Toxicilogical Effects of Aqueous Extract of Hibiscus Sabdariffa on the Liver and Kidney.

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Background: **Hibiscus Sabdariffa(HS) is widely consumed in Nigeria and many parts of the world as a refreshing drink. It is equally used in traditional medicine to treat various diseases such as cough, hypertension, stomach disorders, loss of appetite, upper respiratory congestion, nerve and heart disorders and menstrual difficulties. Some of these ethnomedicinal claims have been confirmed by scientific researches. Some of these researches have shown that HS has protective effect on some body organs while others have demonstrated its toxic effect on some organs.**
Aims and Objectives: **The aim of the present study is to show the effect of graded doses of aqueous extract of HS on major excretory organs(Liver and Kidney) of albino Wistar rats. This may be helpful in determining the safety or otherwise of its consumption at different concentrations.**
Materials and methods: **Sixty male albino Wistar rats weighing 150-250mg and aged 2-3 months were used for this study. Normal rat chow (Guinea feed) and distilled water were provided ad libitum. The rats were divided into five groups (A-E). Groups A-D were the test groups and were administered different doses of the HS extract: 20, 40, 80 and 160mg/kg respectively using oral feeder (Gavage). Group E served as control and was given only distilled water. The extract was administered daily for a period of 12 weeks. After 4 weeks, four rats were picked at random from each group and sacrificed. The histology of the liver and kidney was investigated. At 8^{th} and 12^{th} weeks, the experiments were repeated.**
Results: **There were no significant changes in the histology of the liver throughout the period of HS administration in all the groups. However, there were significant histological changes in the kidney which were more pronounced at higher doses (80 and 160mg/kg). There was shrinkage of glomerular tuft, increase in urinary pole, increase in size of tubular lumen and tubular damage. These effects were more marked as the duration of administration of the extract progressed with greatest effect observed at 12^{th} week.**
Conclusion: **The results of the present study suggest that aqueous extract of HS has no harmful effect on the liver but when consumed in high doses could be harmful to the kidney. Further research aimed at identifying the chemical composition and potential toxic agent(s) in HS is recommended.**

Key words: **Hibiscus Sabdariffa, Toxicity, Liver, Kidney**

INTRODUCTION

The plant hibiscus sabdariffa (HS) belong to the family Malvacae with Hibiscus having about 300 species. It is said to have its nativity in Sudan and is grown widely in West Africa[1]. In Nigeria, it is called different names by

different ethnic groups; it is called Zoborodo in Hausa, Isapa pupa in Yoruba and Aishwe in Tiv. It is called red Sorell in India and Jamaica and Karkade in Switzerland and Arab countries.

The plant utility varies from one country or region to another. Even within a country, the uses may vary from one part to another. In Nigeria, it is used to produce a refreshing drink called zobo. In Arab countries, it is used in making jams, jellies, sauces and wines.

The plant has been used in traditional medicine for treatment of several ailments such as cough, hypertension, stomach/intestinal disorders, dressing of wound and as a diuretic [2]. The plant was also believed to be chologogic, antibacterial and antihelmintic [3]. Extract from the plant was also used to purify blood, improve skin complexion, and promote hair growth and effective for menstrual difficulties [4]. In Egypt, the plant is used for treatment of nerve and heart disorders, cold, upper respiratory congestion and loss of appetite [5].

Scientific researches on these ethnomedical claims have confirmed some of these uses. Its blood pressure lowering effect has been established both in experimental animals and man [2,6,7,8]. Its diuretic effect was also established [9]. A group of researchers in Chung Shan Medical University, Taiwan has also shown that the plant contains antioxidants that can reduce cholesterol and lipid build up in arteries[10] . The plant also has the ability to reduce intestinal motility [11]. Changes in urinary chemical composition have been reported in individuals that consumed HS juice. A decrease in creatinine, uric acid, sodium, potassium and phosphate was reported [11]. HS extract was also shown to possess antifertility actions. It was reported to induce testicular toxicity in rats [13,14]. It was also reported to have toxic effect on some body organs such as kidney, brain and heart [1].

The aim of the present study is to establish the effect of graded doses of aqueous extract of HS on major excretory organs of albino wistar rats (liver and kidney) and thus determine the safety or otherwise of its consumption.

MATERIALS AND METHOD

Sixty male albino wistar rats aged 2-3 months, weighing 150-250g were used in the present study. They were screened for pyrogen and kept at a temperature of 28-32°C with 13: 11 light dark cycle. The rats were kept for two weeks at animal house, University of Nigeria Teaching Hospital, Enugu, to acclimatize before the commencement of the experiment. They were fed with Guinea feed® and distilled water was provided *ad libitum.*

EXTRACTION PROCEDURE: Dry calyxes of HS were purchased from Ogbete main market in Enugu and were identified by the Botany Department, University of Nigeria, Nsukka. Mojiminiyi[9] method of extraction was used. Sixty grams pooled dry calyx was brewed in 400mls of boiled water and allowed to stand for at least 30 minutes. The resulting decoction was filtered using filter paper and the filtrate evaporated to dryness in a water bath maintained at 97°C. The dry residue was scrapped off and stored in a desiccator. The desired quantity from the residue was dissolved in distilled water to make a stock solution of the desired concentration. Four (4) g of the extract was dissolved in 100ml of water and this produced the same colour as the locally prepared aqueous HS drink (zobo) drink. This was stored in a refrigerator at 8°C. Extraction procedure and preparation of stock solution was carried out on weekly basis. The required concentrations were made from the stock solution by using the necessary dilution factors.

DESIGN AND CONDUCT OF THE EXPERIMENT: The rats were grouped into five (A-E) of twelve rats per group. Different concentrations of 20, 40, 80 and 160mg/kg

were prepared from the stock and administered to rats in groups A-D respectively using oral feeder (Gavage). Group E served as control and was given equivalent volume of distilled water. The extract was administered for a total period of 12 weeks. After 4 weeks of administration, 20 rats (4 from each group) were picked at random and sacrificed. The histology of the liver and kidney was investigated. After 8 weeks, another 20 rats (4 from each group) were randomly picked and sacrificed for histological assessment of the liver and kidney. At the end of the HS administration (12 weeks) the remaining 20 rats (4 from each group) were also sacrificed and their livers and kidneys were processed for histological studies.

PROCESSING OF SAMPLES FOR HISTOLOGY: Sections of the liver and kidney were selected and processed for light microscopy. The excised organs were cleared of the adhering connective tissue, fixed for 24hours by immersion in equal parts of 10% buffered formal saline and fluid. Thereafter, fixed tissues were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in liquid paraffin wax.

The tissues were sectioned at 5cm using the Heitz 150 rotary microtome (Cambridge model). The sections were then subjected to Erlich's Haematoxylin and Eosin (H and E) staining technique using Baker and Silverton[15] method.

Sections were examined using swift binocular microscope with in-built lighting system and were photographed. (Figs. I, II).

RESULTS

LIVER: The hepatocytes showed normal parenchyma with mild vacoulation in all the groups(Table I) throughout the duration of HS administration. Frank red blood cells were also found in the central lobular veins in some of the groups(Fig iv).

KIDNEY: After 4 weeks, there were no significant changes in the histology of the kidney. The glomerulus and renal tubules appeared normal in all the groups(Table II). At 8^{th} and 12^{th} weeks there were remarkable changes in the histology of the kidney. There was shrinkage of the glomerular tuft and increase in urinary pole which appeared to be dose-dependent. In addition, there was increase in size of tubular lumen which was more prominent at higher doses (Figsvii-x). Greatest histological changes were observed at 12^{th} week.

Table 1: Histological scoring of the effect of aqueous HS on the liver

		4th Week		8th Week		12th Week	
Group	Dose (mg/kg)	N	V	N	V	N	V
A	20	---	±	---	±	---	±
B	40	---	±	---	±	---	±
C	80	---	±	---	±	---	±
D	160	---	±	---	±	---	±
E	Control	---	---	---	---	---	---

N=Necrosis; V=Vacoulation; - - =Normal features; ±=Mild presence of features

Table 2: Histological scoring of the effect of aqueous HS on the kidney EY

		4th Week			8th Week			12th Week		
Group	Dose (mg/kg)	G	U	T	G	U	T	G	U	T
A	20	---	---	---	---	---	---	---	---	---
B	40	---	---	---	±	±	---	±	±	±
C	80	±	±	±	+	+	++	+	+	++
D	160	++	++	++	+++	++	++	+++	+++	+++
E	Control	---	---	---	---	---	---	---	---	---

G=Glomerulus; U=Urinary pole; T=Tubular size; ---=Normal features; +=Mild effect; ++=Moderate Effect +++ =Severe Effect

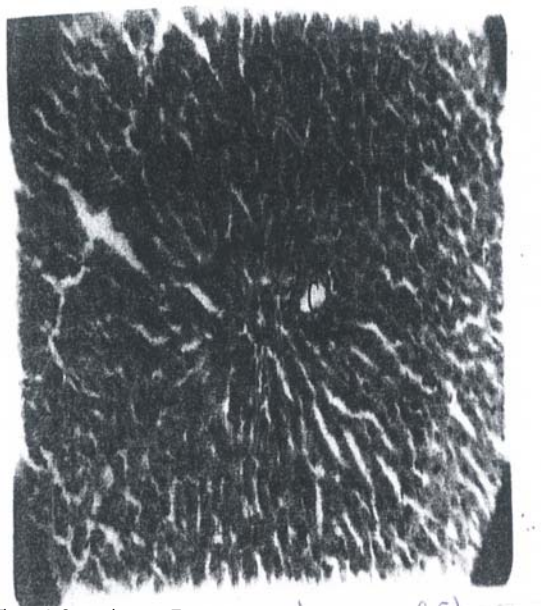

Figure I: Control group E
liver tissue from control group showing centeral vein(C) with mild vacoulation x200

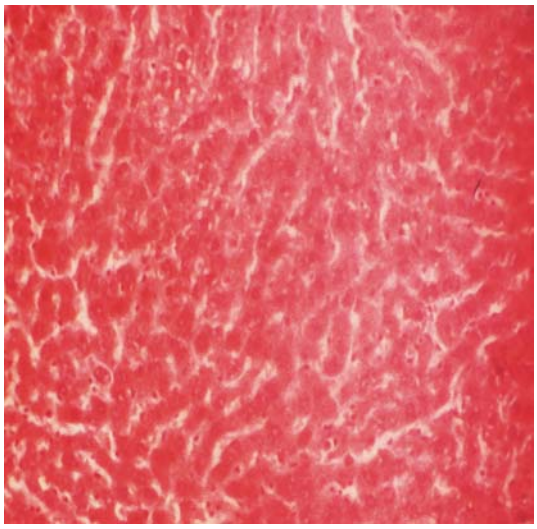

Figure III: Group B
Liver showing mild vacuolation

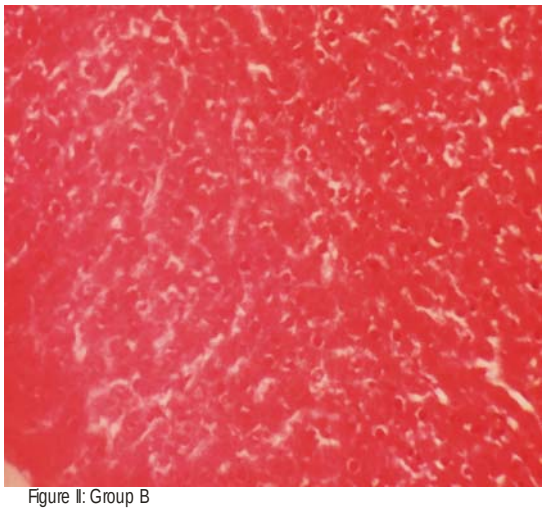

Figure II: Group B
Liver showing mild vacuolation x100

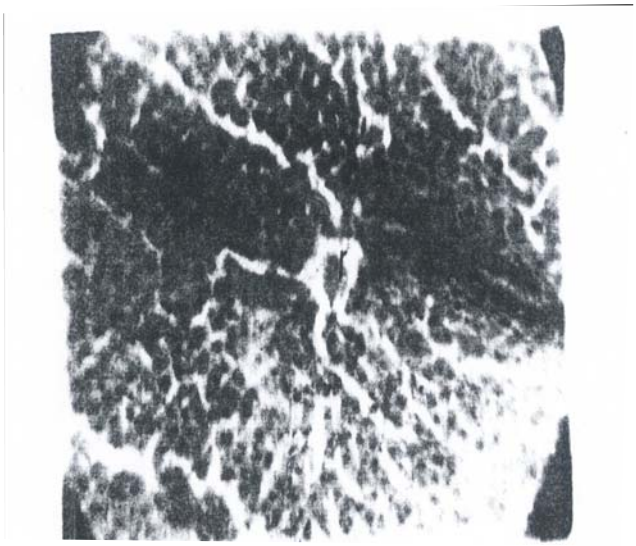

Figure IV: Group D
Liver showing central nlobular vein

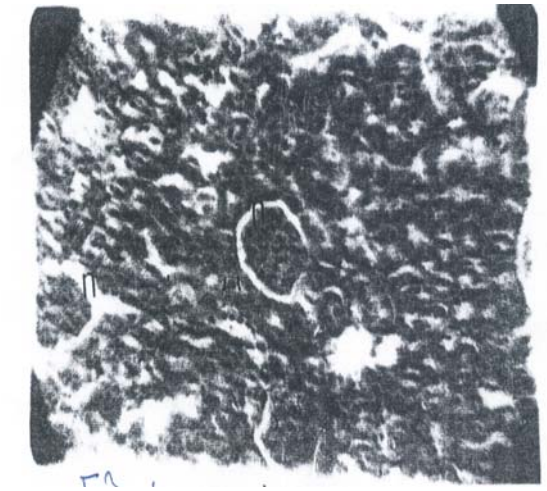

Figure V: Control Group E
Kidney parenchyma showing normal nephron

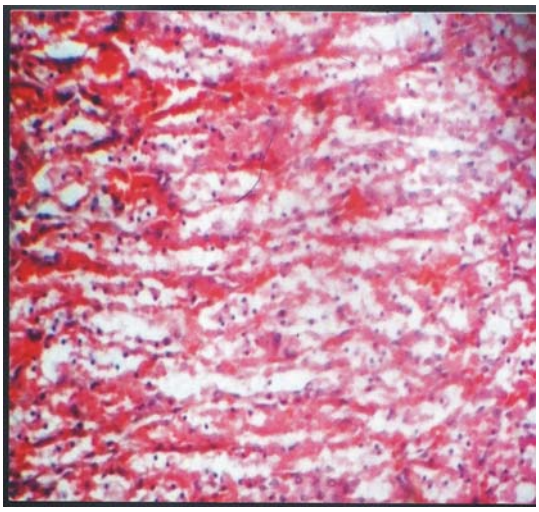

Figure: VII: Test Group C (8th week)
Kidney showing tubular dilation

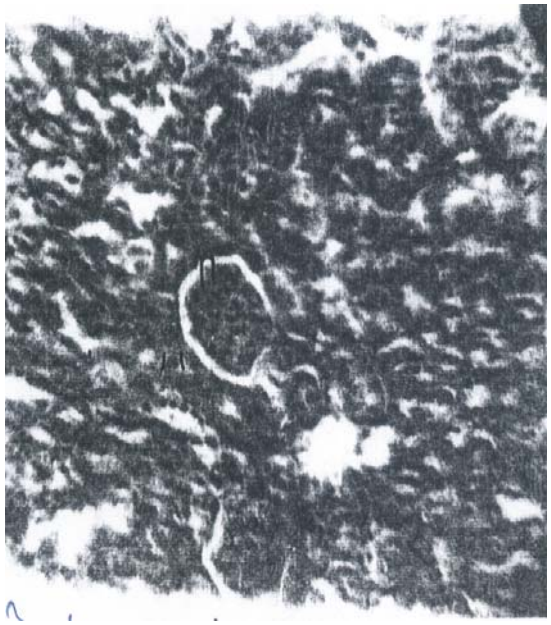

Figure VI: Test Group A (4 weeks)
Kidney parenchyma showing normal nephron

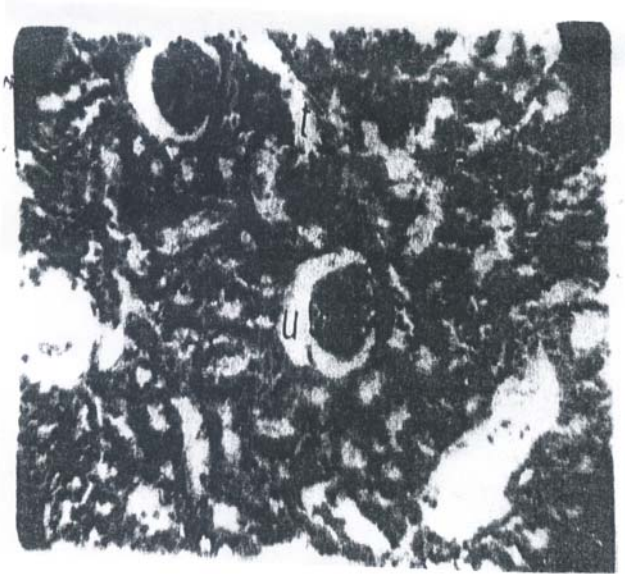

Figure VIII: Test Group C (12th week)
Kidney showing mild increase in glomerular tutf (g), increase in urinary pole (u) and in tubular lumen (l)

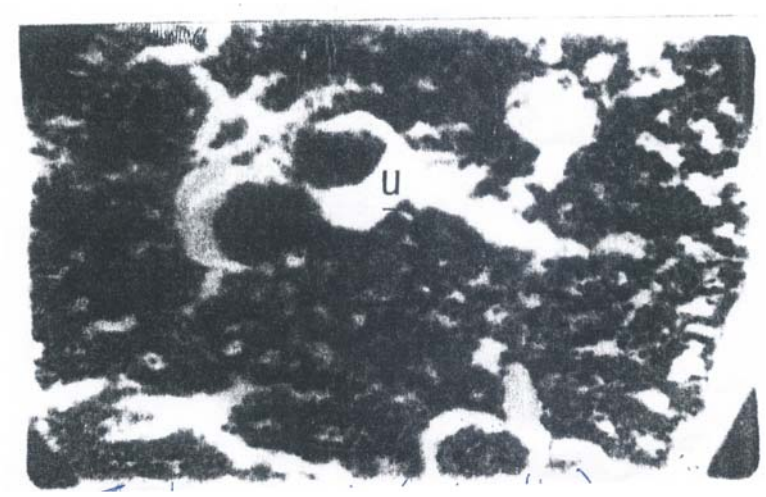

Figure IX: Test Group D (8th week)
Kidney showing remarkable increase in size of urinary pole with tortious tubular disposition x 100

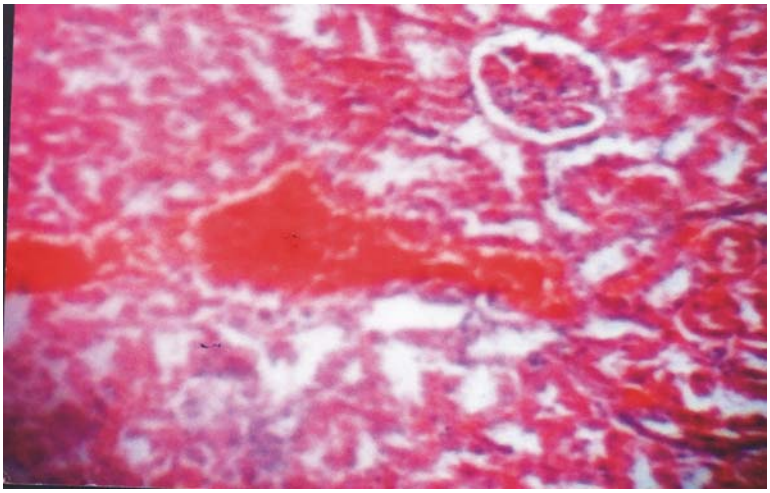

Figure X: Test Group D (12th week)
Kidney showing tubular damage x 100

DISCUSSION

Some authors have reported the toxic effect of Hibiscus Sabdariffa extract on body organs [1,12,13] while others have reported its protective effect on some organs [8] and its ability to lower blood pressure [2,6,16].

The results of the present study show that aqueous extract of HS has some toxic effects on the kidney but not on the liver. The normal histology of the hepatocytes was preserved irrespective of the dosage and the duration of administration(Fig i-iv). There were no inflammatory cells seen. The mild vacoulation

observed (Table I) might be due to the wax and organic solvent xylene used in processing the tissue. The Frank red blood cells seen(Fig iv) might be due to the anesthesia used. This confirms earlier reports that it has no harmful effect on the liver rather it may exert a hepatoprotective action which may be due to the antioxidants contained in it [8].

Several actions of Hibiscus Sabdariffa extract on the kidney have been reported by different authors. Its toxic effect where it caused glomerular damage((Fig ix) and tubular necrosis(Fig x) were reported [1]; its diuretic effect [9] and its ability to change the chemical composition of urine [11] were also reported. The present study confirms the strong influence that Hibiscus Sabdariffa has on the kidney. Extract of HS caused increase in size of renal tubules(Fig vii),increase in glomerular tuft and urinary pole(Fig viii). Damage of the glomerular tuft was observed especially at higher doses(Fig ix) and this agrees with the report by previous workers[1]. The increase in urinary pole and increase in the size of glomerular lumen observed in this study may be responsible for the diuretic action earlier reported [9]. Diuretics are commonly used in the treatment of hypertension and this may constitute a major mechanism of action for its reported hypotensive action both in experimental animals and in humans [4,6,8,16]. Other hypotensive mechanisms of action of HS earlier suggested include inhibition of Ca^{2+} influx through receptor-gated channels and relaxation of vascular smooth muscle mediated via nitric oxide [16].

CONCLUSION

The results obtained from the present study show that aqueous extract of HS (zobo), which is commonly consumed as a local beverage in Nigeria and many other countries, may not be completely safe. Its toxic effect on the kidney suggests that caution should be applied when consuming such natural and unrefined products. It is recommended that further investigations be conducted to identify the chemical composition and the potential toxic agent(s) in Hibiscus Sabdariffa. It is equally recommended that National Agency for Food and Drug Administration and Control (NAFDAC) be involved in the regulation of its consumption and create public awareness on the possible dangers of indiscriminate consumption of such raw natural products especially at high doses.

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