

MORPHOLOGICAL EFFECTS OF CHRONIC CONSUMPTION OF ZOBO DRINKS ON THE KIDNEY OF ADULT WISTAR RATS

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ABSTRACT

The effects of chronic consumption of zobo drinks commonly used as red coloured soft drinks on the kidney of adult wistar rats were investigated. The rats of both sexes (n=20), with an average weight of 200g were randomly assigned into test (n=10) and control (n=10) groups. The rats in the test group was given zobo drinks liberally on a daily basis for thirty days while the control group received water liberally for the same period and through the same route. The rats were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo state, Nigeria. The rats were sacrificed by cervical dislocation method on the thirty-first day of the experiment and the kidney was carefully dissected out, dried, weighed, and quickly fixed in 10% formal saline for further routine histological study. The findings indicated that there was a significant ($P < 0.05$) decrease in weights (g) and increase in relative weights (%) of the test kidneys (right and left) as compared to the control group. The microanatomy (H & E) indicated that the kidney in the test group showed some level of distortion and disruption of the cytoarchitecture of the renal cortical structure, diffuse glomerulonephritis with some congestion of blood and an enlarged Bowman's space as compared to the control group. Chronic consumption of zobo drinks may therefore have an adverse effect on the kidney of adult wistar rats. It is recommended that further studies aimed at corroborating these observations be carried out.

Keywords: Morphological effects, Zobo drinks, Kidney, Wistar Rats.

INTRODUCTION

Among the more than three hundred species of Hibiscus is *Hibiscus sabdariffa* Linn, which has many medicinal uses (Gill, 1992; Morton, 1987). The plant *Hibiscus sabdariffa* Linn belongs to the family Malvaceae (Gill, 1992). It is cultivated for its leaf, fleshy calyx, seed or fibre. Some of these parts are used as herbal remedies (Gill, 1992). In Nigeria a red col-

oured soft drink which is a hot water extract of the red flower of this plant is chilled and marketed as "zobo drinks". Among the chemical constituents of the flower are the flavonoids, gossypetine, hibiscetine, anthocyanin and sabdaretine (Pietta, 2000). Flavonoids are phenolic compounds (Robinson, 1975). Phenolic substances in red wine have been shown to be potent inhibitors of copper catalyzed oxidation

of low density lipoprotein (LDL). Hence they are believed to possess antioxidant activity. There are indications that the extract from the red petals of *Hibiscus sabdariffa* L contains antioxidant principles (Tseng et al., 1997; Wang et al., 2000). It is therefore conceivable that the consumption of the extract may provide natural agents against oxidative tissue damage and other free radical induced disease conditions (Harman, 1984; Wolff et al., 1986). It is generally assumed that the active dietary constituents contributing to these protective effects are the anti-oxidant vitamins. Recent investigations have also revealed that polyphenolic components of plants do exhibit antioxidant properties and do contribute to the anticarcinogenic or cardioprotective actions brought about by the diet (Newman, 1992; Wang et al., 2000; Stainer et al., 2004). Antioxidant vitamins such as vitamins C and E along with flavonoids have been shown to be effective in reducing atherosclerosis along with many other diseases (Jackson et al., 1993; Gaxiane et al., 1994; Amin and Buratovich, 2007).

The various parts of the *Hibiscus sabdariffa* plant serve important uses. The edible calyx is the source of a red beverage known as zobo in Northern Nigeria. It is also used for making jams, jellies, sauces, wines and ice cream due to its rich citric acid content (Duke, 1985). Leaves are used in salads, as a potherb and as a seasoning, in curries, seeds, which have been said to have aphrodisiac properties, may be used in soups and sauces (Duke, 1985). The root which is also edible is a source of fibre and is used in the making of sack cloth, twine and cords (Duke, 1985). In folk medicine, *Hibiscus sabdariffa* has allegedly been used as an antiseptic, astringent, purgative, diuretic, sedative and tonic (Duke, 1985). The plant has also been used as a folk remedy for abscess, cancer cough, dysuria, heart ailments, neurosis and scurvy (Duke, 1985).

The kidney is a paired organ located in the posterior abdominal wall, whose functions include the removal of waste metabolic products from the blood and regulation of water and electrolyte balance in the body. In humans, the majority of drugs administered are eliminated by a combination of hepatic me-

tabolism and renal excretion (Katzung, 1998). Since the kidney is involved in the excretion of many toxic metabolic waste products, including the nitrogenous compounds, it would therefore be worthwhile to examine the effects of long term consumption of zobo drinks on the kidney of adult wistar rats. The purpose of this experiment is to evaluate the possible effects of long term consumption of zobo drinks on the morphology of the kidneys of adult wistar rats.

MATERIALS AND METHODS

Animals: Twenty adult wistar rats of both sexes with average weight of 200g were randomly assigned into two groups: A and B of ten rats each in a group. Group A served as control group (n=10) while group B (n=10) served as the test. The rats were obtained and maintained in the Animal Holding of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. The animals were caged in stainless cages with raised wire floors based on their sex to avoid pregnancy, fed *ad libitum* with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria. The dried *Hibiscus sabdariffa* petals were obtained in Uselu market, Benin City. The dried *Hibiscus sabdariffa* petals were identified and authenticated in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Preparation and consumption of zobo drinks: Dried *Hibiscus sabdariffa* petals were obtained from Uselu Market in Benin City and two hundred grams (200g) of the dried *Hibiscus sabdariffa* petals were soaked in 2000ml of hot water for 30 minutes and then filtered to obtain the red coloured extract and the residue was discarded. The volume of the filtrate obtained was 1415ml and later made up to 1981ml with tap water in such a way that it would be consumed by humans and allowed to cool. This stock solution was stored and then used for this study. The rats in the test group was given zobo drinks *ad libitum* on a daily basis for thirty days while the control group received water *ad libitum* for the same period of thirty days. The rats were sacrificed by cer-

vical dislocation on the thirty-first day of the experiment. The abdominal region was quickly opened and the kidney dissected out, weighed and fixed in 10% formal saline for routine histological techniques.

Histological study: The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 6 microns thick were obtained using a rotatory microtome. The deparaffused sections were stained routinely with haematoxyline and eosin (Drury *et al.*, 1967). Photomicrographs of the desired results were obtained using research photographic microscope in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Edo State, Nigeria.

Statistical analysis: The mean values of the kidney obtained from the control and treatment groups were recorded and compared statistically using the unpaired sample t-test and Symmetric measured test of the Statistical Package for Social Sciences (SPSS). The results were calculated using mean and standard error of mean (SEM) respectively (Adjene and Arukwe, 2009)

RESULTS

The photomicrograph of the kidney in the control group showed normal histological features. The section indicated a detailed cortical parenchyma and the renal corpuscles appeared as dense rounded structures with the glomerulus surrounded by a narrow Bowman’s space (Plate 1 & 2). The findings also indicated that there was a significant ($P < 0.05$) decrease in weights (g) and increase in relative weights (%) of the test kidneys (group B) as compared to the control group (group A) (Table 1, Fig.1, 2, 3 & 4.). The microanatomy (H & E) indicated that the kidney in the test group (group B) showed some level of distortion and disruption of the cytoarchitecture of the renal cortical structure, diffuse glomerulonephritis with some congestion of blood and an enlarged Bowman’s space as compared to the control group (group A) (Plate 1,2,3 & 4).

Table 1: The Mean Weight (g) and Relative Weight (%) of the Kidneys of the animals

Parameters	Groups of Animals	
	Group A Control (n=10)	Group B Test (n=10)
Body Wt. (g)	260 ± 30.19	197 ± 17.19
Right kidney Wt (g)	*0.58 ± 0.14	*0.46 ± 0.06
Relative Rt kidney Wt (%)	0.23 ± 0.07	0.23 ± 0.02
Left kidney Wt (g)	*0.56 ± 0.19	*0.45 ± 0.05
Relative Lt. kidney Wt (%)	*0.22 ± 0.09	*0.23 ± 0.01

*significant ($P < 0.05$)

Values represent mean ± SEM

Wt = Weight; Rt = Right; Lt = Left.

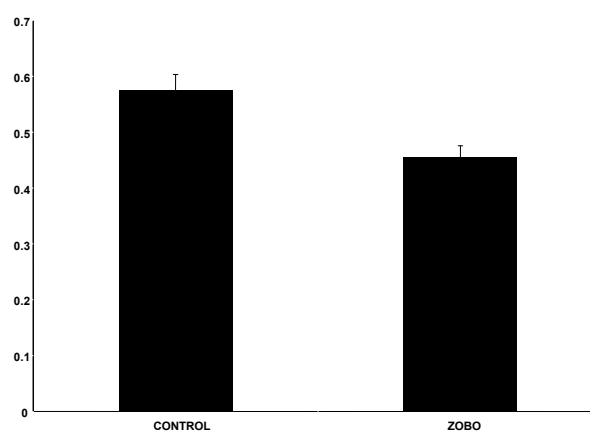


Fig. 1: Bar Chart Showing The Mean Right Kidney Weight (G) Of The Animals

Wt (g) Values represent mean ± SEM

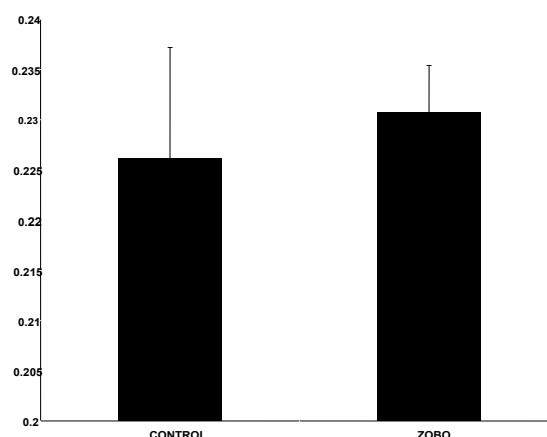


Fig. 2: Bar Chart Showing The Mean Relative Right Kidney Weight (%) of the animals R.Wt (%)

Values represent mean ± SEM

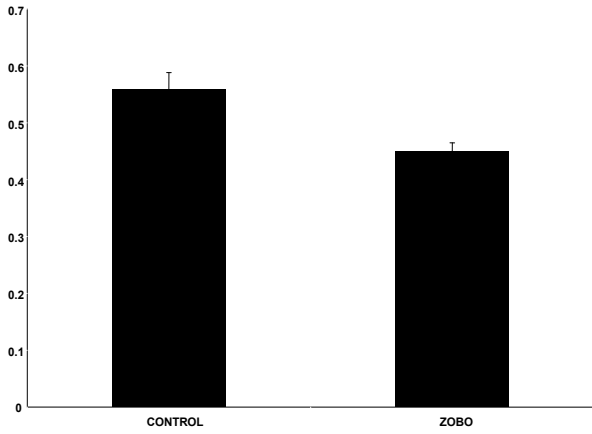


Fig. 3: Bar Chart Showing The Mean Left Kidney Weight (g) of the Animals
Wt (g)
 Values represent mean \pm SEM

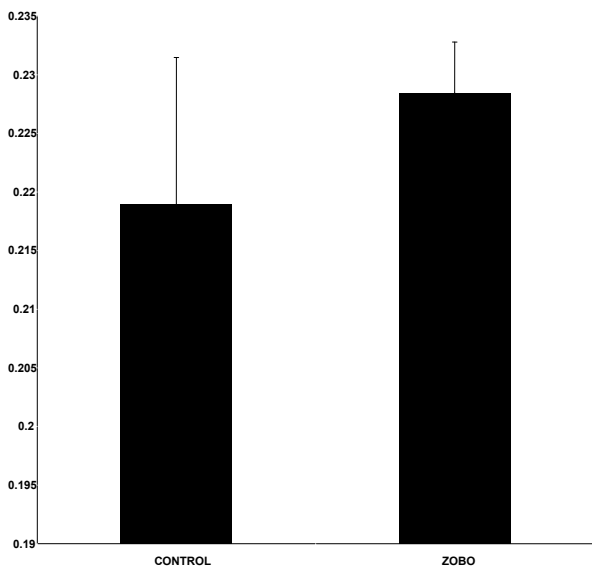


Fig. 4: Bar Chart Showing The Mean Relative Left Kidney Weight (%) of the animals
R.Wt (%)
 Values represent mean \pm SEM
 R.Wt = Relative weight

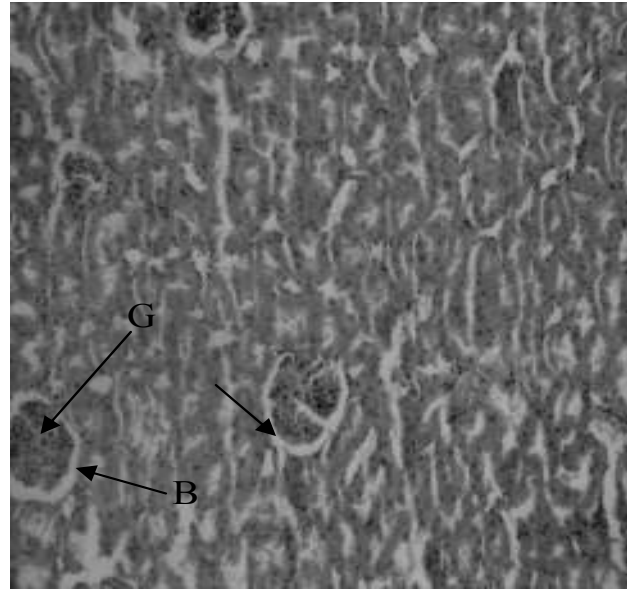


Plate1: Control section of the Kidney showing the Glomerulus (G) and Bowman's space (B) (H & E x100)

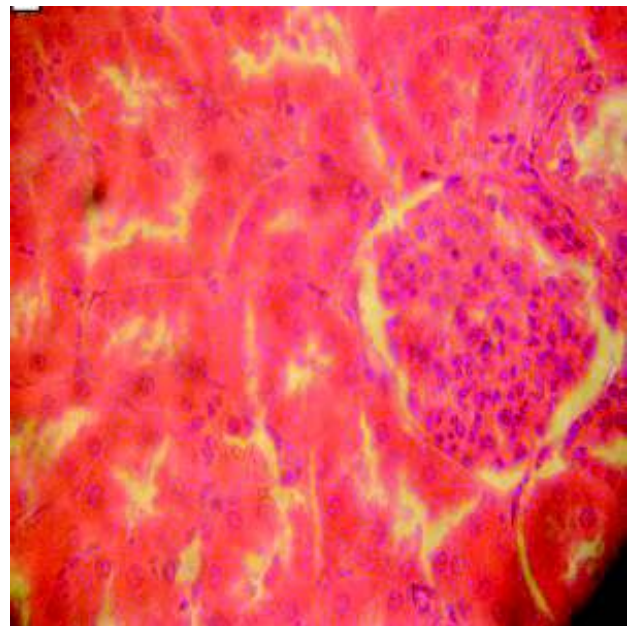


Plate2: Control section of the Kidney showing the Glomerulus (G) and Bowman's space (B) (H & E x400)

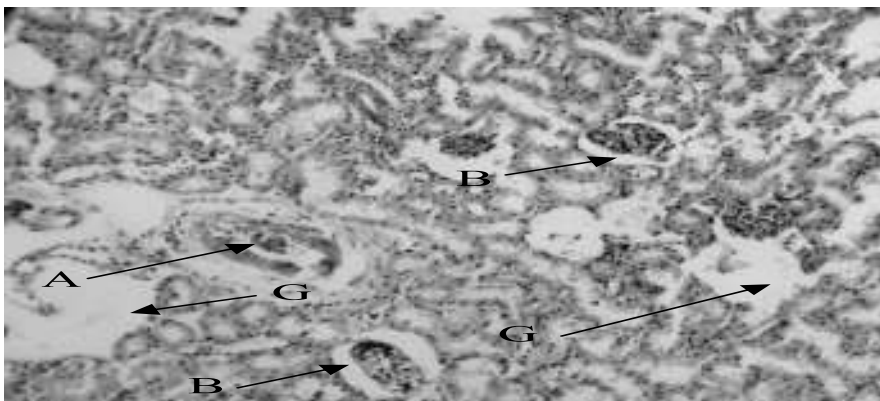


Plate3: Treated section of the Kidney showing diffuse Glomerulonephritis (G), dilated Bowman's space (B) and Blood Congestion (A). (H & E x100)

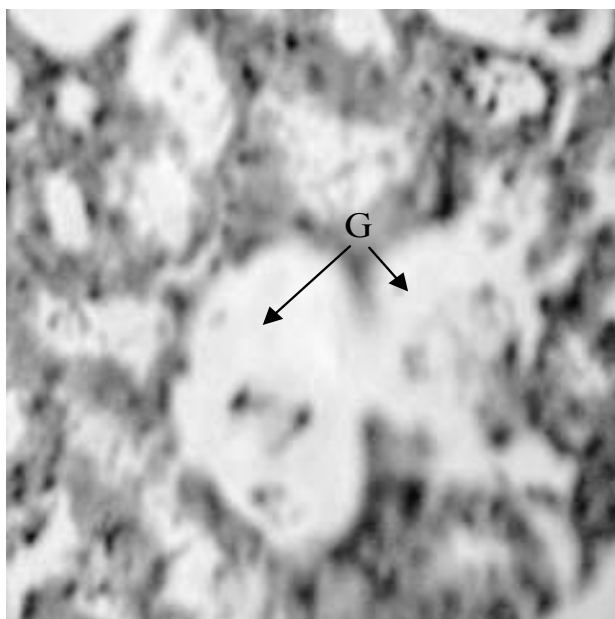


Plate4: Treated section of the Kidney showing diffuse Glomerulonephritis (G) (H & E x400)

DISCUSSION

The findings indicated that there was a significant ($P < 0.05$) decrease in weight (g) and increase in relative weight (%) of the test kidneys (right and left) as compared to the control group. The microanatomy (H & E) indicated that the kidney in the test group showed some level of distortion and disruption of the cytoarchitecture of the renal cortical structure, diffuse glomerulonephritis with some congestion of blood and an enlarged Bowman's space as compared to the control group.

The result obtained in this experiment is probably due to the chronic consumption of the zobo drinks on the kidney. It appeared that chronic consumption of zobo drinks is not as harmless as generally believed. The distortion and disruption of the cytoarchitecture of the kidney observed in this experiment may have been associated with the functional changes that could be detrimental to the health status of the animals. The observed changes in zobo treated kidneys might be due to the cytotoxic effect of zobo drinks on the kidney. As tissue swells or shrinks as seen in this study, the activity of the cellular transporters is approximately modified by the up or down regulations as has been reported in the case of hyponatremia or hypernatremia (Johanson, 1995). Ischemia or pharmacologic disruption of cellular transporters can cause swelling of paren-

chyma of any organ. The pharmacologic disruption of the kidney weights caused by zobo drinks is a cardinal feature of the results of this experiment. There are many different causes of cell swelling or shrinkage, including drug poisoning, water intoxication, hypoxia, and acute hyponatremia (Johanson, 1995). Under such conditions, there is a net shift of water from the extracellular space to the interior of the cells (Johanson, 1995). The significant decrease associated with the weights and significant increase in the relative weights of the kidneys in this experiment usually involves intracellular swellings or shrinkage of the endothelia (Johanson, 1995).

The obvious signs of diffuse glomerulonephritis observed in this experiment may have been due to the zobo drinks cytotoxic effects on the kidney. These findings implicated zobo drinks as a possible precipitant of kidney disease by causing congestion of blood in the microanatomy of the kidney. Pathological or accidental cell death is regarded necrotic and could result from extrinsic insult to the cell as osmotic, thermal, toxic and traumatic effects (Farber *et al.*, 1981). The process of cellular necrosis involves disruption of membranes, as well as structural and functional integrity. Cellular necrosis is not induced by stimuli intrinsic to the cells as in programmed cell death, but by an abrupt environmental perturbation and departure from the normal physiological conditions (Martins *et al.*, 1978). In cellular necrosis the rate of progression depends on the severity of the environmental insults. The greater the severity of the insults, the more rapid the progression of neuronal injury (Ito *et al.*, 1975). The principle holds true for toxicological insult to the brain and other organs (Martins *et al.*, 1978). It may be inferred from the present study that prolonged consumption of zobo drinks may result in increased toxic effects on the kidney. The result obtained in this experiment is in consonance with the work carried out by Enaibe *et al.* (2007) where they reported that administration of camphor resulted in mild edema with glomerulonephritis, glomerular lobulation, tubular necrosis and congestion of blood cell in the kidney of rabbit. It has also been reported that administration of damiana (*Turnera diffusa*) to a matured wistar rats re-

sulted in the distortion of the renal cortical structures, reduced size and number of the renal corpuscles and some degree of cellular necrosis in the histology of the kidney (Enaibe et al., 2007). In this experiment, zobo drinks may have acted as toxin to the cells of the kidney thus resulting in the distortion and disruptions, congestion of blood, enlarged Bowman's space and diffuse glomerulonephritis in the renal cortical structures. The result of this experiment is also in line with the work earlier reported by Adjene et al. (2010) that chronic consumption of soda pop drinks resulted in some varying degree of distortion and disruption of the cytoarchitecture of the renal cortical structures, diffuse glomerulonephritis with some congestion and tubular necrosis in the microanatomy of the treated kidney of adult wistar rats as compared to the control kidney.

CONCLUSION AND RECOMMENDATION

In conclusion, our findings indicated that chronic consumption of zobo drinks resulted in a significant ($P < 0.05$) decrease in weight (g) and increase in relative weight (%) of the test kidneys (right and left) as compared to the control group. The microanatomy (H & E) indicated that the kidney in the test group showed some level of distortion and disruptions of the cytoarchitecture of the renal cortical structures, diffuse glomerulonephritis with some congestion of blood and an enlarged Bowman's spaces as compared to the control group. With these results, it is probable that the functions of the kidney may be adversely affected.

It is recommended that further studies aimed at corroborating these findings be carried out.

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