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Full Length Research Paper

Phytochemical Content And Anti-Inflammatory And Diuretic Activities Of Ethanol Extract Of Amaranthus Hybridus In Experimental Animal Model

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The present study was undertaken to investigate the diuretic and anti-inflammatory activities of Amaranthus hybridus in experimental albino rats. The preliminary phytochemical investigations were carried out to identify the various chemical constituents present in the ethanol extract. It was found that the extract contains alkaloid, flavonoid, saponin, tannins, phenols, hydrocyanic acid and physic. Acute toxicity studies of plant extract were determined and LD50 was found to be 707.1mg/kg. Doses of 1000, 500, 250, 125,62.5 and 31.25mg/kg of the extract were administered intraperitoneally, furosemide (40mg/kg) was used as positive control in diuretic and Aspirin (100mg/kg) was also used as positive control as anti-inflammatory drug. The extract exhibited a significant increase in urine volume and the electrolyte at different concentration of 1000mg to 31.25mg. At 1000mg and 500mg concentration (2.55 ± 0.07 and 2.15 ± 0.07) respectively, exhibit a significant difference (P<0.07). This extract exhibit an increase in sodium (Na+) excreted at higher doses from 1000mg to 250mg (136.50 ± 0.07, to 119.00 ± 1.41), The potassium (K +) level decreases as the doses decreased from 1000mg to 250mg (17.50 0.71 to 14.50 0.71) and chloride concentration of the extract increases as the concentration also increased from 1000mg/kg to 250mg/kg (89.50 ± 0.71, to 112.50 ± 0.71) they are exhibited significant difference (P 0.05). Therefore, there was no significant difference between the treated group of biocarbonatic (HCO-3) at a concentration of 1000mg (25.00 ± 0.000) (P). The average inflammation on the hind paw decreased as the time intervals increased from 30mins to 180mins, which shows 5.75 ± 0.35 to 3.55 ± 0.35, there was a significant anti-inflammatory activity (P< 0.05) exhibited at a various doses of the extract. The % inflammation on the hind paw of rats decreased as the time interval increased from 83.8 0.35 to 05.1 0.35 (P< 0.05). There was increase in % inflammation as dose decreased down in concentration (P<0.05), there was a significant difference. However, the % inhibition of inflammation increases as the time intervals increased from 16.6 ± 0.35 to 34.8 ± 0.36, at all the doses of extract and also exhibited significant difference (P<0.05). This study has demonstrated the anti-inflammatory and diuretic activity of ethanol leaf extracts of Amaranthushybridus in reducing of inflammation and induced diuretic in albino rats and this may be due to the presence of flavonoids and tannins in the plant.

Keywords: Amaranthus, hybridus, anti-inflammatory, diuretic, ethanol extract, Wistar albino rat.

INTRODUCTION

The plant Amaranthus hybriduslinn, is a member of the Amaranthaceae family. In West Africa, the family is represented by fourteen (14) general and thirty-seven (37) species (1). It has a wide variety of uses.The plant is used in the treatment of intestinal bleeding, diarrhea and excessive menstruation (2, 3). It is easy to harvest and very nutritious, but it is rather small, about 1mm in diameter. The genus Amaranthus has received considerable attention in Mexico and in many other countries. It has seven (7) species in West Africa

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Sub-region and five of these species are common in Nigeria (4).

Inflammation is a biochemical and cellular response that occurs in all vascularised tissue whose health and vitality is threatened by either an internal or an external source. Most of the essential components of the inflammatory response can be found in the blood and most of the early mediators (facilitators) of the inflammation function to increase the movement of plasma and infection fighting blood cells from the injured tissue, collectively known as exudates, usually a clear serious fluid, these substances defend the host against infection and facilitate tissue repair and healing.

The inflammatory process involves a series of events that can be elicited by numerous stimuli (e.g. infectious agents, ischemia, antigen-antibody interactions and thermal or other physical injury). The response is usually accompanied by the familiar clinical signs of erythema, eodema tenderness (hyper-analgesia) and pain.

Pyrrole and pyrrolopyrimidine derivatives have attracted the attention of many authors due to their pharmacological effects (5, 6), especially their anti-flammatory activity (8,7). Also, in continuation of previous work about preparation of new simple and efficient synthesis of biologically active pyrroles and fused pyrimidine compounds utilizing inexpensive starting materials (9, 10, 11).

Phu-142731A (12) is a novel anti-inflammatory pyrrol pyrimidine that inhibits the production of cytokines in vivo. This compound is a potent and efficacious inhibitor of eosinophilic lung inflammation and is currently in phase II clinical evaluation for the potential treatment of asthma. The fenametes (13) are a class of non-steroidal anti-inflammatory drugs that share as their common structure N-aryl anthranilic acids. These agents were originally found to be effective anti-inflammatory agents. Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure hypertension, and pregnancy toxaemia (14, 15). Most diuretic drugs have the adverse effect on quality of life, including impotence, fatigue and weakness. Naturally occurring diuretics include caffeine in coffee, tea and Kola, which inhibit Na+ absorption of alcohol in beer (15). Many indigenous drugs have been claimed to have a diuretic effect.

In a literature review, it was found that the parts of the Amaranthushybriduslinn, are used as diuretic antiscorbutic, appetizer, astringent, carminative, laxative, stomachic and tonic, and for jaundice (16,17). Some workers have reported Amaranthushybridus pharmacological activities like anti-bacterial and anti-oxidant activities. However, there are no reports on the diuretic and anti inflammatory activities of the plant. Hence, the present study was designed to evaluate the anti inflammatory and diuretic potential of ethanol extract of Amaranthushybridus and diuretic properties of ethanol extra leaves using experimental animal models.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL AND IDENTIFICATION

A sample of fresh leaves of Amaranthushybridus was collected from the National Root Crops Research Institute farm, Umudike in Abia State in the month of June, 2013. The plant was identified and authenticated by a Taxonomist, Dr.Osuagwu G. C; Department Plant Sciences and Biotechnology Michael Okpara University of Agriculture, Umudike (MOUAU).

ANIMALS

A total of 16 Wistar albino rats of both sexes between the ages of 7-10 weeks old and weighing between 140-180g were used for the study. The animals were kept in well aerated laboratory cages in the Biochemistry Department College of Natural Sciences Micheal Okpara University of Agriculture, Umudike animal house and were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment. They were maintained on standard animal feeds and drinking water during the acclimatization.

METHODS

PREPARATION OF PLANT EXTRACT

The fresh leaves were collected, washed, cleaned and dried at ambient temperature for two weeks. The dried leaves were pulverized to coarse powder using a mechanical grinder.A weighed quantity 200g, of the coarse powder material was extracted by cold maceration in absolute ethanol for 48hr.The extraction mixture was filtered with whatman No 1 filter paper. The filtrate was concentrated in vacuum using a rotary evaporator (at an optimum temperature between 40 and 45^0C to avoid denaturation of the active ingredient) to obtain a dark green semi-solid mass.The extraction yield (%) was estimated gravinmetrically. The extract was stored at $+4^0$C until used.

Acute toxicity and lethality Test (LD50)

Acute toxicity (LD50) of the ethanol leaf extract of the Amaranthushybridus was determined by Lork method (18)

Phytochemical Analysis

The fresh leaves of Amaranthushybridus were subjected to phytochemical analysis according to the method outlined by Harbourne(1998(19)) and Trease and Evans (1989 20). The phytochemical analysis was done to detect the presence of secondary metabolites such as alkaloid, tannins, saponins, resins, flavonoids, steroid, glycosides and terpenoids

Anti-Inflammatory Activity

Anti-inflammatory activity was carried out using the rat paw oedema test according to the method of Okoli C.O and Akah P.A 200O (21) with minor modifications. Rats (140 -180g) of both sexes were divided into eight groups of two animals per group, 0.3ml of fresh undiluted egg albumen was injected into the right hind paw of the rats after pre-treatment with 0.3ml of saline intraperitoneally to group one as a negative control, aspirin 100mg/kg intraperitoneally to group two as positive control while the remaining six groups received 31.25, 62.5, 125, 250, 500 and 1000mg/kg of intraperitoneally respectively. Paw diameters were measured immediately after injection of egg albumen at 30, 60, 90, 120, 150 and 180 minutes after treatment. Percentage oedema inhibition and percentage inflammation were calculated according to the formulae: CO-CT/CO 100/1 and CT/CO 100/1

Where: Ct Average inflammation of treated groups

Co Average inflammation of negative (-) control

Table 1: Result of Toxicity Test

Group	Dose MG/KG	Death	%Mortality
1	125	$^{0}/_{4}$	0
2	250	$^{0}/_{4}$	0
3	00	$^{0}/_{4}$	0
4	1000	$^{2}/_{4}$	50

The preliminary phytochemical screening of the extract showed the presence of alkaloid, flavonoid, saponin, tannins, phenols, hydrocyanic acid and phytic as shown in table 2.

Table 2 Result of phytochemical composition of ethanolic leaf extract of preliminary phytochemical screening of ethanol leaf extract of Amaranthus Hybrids

Phytochemical	Content MG/KG
Alkaloid	3.54
Flavonoid	0.83
Saponin	1.68
Tannins	0.49
Phenols	0.35
Hydrocyanic acid	16.99
Phytic	1.32

Diuretic Effect of ethanol extract of Amaranthus hybridus ethanolic extracts in albino rats. The results from the table above show the mean values of selected parameters of with their

Table 3: Result of diuretic activity of Amaranthus hybridus

Sample	Volume(ml)	Na+ (meq/L)	K+ (meq/L)	Cl- (meq/L)	HCO3- (meq/L)	Na+/K+ ratio
Normal Saline	0.65 ±0.07f	103.50 ±0.71f	5.90 ±0.14f	89.50 ±0.71d	27.50 ±0.71a,b	17.55 ±0.54b
Frusemide	4.00 ±0.28a	153.00 ±1.41a	26.50 ±0.71a	129.00 ±1.41a	28.50 ±0.71a	5.78 ±0.21f
1000mg	2.55 ±0.07b	136.50 ±0.71b	17.50 ±0.71b	112.50 ±0.71b	25.00 ±0.00b,c	7.85 ±0.35e
500mg	2.15 ±0.07c	129.00 ±1.41c	16.50 ±0.71b	102.50 ±0.71c	26.50 ±0.71b,c	7.80 ±0.28e
250mg	1.85 ±0.07d	119.00 ±1.41d	14.50 ±0.71c	89.50 ±0.71d	26.50 ±2.12a,b,c	8.25 ±0.50e
125mg	1.25 ±0.07e	107.00 ±1.41e	10.50 ±0.71d	88.00 ±1.41d,e	27.50 ±0.71a,b	10.20 ±0.58d
62.5mg	0.85 ±0.07f	104.50 ±0.71e,f	7.90 ±0.14e	86.50 ±2.12e,f	25.00 ±1.41b,c	13.200 ±0.14c
31.5mg	0.65 ±0.07f	102.50 ±0.71f	5.05 ±0.07f	84.50 ±0.71f	23.50 ±2.12c	20.30 ±0.14a

standard deviations (Mean ±SD). The ordered alphabets represent the decreasing order of the values, i.e. a > b > c for the groups.
NB: Same alphabet on two or more values of a parameter of the different groups show no significant difference between the groups (i.e. P>0.05).
Urinary excretion by different concentration extracts of Amaranthus hybridus linn.

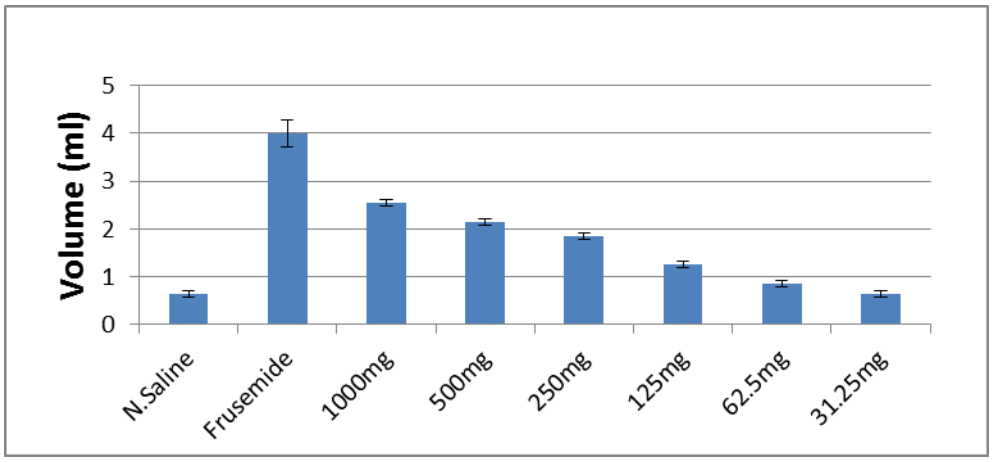

Figure 2: Urine volume at different concentrations

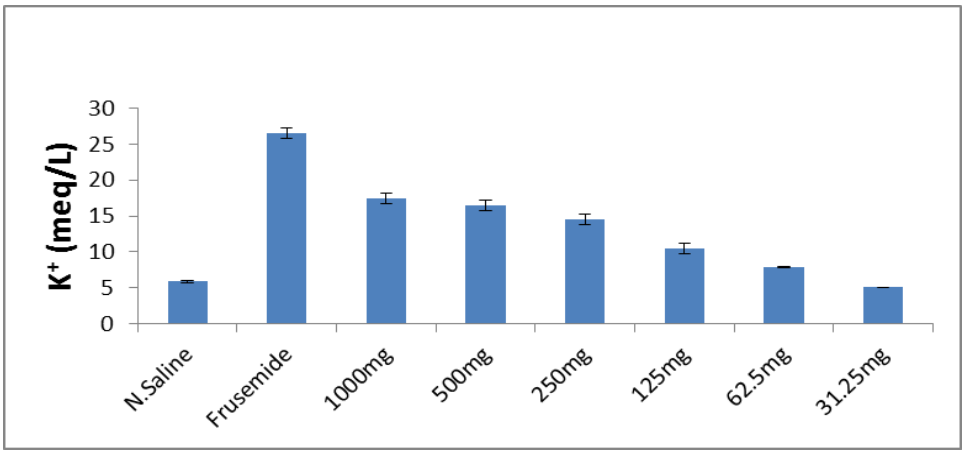

Figure 3: Sodium (Na+) excretion in the urine by different concentration

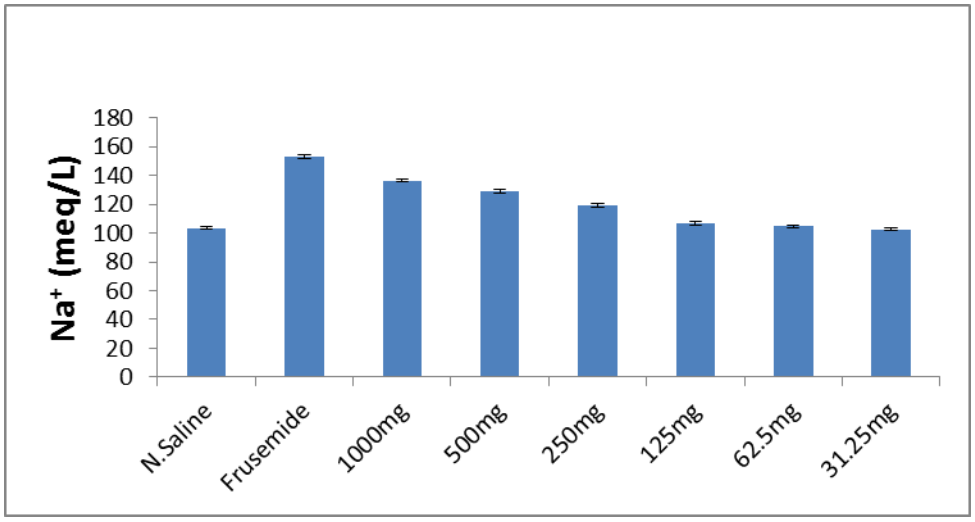

Figure 4: Potassium (K+) excretion in the urine by different concentration

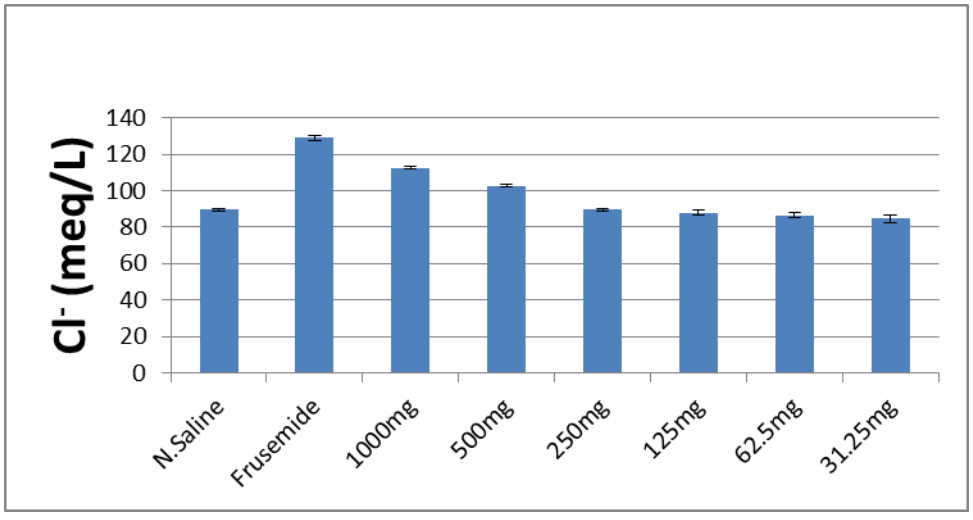

Figure 5: Chlorine (cl-) excretion in the urine by different concentration

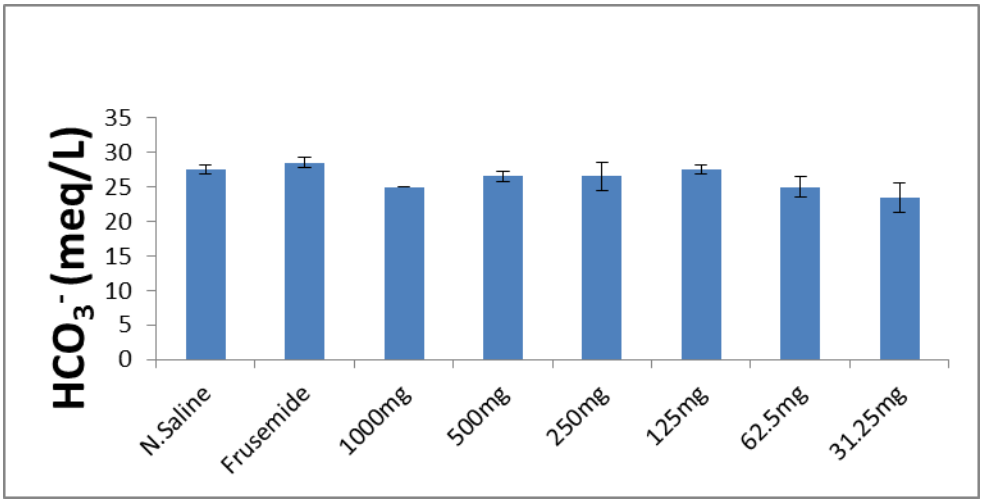

Figure 6: Bicarbonate (HCO3-) excretion in the urine by different concentration

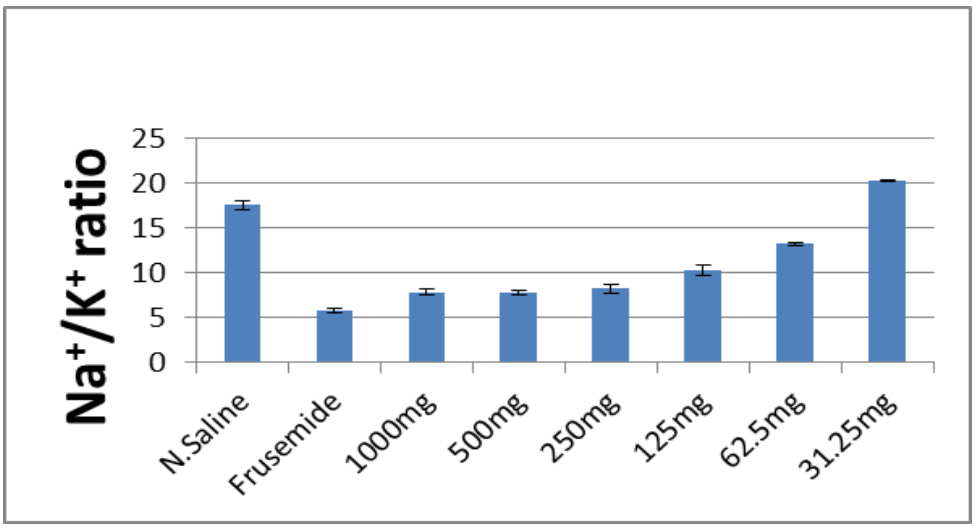

Figure 7: Ratio of sodium and potassium ions (Na+ / K+) showed in excretion in the urine by different concentration.

Evaluation of Diuretic Activity

Both sex albino rats weighing between 140-180g, deprived of water for 16-18hrs before the test drugs are administered. The animals were pre-treated with normal saline at an intraperitoneally dose of 0.5mg body weight to impose a uniform water and salt load. Two animals were kept in each group.
Group 1: treated with normal saline (which is the negative control)
Group 2: treated with Furosemide (which is the positive control)
Group 3: treated with ethanol extract of Amaranthushybridus at a dose 1000mg/kg.
Group 4: treated with ethanol extract of Amaranthushybridus at a dose 500 and 500mg/kg.
Group 5: treated with ethanol extract of Amaranthushybridus at a dose 250 and 250mg/kg
Group 6: treated with ethanol extract of Amaranthushybridus at a dose 125mg/kg
Group 7: treated with ethanol extract of Amaranthushybridus at a dose 62.5mg/kg
Group 8: treated with ethanol extract of Amaranthushybridus at a dose 31.25mg/kg
Immediately after the administration of the drugs, animals were placed in metabolic cages individually to allow the separation of urine and feces and maintained at room temperature throughout the experiment. During this period, no water and food was made available to the animals.

Results

Acute Toxicity Studies

Signs of toxicity were first noticed after 4 -5 hours of the extract administration. There were decreased locomotor activity and sensitivity to touch and pain, including decreased feed intake, tachypnoea and prostration after 12 hours of administration. There were deaths in the group administered with 1000 mg/kg with 50% mortality recorded as shown in table 4.1.
The LD50 was thus calculated as

$$\sqrt{\ } Highestnonlethaldose \times lowestlethaldose = \sqrt{1000\ x\ 500} = 707.1mg/kg$$

Anti-Inflammatory Activity

Egg albumen was used to induce inflammation in the hind paws of rats divided into eight (8) groups. The activity of leaf extract was compared to that of aspirin used as standard.

Table 4 – 6 and figure 8 and figure 9 show the results of calculating percentage inflammation and percentage inhibition of inflammation respectively per time interval.

Table 4: Result of inflammation of the hind paw (oedema) using 2mg/ml of fresh egg albumen in diameter (mm).

Discussion

The ethanol extract of Amaranthus hybridus exhibited a dose-dependent in urine excretion. With respect to the ethanol extract of Amaranthus hybridus, the maximum increase in urinary excretion was produced at 1000mg/kg and 500mg/kg. This is comparable with the loop diuretic (furosemide). The diuretic effect of the Amaranthus hybridus was generally high and qualitatively almost similar to that of furosemide which clearly shows that the ethanol extract of Amaranthus hybridus has a potential to induce diuretic markedly as those of known synthetic diuretic like furosemide. The ethanol extract of Amaranthus hybridus induces the urinary output accompanied by a corresponding significant increased in Na+, k+, cl-, Na+/k+ ration ($P < 0.05$). Collectively, these observations suggest that it is acting as an osmotic diuretic.

Anti-diurectic hormone (ADH) plays a vital role in the regulation of urine output. The extract of Amaranthus hybridus may stimulate dieresis by inhibiting an anti-diuretic hormone (ADH) release or its action on the uriniferous tubules or it could produce diuresis by stimulating the release of endogenous natriuretic peptides, which promote sodium and water (H20) secretion. It promotes an increase in natriuresis and kaleuresis with its diuretic action (22, 23).

Therefore, the plant was acting as a loop diuretic. Loop diuretics are the most powerful of all diuretics and they inhibit the Na+, K+, 2cl- co-transporter system of the ascending limb of Henle's loop. The ethanol extract is not associated with a reduction in urinary k+ levels, unlike some plant extracts that have been reported to have an interesting k+ saving effect, suggesting that this plant was not acting as potassium (K+) sparing diuretic (24,25).

In ethanol extract of Amaranthus hybridus, it should be pointed out that the water (H20) soluble salts are not present in the extract in sufficient amount as they have poor solubility in such solvent. Hence, such water soluble solutes do not interfere with the urinary excretion. Thus the notable diuretic effect produced by the ethanol extract of Amaranthus hybridus was reaffirmed that the diuretic activity of Amaranthus hybridus was not due to its content of potassium salt rather it was due to the intrinsic ability of the plants phytoconstituents to exert the effect.

Diuretic effect may be produced by stimulation of regional blood flow or initial vasodilation or by producing inhibition of tubular reabsorption of water and anions (24, 25).

Inflammation can be defined as a reaction of a living cell or tissue to injury, infection or irritation /infiltration. Inflammation is characterized by pains, swelling, redness and heat/fever. Inflammation could be induced by conditions that bring about the release of inflammatory mediators such as histamine, prostaglandians, nitric oxide, serotonin, cytokines, leukotrienes, platelet activating factors and substance (26).

Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents. The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymerphonuclear leukocytes to sites of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase to prostaglandins, which are major components that induce pain and inflammation (27, 28).

Most of the anti-inflammatory drugs exert their beneficial effects by inhibiting either release of these enzymes or by stabilizing lysosomal membrane which is one of the major events responsible for the inflammatory process (29). So, we can assume that ethanol extract of Amaranthus hybridus might be acting by either inhibiting the lysosomal enzymes or stabilizing the membrane.

Anti-inflammatory effect of the ethanol extract of Amaranthus hybridus exhibited may be due to its phytochemical constituents present in the plant such as tannins,flavonoids, saponins were proven to have anti-inflammatory effects activity (30).

Table 4.4 above shows the effect of the different treatment groups with regards to the mean hind paw diameter, anti-inflammatory data for the leaf extract of Amaranthus hybridus exhibits reduction in the induced edema which was significant ($P < 0.05$).

From the above study of Amaranthus hybridus, it is quite apparent that the ethanolic extract of Amaranthus hybridus possesses significant anti-inflammatory activity.

Conclusion

The present study has demonstrated the anti-inflammatory and diuretic activities of ethanol leaf ethanol extract of Amaranthus hybridus in reducing inflammation and induced diuretic in wistar rats. These effects may be due to the presence of flavonoids and tannins in the plant extract.

Table 4: Result of inflammation of hind paw (oedema) using 2mg/ml of fresh egg albumen in diameter (mm)

DOSES	30 MINS	60 MINS	90 MINS	120 MINS	150 MINS	180 MINS
500mg	5.75	5.00 0.00	4.25 0.35	4.05 0.35	3.85 0.35	3.55 0.35
250mg	6.15 0.07	5.50 0.00	4.65 0.07	4.50 0.00	4.30 0.14	4.05 0.07
125mg	6.25 0.07	6.05 0.07	5.75 0.07	5.35 0.07	5.15 0.07	4.70 0.14
62.5mg	6.45 0.07	6.35 0.07	6.05 0.21	5.90 0.28	5.70 0.42	5.00 0.00
31.25mg	6.50 0.07	6.45 0.07	6.25 0.07	6.10 0.14	5.90 0.14	5.25 0.07
15.625mg	6.70 0.00	6.55 0.07	6.40 0.00	6.30 0.00	6.15 0.07	5.50 0.14
Normal saline (-)	6.90 0.00	6.75 0.07	6.55 0.07	6.45 0.07	6.30 0.00	5.45 0.07
Aspirin (+)	5.25 0.35	4.75 0.35	4.65 0.21	4.50 0.00	4.25 0.07	3.65 0.21

Table 5: Result of % inflammation (oedema) per time intervals.

Dose	30 Mins	60 Mins	90 Mins	120 Mins	150 Mins	180 Mins
A500mg	83.3	74	64.8	62.7	61.1	65.1
B250mg	89.1	81.4	70.9	69.7	68.2	74.3
C125mg	90.7	89.6	87.7	82.9	81.7	87.1
D62.5mg	93.4	94	92.3	91.4	90.4	91.7
E32.5mg	94.3	95.5	95.4	94.5	93.6	96.3
F15.5mg	97.1	97	97.7	97.6	97.6	100.9
Aspirin	76	70.3	69.6	69.7	67.4	66.9

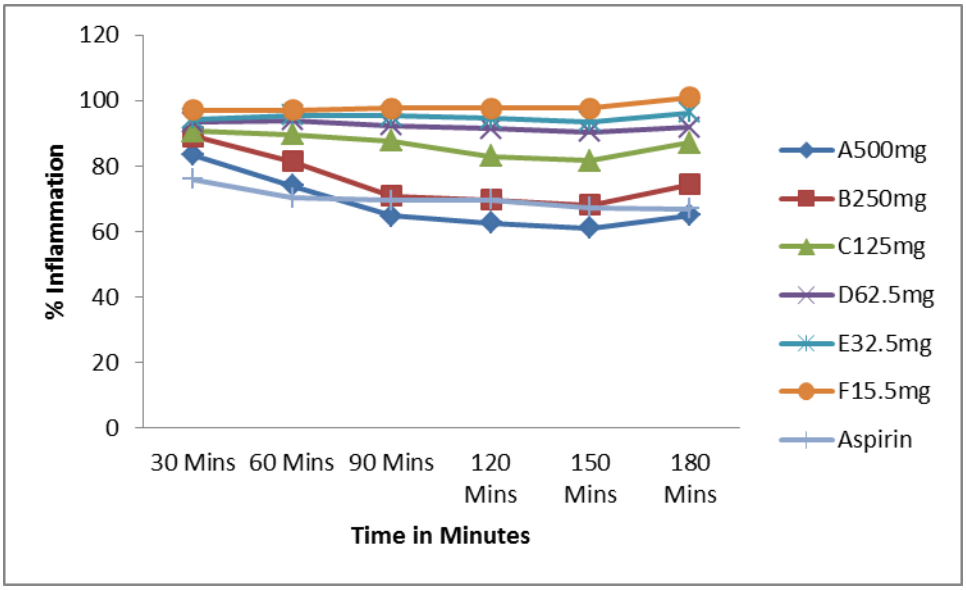

Figure 8: Percentage inflammation against time in minutes

Table 6: Result of % inhibition of inflammation (oedema) per time intervals.

Dose	30 Mins	60 Mins	90 Mins	120 Mins	150 Mins	180 Mins
A500mg	16.6%	25. 3%	35.1%	37.2%	38.8%	34.8%
B250mg	10.8%	17.9%	29%	30.2%	31.7%	25.6%
C125mg	9.42%	10.4%	12.2%	17%	18.2%	13.7%
D62.5mg	6.52%	5.2%	7.6%	8.5%	9.5%	8.3%
E32.5mg	5.79%	3.7%	4.5%	5.4%	6.3%	3.7%
F15.5mg	2.89%	2.2%	2.2%	2.3%	2.3%	0%
GAspirin(100mg)	23.9%	29.1%	29%	30.2%	32.5%	33.6%

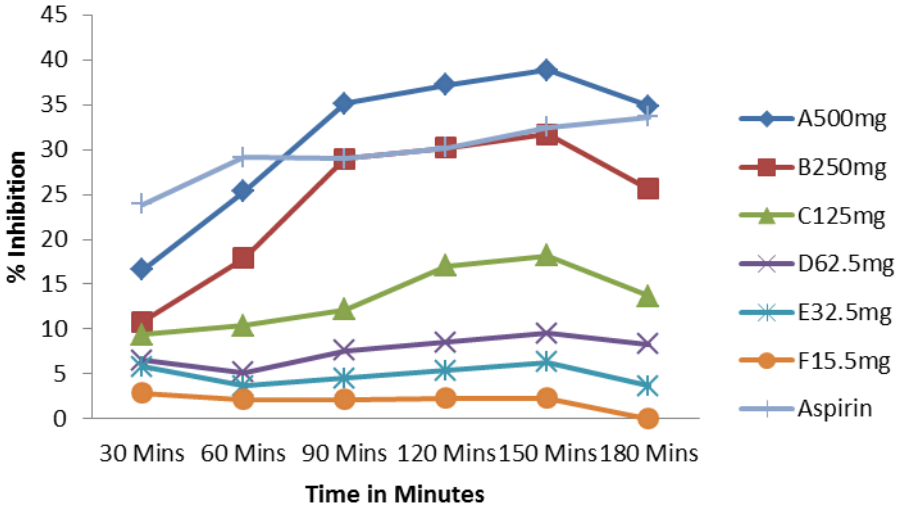

Figure 9: Percentage inhibition of inflammation against time in minutes.

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