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Research paper

Toxicological implications of *Margaritaria discoidea* aqueous seed extract on Na^+, K^+-ATPase specific activity, glucose and serum electrolytes level in wistar rats

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Abstract

The effect of aqueous seed extract of *Margaritaria discoidea* on Na^+ K^+ ATPase activity and toxicity studies of its seed extract were investigated in albino rats. The study involved oral administration of different doses of the aqueous extract to groups of male albino rats at 25, 50,100, and 200mg/kg body weight resulted in significant ($p<0.05$) decrease in sodium, potassium and glucose levels while chloride and calcium ions showed a significant ($p<0.05$) increase relative to control. The seed extract also showed significant ($p<0.05$) decrease in Na^+ K^+ ATPase specific activity in a dose dependent manner relative to control. The median lethal dose (LD_{50}) of this extract in mice was established at 316.2mg/kg body weight using probit of mortality. The results showed that the acute toxicity potency of the aqueous extract of the seeds was practically non-toxic. The study showed that seeds of *Margaritaria discoidea* could be toxic if not consumed in moderate quantities.

Keywords: *ATPase activity; Probit; Lethal Dose (LD_{50}); Margaritaria discoidea.*

1. Introduction

Plants are the oldest known sources of human and livestock healthcare, and an important component of global biodiversity (Lambert et al., 2005). Medicinal plants are of great importance in traditional medical practice because they are the main ingredients used for preparing the remedies administered to patients who patronize the medical practice (Amusan et al., 2005b, 2007). Apart from being the major component of ethnomedicine, medicinal plants have also been major source of drugs used in the orthodox medical practice (Fansworth, 1990). Knowledge of the chemical constituent of plants is helpful in the discovery of therapeutic agents as well as new source of economic material (Manasi et al., 2008). *Margaritaria discoidea* is a specie in the family of Euphorbiaceae which is native to South East parts of Nigeria, Gambia, Senegal and Swaziland (Burkill, 1994). The seeds of *Margaritaria discoidea* are used by traditional medical practitioners in the treatment of headaches, pain, inflammation, bacterial infection, purging and in management of hypertension. Amusan et al (2005b) reported that the roots of *Margaritaria discoidea* contain flavonoids, tannins, alkaloids and phenols. In fact, there is no documented evidence for its use in the treatment of these ailments and its toxicological implications. The aim of the study is to determine the toxicity of the seeds and its toxicological effects on Na^+K^+ATPase specific activity and serum electrolytes in rats. This forms the basis of the research study.

2. Materials and methods

2.1. Plant materials

Fresh and matured seeds of *Margaritaria discoidea* were purchased from Umuahia main market, Abia State in Nigeria. The seeds of this plant were identified by Mr. Ibe .K. Ndukwe a taxonomist at the herbarium unit of Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State. The husk covering the seed was removed to get the soft part. It was grounded and subjected to aqueous extraction which was done by soaking 140g in 420 ml of distilled water and kept for 48 hours at room temperature. The solution was filtered using Whatman No.1 filter paper. 10ml of the filtrate was concentrated to determine the value of the dry residue and the value was used to calculate the concentration of the filtrate.

2.2. Animal grouping

Twenty five male albino rats weighing 90-160 g were purchased from the animal breeding unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. They were carefully handled and transported to the Animal House Unit in the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike. The animals had free access to tap water and standard pellet diet (Vital Growers Mash) *ad libitum* during acclimatization and housed in stainless steel cages with plastic base under humid tropical conditions. The protocol used in animal handling conforms to the guidelines of the National Institute of Health (NIH publication, 2011) for laboratory animal care and use.

2.3. Acute toxicity studies

The acute toxicity of *Margaritaria discoidea* aqueous extract was determined according to the method of Miller and Tainter (1944). Twenty Swiss albino mice (12–25 g) were bred at the Department

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of Biochemistry and were randomly divided into five groups of four mice each. Graded doses of the extract (100, 250, 500, 750 and 1000mg/kg) were injected into the different groups respectively. The number of dead animals after injection of the extract in each group was recorded. The log dose was plotted against probits of the percentage dead. The dose corresponding to probit 5 was the Lethal Dose (LD_{50}).

2.4. Sub-acute toxicity studies

Twenty five albino rats were divided into five experimental groups with five animals each. The control group (group E) received distilled water while the experimental groups (A-D) received aqueous extract at 25mg/kg, 50mg/kg, 100mg/kg and 200mg/kg body weight respectively, administered orally for 28 days. All animals were weighed weekly for 28days.

2.5. Biochemical analysis

The blood collected into non-heparinized tubes was centrifuged at 3000 rpm for 10minutes. The serum was separated and used for the analysis of electrolyte and glucose while the small intestine homogenates was used for the analysis of Na^+,K^+-ATPase. Bicarbonate and chloride were estimated by titrimetric method as described by Harold (1988). Potassium level was estimated by Na-cobalti nitrite method as described by Jacobs and Hoftman and modified by Lochhead and Purnell (1951). Sodium content was determined by Zinc uranyl acetate method as described by McCance and Shipp (1931). The calcium content was estimated by titrimetric method by Harold (1988).The glucose -hexokinase method as described by Tietz (1976) was used for estimation of glucose.

2.6. Na^+ K^+ ATPase activity

This was carried out using the method of Leong and Manahan (2006). The intestines collected were weighed using a top loading balance and macerated in 5ml of normal saline and the content centrifuged for 10-15mins repeatedly until clear and the supernatant carefully collected. The assay medium contain 0.1ml of NaCl, 0.2ml of KCl, 0.2ml of $MgCl_2$, 0.2ml of EDTA, 0.2ml of Tris buffer and 0.2 ml of ATP. The content was mixed together followed by addition of 2 ml of the sample and the content mixed thoroughly. The mixture was allowed to stand for 15 min and 1ml of perchloric acid was added to stop the reaction. 0.1ml of the mixture was taken in triplicate in a centrifuge tube and 2.4ml of 10% TCA added. The content was centrifuged and 1ml of supernatant collected. 1.85ml of distilled water was added followed by addition of 0.1ml of sulphuric acid Molybdate reagent and 0.05ml of dilute stannous chloride solution. The absorbance was read at 650 nm against a blank. Also from the same assay medium, the protein concentration was estimated using the method of Lowry et al. (1951) as modified by Macdonald and Chen (1965).

$$\text{Na}^+, \text{K}^{+-}\text{ATPase specific activity (pm/hr per mg Protein)} = \frac{\text{Na}^+,\text{K}^{+-}\text{ATPase concentration} \times 3.7820 \times 1{,}000{,}000}{\text{Protein concentration} \times \text{molar mass of } PO_4\text{-}(87)}$$

2.7. Statistical analysis

Results were expressed as Mean ± SD. Statistical analysis was done using SPSS for Windows 20.0. Analysis of Variance was done at $p<0.05$ significance level. Least significant difference (LSD) was used to locate significances between means.

Results and Discussion

Table 1 shows the acute toxicity studies in mice which strongly indicated that the extract of *Margaritaria discoidea* would be toxic if not moderately consumed both in humans and rats. This is indicated by the median LD_{50} estimated at 316.2mg/kg body weight. The behavioural changes noted in these animals following administration of seed extract was dullness, although the animals became active after some time. According to Schorderet (1992), a substance with LD_{50} value greater than 5000mg/kg body weight are classified as substances with low toxicity, thus the seed extract of *Magaritaria discoidea* could be considered as a substance with low toxicity. Oral administration of the aqueous extract in mice shows behavioural changes such as dullness and immediate water intake.

Figure 1 shows the mean specific activity of Na^+ K^+ ATPase activity in rats. The specific activity of Na^+ K^+ ATPase decreased ($p<0.05$) significantly in a dose dependent manner relative to the control.

The aqueous extract was recorded to cause a significant reduction in Na^+,K^{+-}ATPase activity relative to the control in figure 1. Kreydigyeh and Usta (2007) reported that such reduction would decrease apical cellular Na^+ reabsorption, lower K^+ secretion, increase K^+ concentration in the intercellular space and consequently would inhibit passive K^+ influx across the tight junctions. This would eventually lead to a reduction in Na^+ and K^+ reabsorption leading to an osmotic water flow into the lumen and diuresis. The Na^+,K^{+-}ATPase converts 20-30% of the ATP production in mammals to active transport of Na^+ and K^+ in kidney during rest (Jorgensen and Pedersen, 2001). The alteration of Na^+,K^+ATPase properties and the resultant changes in control of Na^+ homeostasis and membrane potential are key factors in regulation of vascular tone and blood pressure. These are the underlying factors in hypertension (Sweadner et al., 1994, Magyar et al 1995, Trouve et al., 2000). Endogenous cardiotonic steroids (CTS) such as digitalis and ouabain are inhibitors of Na^+,K^{+-}ATPase and they increase the force of contraction of heart muscles by altering the excitability of the tissue (Davidson, 2005 ;Devlin, 2006). The reduction of Na^+,K^+ATPase activity in the outside leads to accumulation of Na^+ ion in the cells and thus measures to reduce it including the opening of Ca^+-$3Na^+$ exchanger. (Davidson, 2005; Devlin, 2006). The resultant increase in calcium in the sarcoplasmic reticulum of heart muscles cells leads stronger contraction causing relaxation of smooth muscles. Thus this is the basis for the use of cardiotonic steroids in management of hypertension. (Devlin, 2006, Guyton and Hall,1996). The study also recorded significant reduction of Na^+,K^{+-}ATPase, increase in calcium and gradual restoration of Na^+ concentration suggesting the possible use of the extract in hypertension management.

Previous studies using various models of hypertension have suggested that preservation of lower blood pressure is associated with an increase in the affinities of the Na^+ binding site of the Na^+ K^{+-} ATPase (Vrbjar et al., 1999a,b, 2002, Lavorkorae et al., 2003). Polyphenols and phenols suppress the damage to the Na^+ binding site observed in hypertension. Application of phenols during the development of hypertension induces a partial protection of the Na^+,K^{+-}ATPase probably by inhibiting the increase of systolic blood pressure via a nitric oxide – dependent mechanism (Veronika et al., 2003; Javorková et al., 2004).

Figure 2 depicts the mean values of aqueous seed extract of *Margaritaria discoidea* on serum electrolytes. The figure shows a significant ($p<0.05$) decrease of sodium at doses of 25 and 50mg/kg relative to control. The study recorded significant reduction of Na^+ and K^+ concentration as shown in figure 2. Excessive sodium and potassium loss results in diarrhoea and in purgative abuse state. The Crypt of Lieberkuhn is an immature epithelia cell that secretes small quantities of Na^+ and Cl^- ions in the small intestine lumen. In acute laxative abuse state, they are greatly stimulated that this secretion overpowers the absorption in the small intestine due to reduced activity of Na^+,K^{+-}ATPase. This leads to loss of electrolytes (Guyton and Hall 1996). This might suggest the use of the extract by traditional medical practitioners as a purgative.

Gill (1992) reported that many species of Euphorbiaceae have been recorded to induce purgation due to high alkaloid and tannin content. The increased calcium concentration as shown in figure 2 becomes necessary because of the Ca^{+}-$3Na^{+}$ counter transport that regulates calcium and sodium level in reduced activity of Na^{+},K^{+-} ATPase pump. The study also recorded significant increase in bicarbonate at doses of 25 and 50mg/kg with mean concentrations of 4.39 ± 0.23 and 4.50 ± 0.27 when compared with the control (4.25 ± 0.18). as shown in figure 2. However at high doses of 100 and 200mg/kg, there was no significant ($p>0.05$) difference with the control. Chloride ion re-absorption is associated with excretion of bicarbonate ion. As the plasma chloride ion increases, the bicarbonate ion decreases to keep total concentration constant (Davidson, 2005). The results in figure 2 also showed increase in chloride concentration which might be what led to no significant effect in bicarbonate at high doses.

Figure 3 depicts the glucose concentration at different doses in albino rats. The glucose concentration showed a significant ($p<0.05$) decrease relative to the control at 0 mg/kg. Figure 3 depicted that the aqueous extract decreased serum glucose concentration significantly at $p<0.05$. Glucose is co-transported with sodium because of the favourable Na^{+}-gradient maintained by the ATP-driven Na^{+},K^{+-}ATPase (Delvin 2006, Davidson, 2005). The Na-glucose symporter uses the potential energy released as Na^{+} enter the cell down their electrochemical gradient to drive the uphill movement of glucose in the same direction (Davidson, 2005). The major transport of Na^{+} across the cell is through the Na^{+},K^{+-}ATPase mechanism, thus reduced activity of the enzyme might be the possible contributor to the hypoglycaemic effect of the extract. Badole et al (2006) reported that alkaloid fractions prepared from plants showed considerable anti-hyperglycaemic activity in diabetic rats.

3. Results

Table 1: LD_{50} Determination of aqueous seed extract of *Margaritaria discoidea* in mice.

Group	Total Mice	Dose	No of Dead	% Dead	Probit	Log Dose
1	4	100	0	0	3.27	2.00
2	4	250	0	0	3.27	2.39
3	4	500	2	50	5.00	2.69
4	4	750	3	75	5.42	2.88
5	4	1000	4	100	8.09	3.00

LD_{50} = Lethal Dose

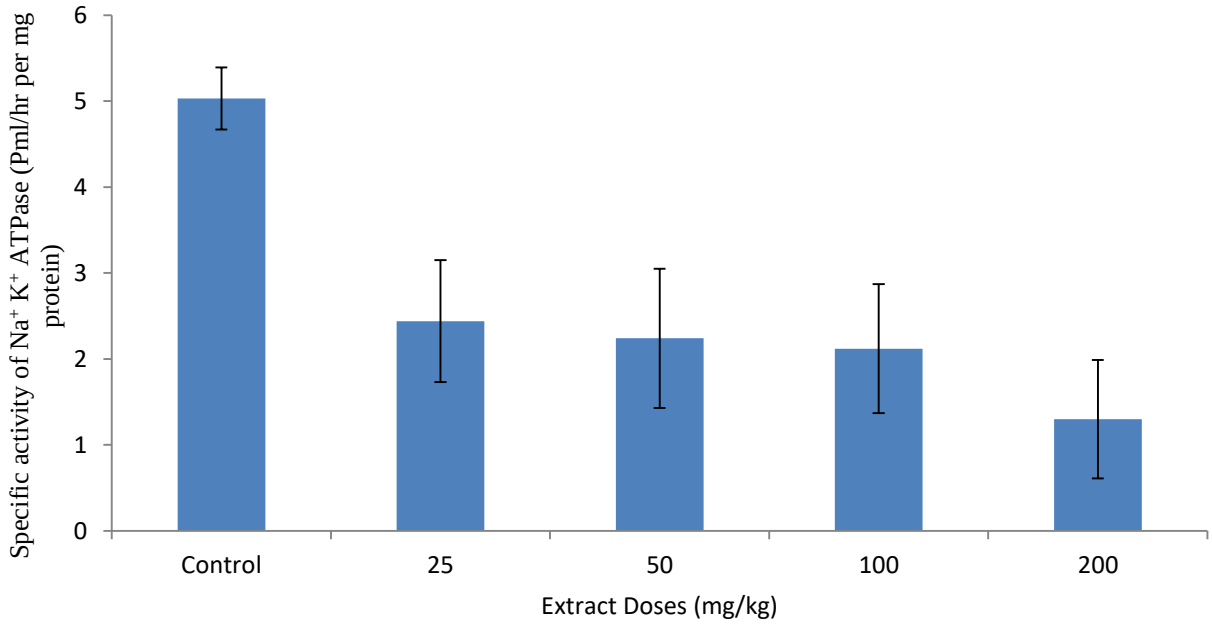


Fig. 1: Effect of aqueous extract of *Magaritaria discoidea* on Na^{+}, K^{+} ATPase activity in rats.

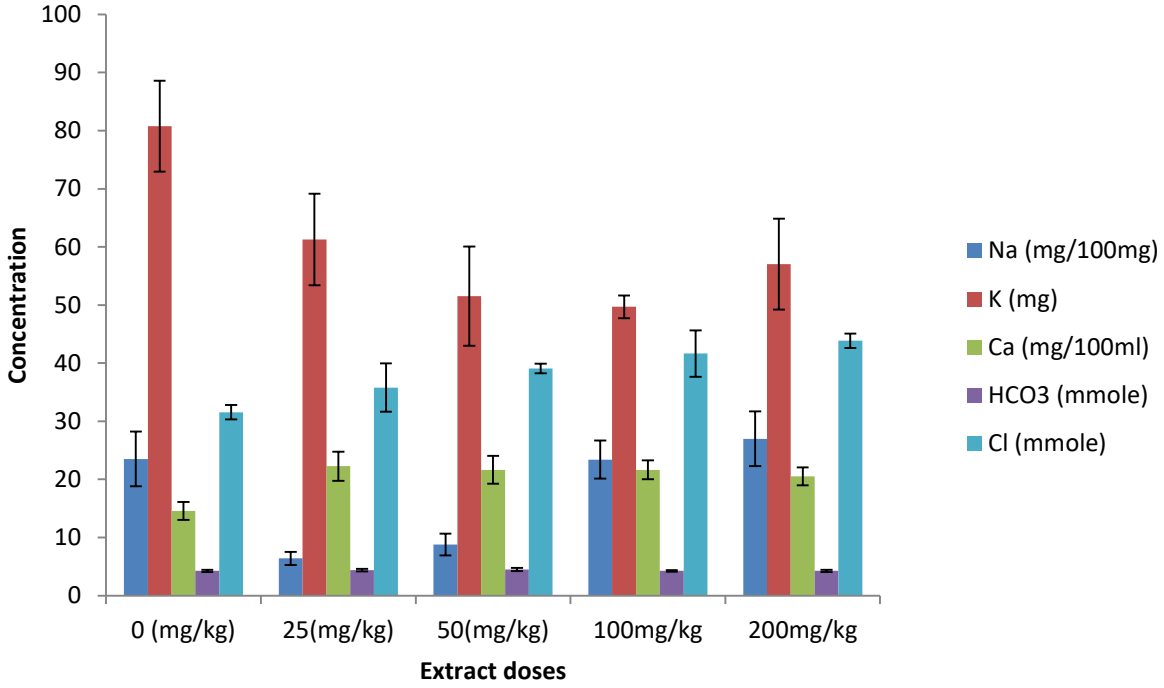


Fig. 2: Effect of aqueous seed extract of *Margaritaria discoidea* on serum electrolytes at different doses.

Na- Sodium
K- Potassium
Ca- Calcium
HCO_3- Bicarbonate
Cl- Chlorine

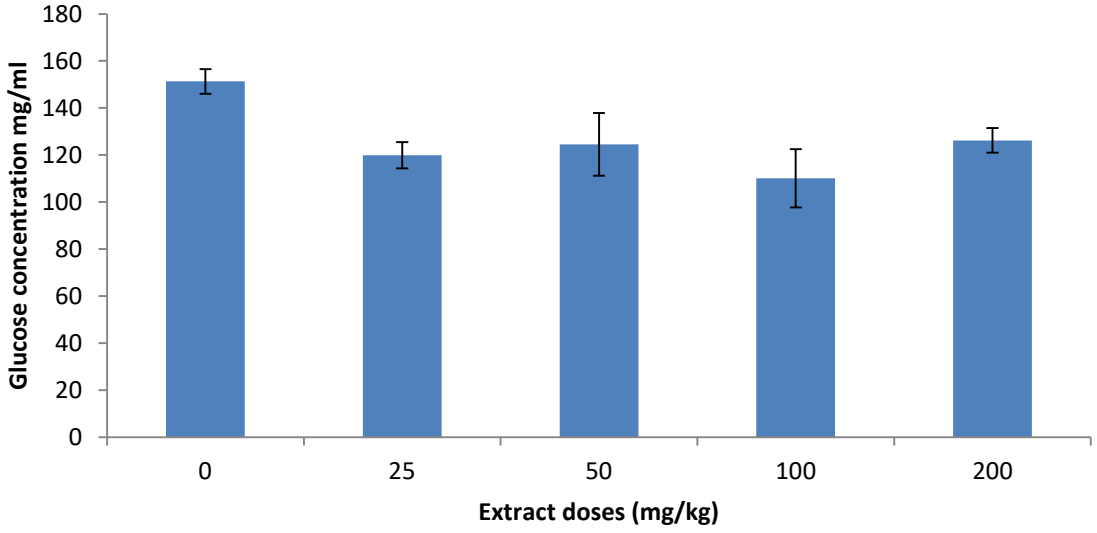


Fig. 3: Effect of aqueous seed extracts of *Margaritaria discoidea* on glucose concentration at different doses.

4. Conclusion

The experimental evidence obtained in the present laboratory animal study suggests that the seeds of *Margaritaria discoidea* decreases the specific activity of Na^+, K^+- ATPase, and could have hypoglycemic activity. This property could be the basis for the use of *Margaritaria discoidea* plant in treating various ailment and disease condition. However caution should be taken in using this plant for medicinal purpose because of the toxicological implication.

5. Conflicts of interests

All authors declare no conflicts of interest.

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