Original Article

Anti-inflammatory and Diuretic Activities of Ethanol Extract of *Dioscorea bulbifera* Leaf

Omodamiro O.D.*

Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria

Address for Correspondence

Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.
E-mail: majekdamiro@yahoo.com

ABSTRACT

Objective: The present study was designed to determine the anti-inflammatory and diuretic activities of the plant *Dioscorea bulbifera*. In the anti-inflammatory study, thirty two animals were used and they were randomly distributed four animals per group.
Method: Administered concentration of *Dioscorea bulbifera* were 500mg/kg, 250mg/kg, 125mg/kg, 62.5mg/kg and 31.25mg/kg, 15.5mg/kg were given to the test group labeled group A to F. A negative control receiving normal saline at 0.9mg/kg and a positive control receiving aspirin at 100mg/kg were as control groups for the study. Egg albumin was used as the inflammatory agent.
Result: The negative control showed no inhibition. Further results showed that the concentration at 500mg/kg of the ethanol extract had the highest anti-inflammatory effect at 20.4 ± 0.14. In the diuretic study, the negative control containing the normal saline and the positive control containing furosemide were used. The albino rats were administered the extracts at conc. at 1000mg/ml, 500mg/ml, 250mg/ml, 62.5mg/ml and 31.25mg/ml. the albino rats administered the positive control had the highest volume of urine but were low in Na^+/K^+ at 25.79 ± 1.124.
Conclusion: This result demonstrates that *Dioscorea bulbifera* has potential anti-inflammatory effects and little diuretic activities.

Keywords: *Dioscorea bulbifera*, Wistar albino rats, LD_{50}, Aspirin, Furosemide, Sodium.

INTRODUCTION

Diuretic activities refer to mechanisms that increase urine excretion of both water and electrolytes, and diuretics are commonly called 'water tablets'. In general, they inhibit electrolyte reabsorption from the lumen of the nephron, increasing osmolarity and enhancing water excretion. The sub-classes of diuretics include; thiazides, loop diuretics (e.g furosemide, bumetanide, torasemide, potassium sparing diuretics which are the weak diuretics, whereas spironolactone and eplerenone are used in

the treatment of hypertension, oedema of liver failure, and loop or thiazide diuretics[1].

Inflammation is part of the complex biological response of vascular tissue to harmful stimuli such as pathogens, damaged cells, or irritant[2]. Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Ant-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to oploids, which affect the central nervous system.

Dioscorea bulbifera, the air potato, is a true yam species in the *Dioscorea* or true yam. It is a perennial vine with broad leaves and two types of storage organs. The plant form bulbils in the leaf axils of the twining stems, and tubers beneath the ground. Air potato can grow extremely quickly, roughly 8 inches per day, and eventually reach over 60 feet long. It typically climbs to the top of trees and has a tendency to take over nature plants.

Dioscorea bulbifera is a monocotyledonous, dioecicus, herbaceous perennial vine. Its annual stems, which arise from tubers, twine counter clockwise[3].

Dioscorea bulbifera contains steriod, disogenin and has been used as starting material of their manufactures for diverse artificial steroidal hormones such as hormonal contraception and other sex hormones. It also contains a number of flavonoids and isoflavonoids, which have oestrogenic, heart protective, antioxidant and anti-cancer properties. Those contained in the aerial potatoes are particularly potent free radical scavengers[4]. The tubers were found with a high amount of protein, a good proportion of essential amino acids and appeared as a fairly good source of many dietary minerals[5].

In this study, we determine the ant-inflammatory and diuretic activities of *Dioscorea bulbifera* using wistar albino rats. Inflammation which occurs on daily basis and the demand for plant derive material to combat inflammation is on the increase, thus we decided to study the plant for anti-inflammatory activities.

MATERIALS AND METHODS

Plant material

The plant sample *Dioscorea bulbifera* was obtained from Ubakala in Umuahia area of Abia State, Nigeria. It was obtained from a farm in the area where water supply is consistent. It was authenticated by Dr. Osuagwu G. of the Department of Plant Science and Biotechnology, College of Natural Sciences, Micahel Okpara University of Agriculture, Umudike, Abia State, Nigeria and was given the herbarium number MOUAU/PSB/2013/10/21.

Experimental animal

Thirty four wistar albino rats used for this study was obtained from the Veterinary Department of the University of Nigeria, Nsukka, Enugu State, Nigeria and was conveyed to the animal house, of the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Abia state. The animals were allowed one week acclimatization period; they were housed in plastic cages and were provided with clean water and rat pellets before the experimental period. Ethical permission was sought from the ethical committee of the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Ethical standards set by the board was also followed.

Preparation of plant extract

The plant material *Dioscorea Bulbifera* was air dried under room temperature for 10days, it was then milled with Thomas Wiley mill model Ed-5 milling machine and stored in air tight container,

free from sunlight and oxygen. Fifty grams (50g) of the fine powder of *Dioscorea bulbifera* was weighed then dissolved in 200ml of ethanol in a beaker. It was then allowed to stand for 72 hours at room temperature and then filtration was done using the whatmann No 1 filter paper. The filtrate was then concentrated at temperature of 50^0c with the use of the Uniscope water bath to allow the ethanol to evaporate.

Acute toxicity studies (LD_{50})

A total of 20 white Albino mice were used. The animals had an average weight of 30-33g. The animals were divided into five groups consisting of four animals each. The plant extract was administered intraperitoneally (I.P) to the animals at doses of 62.5mg/kg, 125mg/kg, 250mg/kg, 500mg/kg, 1000mg/kg body weight respectively. The animals were then observed at a period of one to twenty four hours after administration of plant extract, mortality rate noted at specific concentration. The test was carried out as suggested by Ganapathy[6].

Experimental design

For anti-inflammatory activities, sixteen animals were randomly distributed into eight groups, with each group consisting of two animals each. For diuretic activity, the remaining eighteen animals were randomly distributed into nine groups. Ethical rules guiding the use of Laboratory animals as approved by the Board of Biochemistry Department, Michael Okpara University of Agriculture, Umudike, strictly followed.

Evaluation of anti-inflammatory activity

This was achieved using the method described by Vinegar *et al.,*[7]. The animals were deprived of feed for 12hours prior to the experiment but were allowed access to pure drinking water during the experiment. Group A served as the negative control group, was induced inflammation and did not receive any of the treatment, Group B received 500mg/kg plant extract, Group C received 250mg/kg plant extract, Group D received 125mg/kg of plant extract, Group E received 62.5mg/kg of plant extract, Group F received 31.25mg/kg plant extract, Group G received 15.5mg/kg of plant extract while Group H served as the positive control which received Aspirin. The plant extract and aspirin were administered intraperitoneally. The animals were administered the extract and aspirin in concentration according to the group first then after 30mins 1ml of fresh egg albumin was then injected into the sub-plantar of the right hand paw of each of the rat. Using a vernier caliper, the diameter of the right hand paw was measured at 30mins interval of 30mins, 60mins, 90mins, 120mins, 150mins, and 180mins respectively. Percentage inflammation and inhibition of inflammation were calculated with the formular below;

$$\% \text{ inflammation} = \frac{Ct \times 100}{Co}$$

$$\% \text{ inhibition} = \frac{Co-Ct}{Co} \times \frac{100}{1}$$

Evaluation of diuretic activity

The method of Lipschitz *et al*,[8] was employed for the assessment of diuretic activity. The animals were deprived of food and water for hours prior to the experiment, were divided into nine groups of two animals each. The Group labelled 1seved as control group which received normal saline, Group 2 received, Group 3- 9 received plant extract at varying concentration respectively (15.5, 31.25, 62.5, 125, 250, 500, and 1000 mg/ml).

The urine and feaces were separated using a syringe kept at 25^0c ± 0.6^0c. The volume of the urine collected was measured at the end of 24hrs. During this period, no

food and water was made available to the animals. The parameters, taken were total urine volume, concentration of sodium ion, potassium ion, chloride and bicarbonate in the urine.

Sodium and potassium ion concentration were determined by flame photometer[9], chloride concentration and bicarbonate was estimated by titration with silver nitrate solution using three drops of 5% potassium chloride solution as indicator.

Statistical analysis

Data obtained from the experiment were statistically analysed using statistical package for social sciences (SPSS) (Version 16). Differences between means were separated by one-way ANOVA and multiple comparisons method. The values of the result are expressed as mean ± standard deviation and probability value taken at 95% significance level.

RESULT

Result for LD_{50}

See table 1.

Diuretic activity

See table 2&3 and figure 1-5.

Result of Anti-inflammatory activity

See table 4-6 and graph 1&2.

DISCUSSION

The most widely used primary test to screen ant-inflammatory agent measures the ability of a compound to reduce local oedema induced in the rat paw by injection of an irritant agent[10]. Egg albumin induced oedema has been commonly used as an experimental animal model for inflammation. Table 1 shows the effect of the various treatment groups with regards to the mean paw circumference. There is significance difference ($P < 0.05$) between the mean paw circumferences of group treated with 500mg/mg concentration of the ethanol extract and the negative control.

The development of oedema in the right paw of the rat after injection of egg albumin is due to release of pro-inflammatory mediators like histamine and prostaglandin[11]. The significant inhibitory activity shown by the extract of *Dioscorea bulbifera* (500mg/kg, 250mg/kg, 125mg/kg, 62.5mg/kg, 31.25mg/kg and 15.5mg/kg concentration) at the final 30 minutes interval was higher to that exhibited by the group treated with aspirin. The highest percentage inhibition activity was found in the 500mg/kg concentration of the ethanol extract. In table 3 there is significant difference ($P < 0.05$) between the mean paw circumferences of the treated group. The former showed higher anti-inflammatory activity due to a greater reduction of oedema paw circumference in 180 minutes.

In diuretic study, the ethanol extract of *Dioscorea bulbifera* leaves has low significant effect on the urinary output as well as the urinary electrolyte concentration at higher dose tested (1000mg/ml).

The 1000mg/ml fraction was found to be the most potent in increasing the urinary output: the effect was comparable to that of the standard drug (furosemide), whereas the normal saline was found to be the least potent.

Determination of the urinary electrolyte concentration revealed that, 1000mg/ml was most effective in increasing urinary electrolyte concentration for all the four ions tested (Na^+, K^+, Cl^-, HCO_3^-).

All fractions except the control cost similar type of increase in urinary electrolyte concentration but to a lesser extent the control fraction did not increase urinary electrolyte concentration. 1000mg/ml fraction slightly increased urinary output and also electrolyte concentration .Slight increase in the ratio of excreted sodium and potassium ions indicate that the extract slightly increased sodium ion to greater extent than potassium, which is a

very essential quality of a good diuretic with lesser hyperkalaemia side effects.

CONCLUSION

The experimental evidence obtained in the present study indicates that *Dioscorea bulbifera* has significant anti-inflammatory properties and low diuretic activities. The results support the traditional use of this plant (*Dioscorea bulbifera*) in inflammatory conditions and suggest the presence of biologically active components which may be worth further investigations and research.

Competing interest

The authors declare that there is no competing interest, and all authors participated actively in the work. All authors read and approved final version of manuscript for publication.

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Table 1. Showing mortality rate of mice after administration of plant extract

Conc. In mg	No. of animals	No. of mortality
1000	4	2\4
500	4	1\4
250	4	-
125	4	-
62.5	4	-

Average weight of mice = 25g

Table 2. Effect of *Dioscorea bulbifera* on urine volume

Sample	Volume (ml)
Normal saline	0.065 ± 0.07^{c}
Furosemide	4.00 ± 0.28^{a}
1000mg	0.95 ± 0.07^{b}
500mg	$0.75 \pm 0.07^{b,c}$
250mg	0.65 ± 0.07^{c}
125mg	$0.55 \pm 0.07^{c,d}$
62.5mg	$0.35 \pm 0.07^{d,e}$
31.25mg	0.15 ± 0.07^{e}

In the diuretic study, the result from the table below shows the mean values of selected parameters with their standard deviations (Mean ±SD). The ordered alphabets represent the increasing orders 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)

Table 3. Effect of *Dioscorea bulbifera* on urinary electrolyte excretion

Sample	Na^{+} (meq/L)	K^{+} (meq/L)	Cl^{-} (meq/L)	HCO3 (meq/L)	Na/K ratio
N. Saline	103.50 ± 0.71^{d}	5.90 ± 0.14^{c}	89.50 ± 0.71^{b}	27.50 ± 0.71^{a}	17.55 ± 0.54^{b}
Frusemide	153.00 ± 1.41^{a}	26.5 ± 0.71^{a}	129.00 ± 1.41^{a}	28.50 ± 0.71^{a}	5.78 ± 0.21^{c}
1000mg	119.50 ± 0.71^{b}	7.8 ± 0.07^{b}	90.5 ± 0.71^{b}	27.5 ± 0.71^{a}	15.22 ± 0.23^{c}
500mg	113.00 ± 1.41^{c}	7.3 ± 0.14^{b}	89.5 ± 0.71^{b}	24.5 ± 0.71^{b}	15.49 ± 0.49^{c}
250mg	104.50 ± 0.71^{d}	6.10 ± 0.14^{c}	87.00 ± 1.41^{c}	$23.5\pm0.71^{b,c}$	17.14 ± 0.52^{b}
125mg	104.5 ± 0.71^{d}	5.9 ± 0.14^{c}	85.5 ± 0.71^{c}	$23.0\pm1.41^{b,c}$	17.72 ± 0.30^{b}
62.5mg	$101.5\pm0.71^{e,f}$	5.5 ± 0.14^{c}	82.5 ± 0.71^{d}	22.0 ± 0.14^{c}	18.46 ± 0.35^{b}
31.25mg	100.5 ± 0.71^{f}	3.9 ± 0.14^{d}	79.5 ± 0.71^{e}	24.5 ± 0.71^{b}	25.79 ± 1.12^{a}

Table 4. Result of anti-inflammatory activities of ethanol extract of *Dioscorea bulbifera*

Dose	30 mins	60 mins	90 mins	120 mins	150 mins	180 mins
Negative Control	6.25±0.35	6.15±0.21	6.15±0.07	5.90±0.14	5.60±0.14	5.40±0.14
500mg/kg	5.25±0.35	5.20±0.28	5.05±0.07	4.85±0.21	4.50±0.14	4.30±0.14
250mg/kg	4.85±0.07	4.60±0.14	4.45±0.21	4.30±0.14	4.20±0.14	3.90±0.14
125mg/kg	4.65±0.07	4.45±0.07	4.30±0.14	4.10±0.14	3.80±0.14	3.60±0.14
62.5mg/kg	4.40±0.14	4.25±0.07	4.05±0.07	3.75±0.21	3.60±0.14	3.30±0.14
31.25mg/kg	4.35±0.07	4.05±0.07	3.80±0.14	3.65±0.21	3.45±0.21	2.90±0.14
15.5mg/kg	4.10±0.14	3.85±0.07	3.65±0.07	3.45±0.21	3.20±0.14	2.60±0.14
15.5mg/kg	4.10±0.14	3.85±0.07	3.65±0.07	3.45±0.21	3.20±0.14	2.60±0.14
Aspirin (100mg/ml)	5.25±0.35	5.25±0.35	4.25±0.35	4.00±0.14	3.75±0.21	3.35±0.21

The extracts were tested at six different dose levels. The results shows that ethanol extract administered at 500mg/kg concentration had higher anti-inflammatory effect than the rest of the extract concentration.

Table 5. Result of percentage inflammation (Oedema) per time intervals using non-treated animals as control

Dose	30 mins	60 mins	90 mins	120 mins	150 mins	180 mins
500mg/kg	84.0±0.35	84.6±0.28	82.9±0.21	82.2±0.21	80.4±0.14	79.6±0.14
250mg/kg	77.6±0.07	74.8±0.14	74.8±0.21	72.9±0.14	75.0±0.14	72.2±0.14
125mg/kg	74.4±0.07	73.17±0.07	73.17±0.14	69.5±0.14	67.9±0.14	66.7±0.14
62.5mg/kg	70.4±0.14	69.1±0.07	65.9±0.07	63.6±0.21	64.3±0.14	61.1±0.14
31.25mg/kg	69.6±0.07	66.7±0.07	61.8±0.14	61.9±0.21	61.6±0.07	53.7±0.14
15.5mg/kg	65.6±0.14	62.7±0.07	59.5±0.07	58.5±0.21	57.1±0.14	48.2±0.14
Aspirin 100mg/ml	84.0±0.35	85.4±0.35	69.1±0.35	67.8±0.14	66.9±0.21	62.0±0.21

Table 6. Result of percentage inhibition of inflammation (Oedema) per time intervals

Dose	30 mins	60 mins	90 mins	120 mins	150 mins	180 mins
500mg/kg	16.0±0.35	15.5±0.28	17.9±0.21	17.8±0.21	19.6±0.14	20.4±0.14
250mg/kg	22.4±0.07	25.2±0.14	27.6±0.21	27.1±0.14	25.0±0.14	27.8±0.14
125mg/kg	25.6±0.07	27.6±0.07	30.1±0.14	30.5±0.14	32.0±0.14	33.3±0.14
62.5mg/kg	29.6±0.14	30.9±0.07	34.2±0.07	36.4±0.21	35.7±0.14	38.9±0.14
31.25mg/kg	30.4±0.07	34.9±0.07	38.2±0.14	38.1±0.21	38.4±0.07	46.3±0.14
15.5mg/kg	34.4±0.14	37.4±0.07	40.7±0.07	41.5±0.21	42.9±0.14	51.9±0.14
Aspirin 100mg/ml	16.0±0.35	14.6±0.35	30.9±0.35	32.2±0.14	33.0±0.21	37.9±0.21

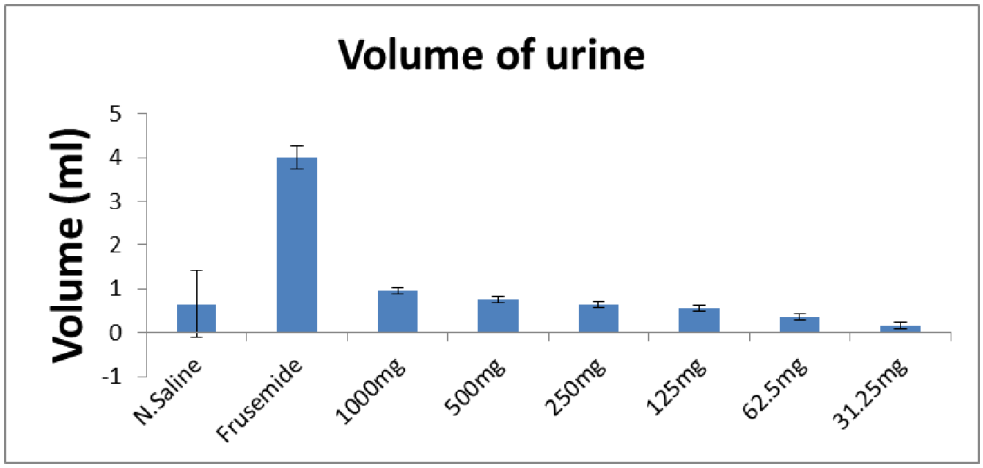


Figure 1. The mean concentration (ml) of urine volume

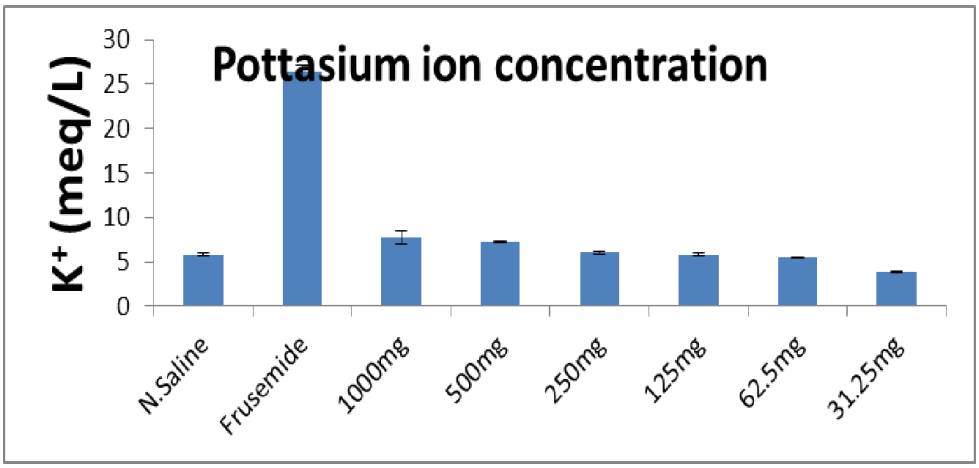


Figure 2. The mean concentration (meq/L) of Potassium ion (K^{+})

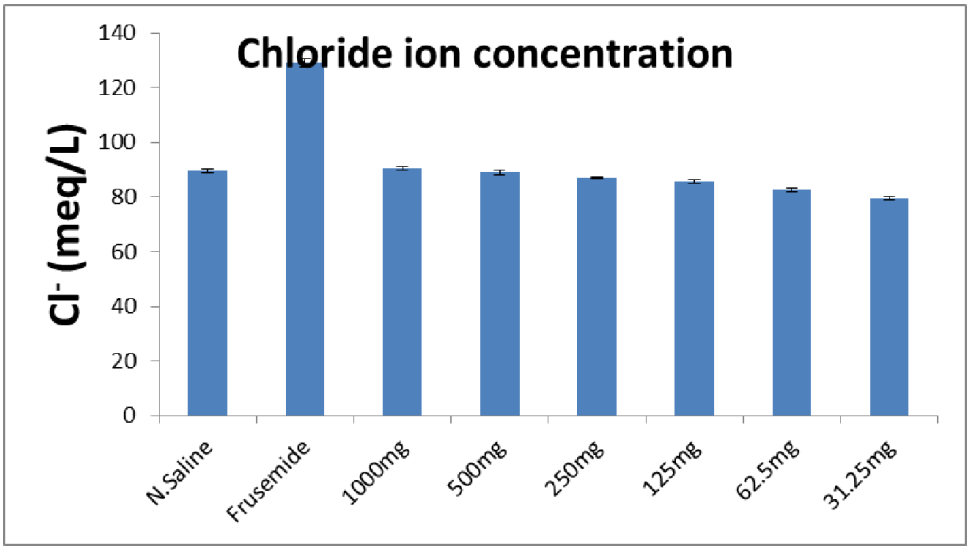


Figure 3. The mean concentration (meq/L) of Chloride ion (Cl^{+})

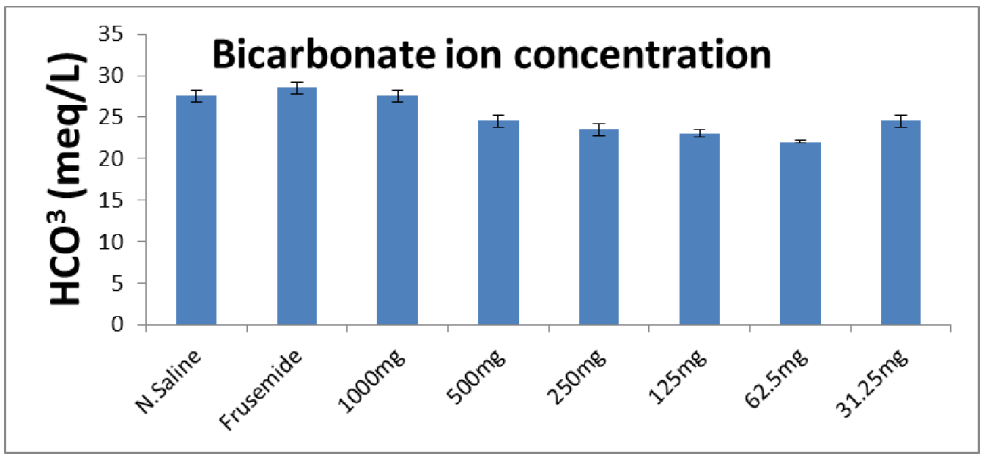


Figure 4. The mean concentration (meq/L) of Tricarbonate ion (HCO_3^-)

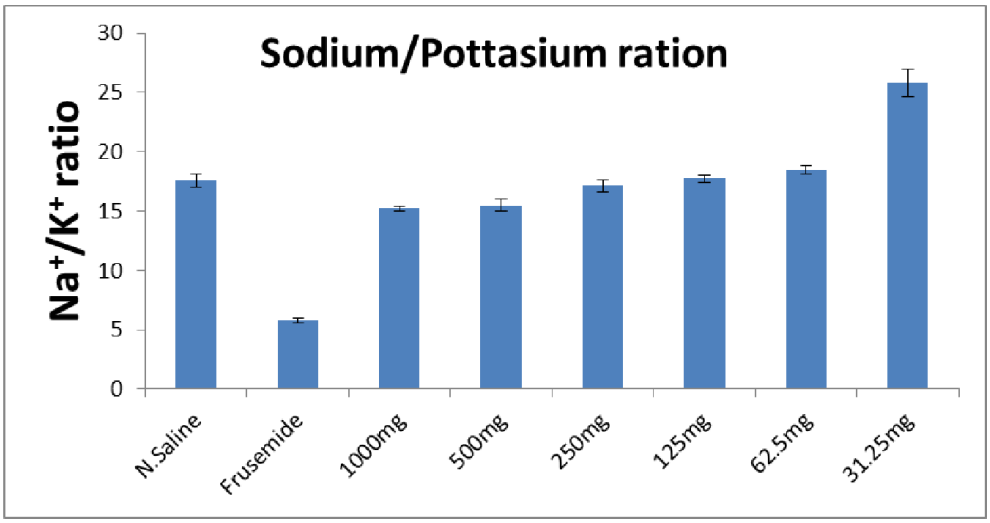
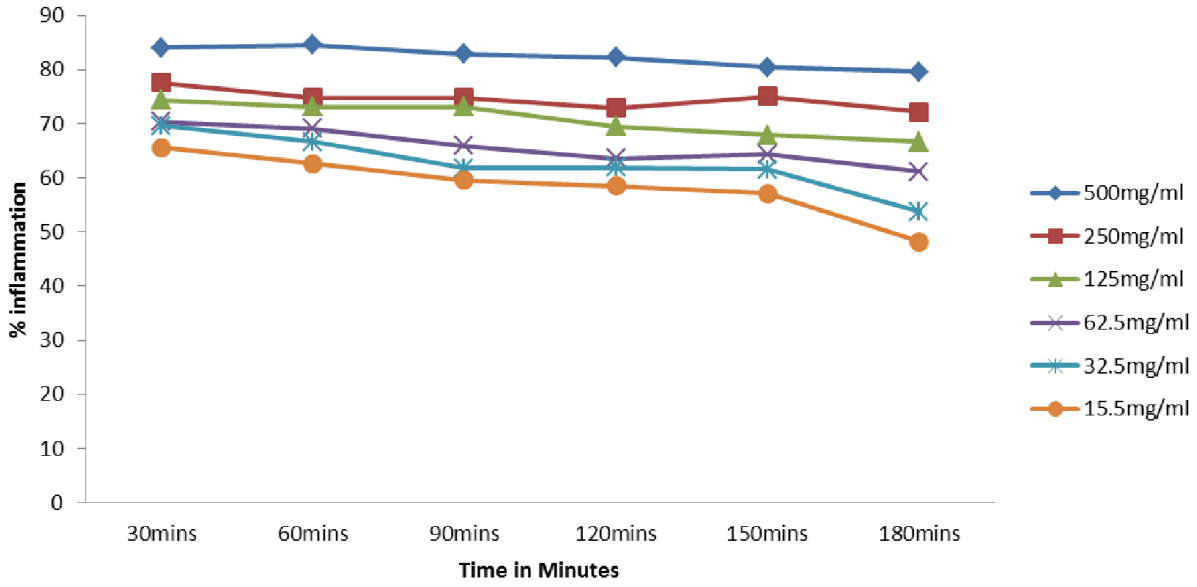
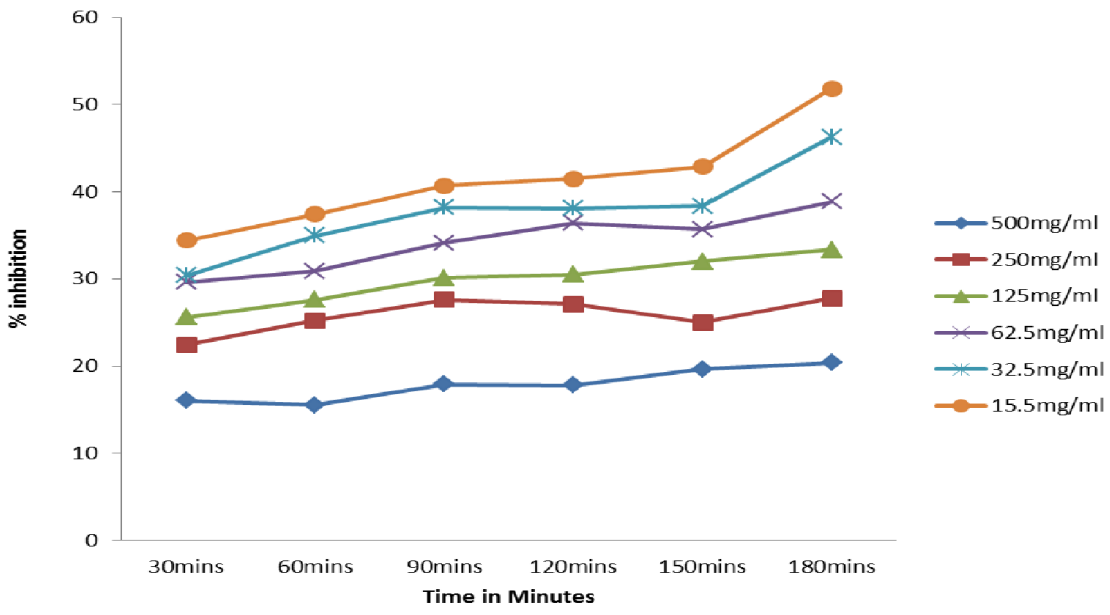


Figure 5. The mean concentration of sodium / Potassium ion ratio (Na^+ / K^+)



Graph 1. % inflammation with time



Graph 2. % inhibition of inflammation with time

Results show that the concentration at 500mg/kg of the ethanol extract had the highest anti-inflammatory effect because the rate of percentage inhibition was the greatest at the final 30 minutes interval.