The inhibition occurred in dose-dependent manner, increasing with increased concentration of the extract and was comparable with that of indomethacin (200µg/ml) which produced percentage inhibition of 80 %.

Table 5: Effect of *Cissu saralioides* Extract on Hypotonic-Induced Haemolysis of HRBCs

Treatment	Dose (µg/ml)	Mean absorbance ± S.D		% Inhibition of haemolysis
		Hypotonic solution	Isotonic solution	
Control	-	0.67±0.01	0.05±0.01	
Extract	100	0.65±0.02	0.08±0.01	3
	200	0.63±0.02	0.12±0.03	7
	400	0.62±0.06**	0.40±0.03	19
	600	0.61±0.01**	0.56±0.03	55
	800	0.60±0.03**	0.58±0.01	78
Indomethacin	200	0.19±0.03***	0.07±0.02	80

Level of significance * = P<0.05, ** = P<0.01, *** = P<0.001. Percentage inhibition was calculated relative to the control. n= 2

DISCUSSION

Inflammation is a protective attempt by the organism to remove injurious stimuli and to initiate the healing process [14]. The primary mediators of inflammation are vaso-active amines and eicosanoids (prostaglandins), released from mast cells. These can cause vasodilation, fever and pain evident in many disorders [15].

Oedema induced by phylogistic agent (egg albumin) is a widely accepted model for the evaluation of anti-oedema and anti-inflammatory effect of drugs [8]. Egg albumin-induced oedema has been commonly used as an experimental model for acute inflammatory studies and it is believed to be biphasic. The early phase (1-2 hour) of the oedema is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The later phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells, and prostaglandins produced by tissue macrophages [16]. Results of this anti-inflammatory study indicated that *Cissus aralioides* extract at the dose of 400mg/kg acted potently in the later phase probably by inhibiting prostaglandin synthesis involving arachidonic metabolites. Phytochemical screening of *Cissus aralioides* have shown that the extract possesses flavonoids, terpenoids, tannins and steroids which may be responsible for the inhibition of the enzymes involved in the production of the chemical mediators of inflammation. The extract exhibited significant (p<0.05) inhibition of the egg albumin-induced inflammation in a dose-dependent manner with percentage

inhibition of oedema and inflammation of 88, 90 and 94 % at the doses of 100, 200 and 400 mg/kg respectively at the 5th hour. This result is comparable to that of indomethacin (50 mg/kg) which produced 93% inhibition of rat paw oedema. Across time at respective doses, there was inhibition of paw oedema with higher inhibitions occurring at the administration of 400mg/kg dose. It was also observed that the highest level of inhibition occurred at the 5th hour followed by the 4th hour. This pattern of activity is in tandem with the acute nature of the egg albumin induction of inflammation and paw oedema.

The anti-inflammatory activities of methanol extract of *Cissus aralioides* on rat paw oedema induced by egg albumin can be attributed to phytochemical constituents in the extract such as flavonoids, tannins, saponins and steroids. Tannins function mainly as an astringent, since it can bind albumin existing in the skin and mucous membranes of the body thereby forming a protective layer which is insoluble and can resist disease. Tannins heal affected areas when treated with them by protecting the areas and at the same time can reduce inflammation. This accentuates the use of herbs containing tannins as compress for cuts and wounds, haemorrhoids, varicose veins, for diarrhoea, catarrh, heavy menstrual flow and inflammatory conditions of the digestive tract [17]. Saponins are glycosides found widely among medicinal plants which foams like soap on mixing with water. They possess a wide range of therapeutic actions in the body including anti-inflammatory, expectorant, diuretic, anti-malarial and haemolytic effects on red blood cells [17]. Many steroids such as glucocorticoids reduce inflammation or swelling by binding to glucocorticoid receptors. These drugs are often referred to as corticosteroids.

The various doses of the *Cissus aralioides* extract evoked significant ($p<0.05$) inhibition of agar induced leukocyte migration into the peritoneum. At the onset of an inflammation, a number of different cells become activated and are recruited into an inflammatory area, where they release inflammatory mediators that cause vasodilation and increased permeability of plasma proteins and fluids into the tissues. The vessel wall become engorged and dilated allowing large numbers of neutrophils to extravagate and appear within the junctional epithelium and underlying connective tissue [18]. These cells are responsible for the inactivation and removal of invading infectious agents and damaged tissues. Chemotactic movement of leukocytes towards the foreign body is the first and most important step in phagocytosis [19]. Leukocytes are rapidly mobilized from the bone marrow into the blood during infections, acute inflammatory reactions and in the superficial surface of a lesion during sub-acute or chronic inflammation. They function as phagocytes of bacteria, fungi and viruses, and detoxifiers of toxic proteins that may result from allergic reactions and cellular injury [20]. The anti-inflammatory effect of *C. aralioides* might have been possible through the alteration of the activation of inflammatory cells. The neutrophils being higher in percentage than the lymphocytes probably might have led to the engulfing and elimination of the foreign body and to the alteration in the migration of the inflammatory cells. Generally, neutrophils are the first line of defence of the immunological system against pathogens. However, in an inflammatory disease such as rheumatoid arthritis, they represent a potential cause of tissue damage [21]. The interaction of recruited neutrophils in the site of inflammation with resident cells, local inflammatory mediators and extracellular matrix may lead to the production of several other mediators including cytokines, degrading enzymes, oxygen and nitrogen species that may further amplify the inflammatory response and further injure surrounding tissues [19]. Innate and adaptive mechanisms of the immune system could be modified by substances to either enhance or suppress their ability to resist invasion by antigens (pathogens) [22]. The inhibition of leukocyte migration by *Cissus aralioides* extract therefore showed that the extract could alter the action of the endogenous factors that are involved in the migration of these cells to the site of inflammation, thereby reducing the inflammatory process.

The anti-inflammatory activity of methanol extract of *Cissus aralioides* was also assessed by acetic acid-induced vascular permeability in rats. Vascular permeability change is a pathophysiological event of inflammation with leakage of vascular contents into the interstitial tissue as is observed by the amount of Evans blue dye which leaked into the peritoneal fluid. Methanol extract of *Cissus aralioides* significantly ($p<0.05$) reduced the extent of vascular permeability. Inflammatory response is a physiologic characteristic of vascularised tissues [23]. Increased vascular permeability occurs as a result of contraction and separation of endothelial cells at their boundaries to expose the basement membrane, which is freely permeable to plasma proteins and fluids [24]. This leads to exudation of fluid rich in plasma proteins including antibodies (immunoglobulins), coagulation factors and inflammatory cells into the injured tissue. Exudation is a consequence of increased vascular permeability and it is considered a major feature of acute inflammation. Acetic acid-induced vascular permeability causes immediate and sustained reaction that is prolonged over 24 hours and its inhibition would indicate that *Cissus aralioides* extract might effectively suppress the exudative

phase of acute inflammation in a concentration-dependent manner by activation of cyclooxygenase and lipoxygenase pathways.

In this study, the methanol extract of *C. aralioides* at concentrations of 100-800µg/ml significantly ($p<0.05$) protected the human red blood cell against lysis induced by hypotonic solution and heat. Indomethacin (200µg/ml) also offered a significant protection of red blood cell against the damaging effect of hypotonic solution and heat. During inflammation, there are lysis of lysosomes which release their component enzymes that produce a variety of disorders. Non-steroidal anti-inflammatory drugs (NSAID'S) exert their pharmacological activity by either inhibiting the release of lysosomal enzymes or stabilizing the red cell membranes. Exposure of red blood cells to injurious substances such as hypotonic solution, heat, methylsalicylate or phenylhydrazine results in the lysis of the membrane accompanied by haemolysis and oxidation of haemoglobin. Since human red blood cell membranes are similar to lysosomal membrane components, the inhibition of hypotonicity and heat-induced red blood cell membrane lysis was taken as a measure for the anti-inflammatory activity of *Cissus aralioides* extract. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluids within the cell resulting to rupturing of its membrane. Injury to red blood cell membrane will render the cell more susceptible to secondary damage through free radical induced lipid peroxidation [25].

Compounds with membrane stabilizing properties are well known for their ability to interfere with the early phase of inflammatory reactions such as the prevention of the release of phospholipases that trigger the formation of inflammatory mediators resulting in the leakage of serum proteins and fluids into the tissues during periods of increased permeability caused by inflammatory mediators. *Cissus aralioides* extract perhaps stabilized the red blood cell membrane by preventing the release of lytic enzymes and active mediators of inflammation. Another possible mechanism for the membrane stabilizing activity of *C. aralioides* extract could be due to an increase in the surface area/volume ratio of the cell, which could be brought about by expansion of membrane or shrinkage of the cell and interaction with membrane proteins [26]. Moreover, it has also been reported that the cell volume of erythrocytes are closely related to the intracellular content of calcium [13]. Hence, it could be speculated that the cytoprotective effect of the extract on the erythrocyte membrane might be due to the ability of the extract to alter the influx of calcium into the erythrocyte.

CONCLUSIONS

From the results in this study, it can thus be concluded that methanol extract of *Cissus aralioides* demonstrated potent anti inflammatory properties and stabilized erythrocyte membrane against lysis induced by hypotonic solution and heat.

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