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Morphological Effects of Chronic Consumption of Extracts of *Hibiscus sabdariffa* Linn Drinks on the Liver of Adult Wistar Rats

[1]*JO ADJENE, [2]J A AVBUNUDIOGBA, [1]CF AWOLO

[1]Department Of Anatomy, School Of Basic Medical Sciences University Of Benin, Edo State, Nigeria.

[2]Department of Pharmaceutics & Industrial Pharmacy, Faculty of Pharmacy, Delta State University, Abraka, Delta State, Nigeria.

*Author for Correspondence

ABSTRACT

The effects of chronic consumption of hot water extracts of the flower of *H. Sabdariffa* (zobo) drinks on the liver of adult wistar rats was 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 liver was carefully dissected out, dried, weighed, and quickly fixed in 10% formal saline for further routine histological study. The microanatomy (H & E) indicated that the liver in the test group showed some level of distortion and disruption of the cytoarchitecture with some marked congestion of blood at the central vein of the treated liver. There is an obvious reduction in the size of the hepatocytes and distortion of the collagen tissue of the treated liver as compared to the control group. The findings also indicated that there was a significant ($P < 0.05$) decrease in weights (g) of the test liver (group B) as compared to the control group. Chronic consumption of zobo drinks may therefore have an adverse effect on the liver of adult wistar rats. It is recommended that further studies aimed at corroborating these observations be carried out.

Key words: Morphological effects, Zobo drinks, Liver, Wistar Rats.

Among the more than three hundred species of Hibiscus is *Hibiscus sabdariffa Linn*, which has many medicinal uses (Gill 1992, Morton 1987). 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 coloured 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 posses 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, Stainner *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 (Jamaica in the South American and Karkade in Arabic). 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 liver is a large glandular organ located in the abdominal region. It is involved in the detoxification of most substances (Burkitt and Health, 1993). It is lined by endothelial cells that contain the kupffer cells, which are typical macrophages (Junqueira *et al* 1998). In addition to the endothelial cells, the sinusoids contain phagocytic cells known as the kuffer cells which are found in the surface of the endothelial cells (Junqueira *et al* 1998). In humans, the majority of drugs administered are eliminated by a combination of hepatic metabolism and renal excretion (Katzung 1998). Since the liver is susceptible to injuries particularly in situations of toxicity, it would therefore be worthwhile to examine the effects of long term consumption of zobo drinks on the liver of wistar rat to be able to make possible inferential statements regarding man. The purpose of this experiment is to evaluate the possible effects of long term consumption of zobo drinks on the morphology of the liver 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 were 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 cervical dislocation on the thirty-first day of the experiment. The abdominal region was quickly opened and the liver 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* 1976). 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 liver obtained from the control and test 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)

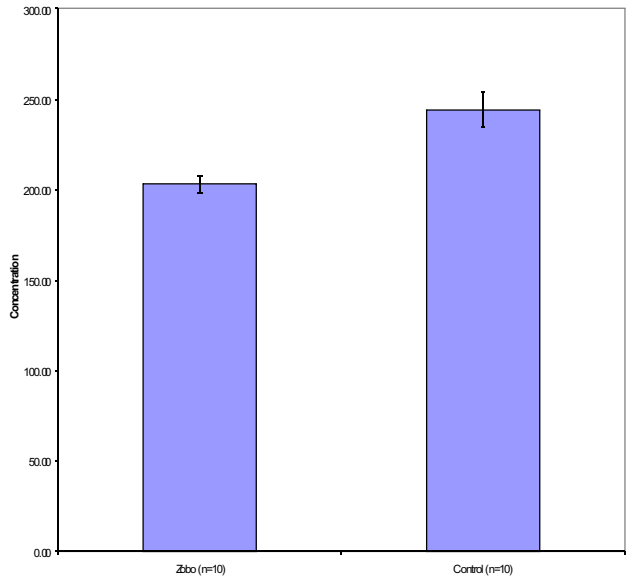
RESULTS

The findings indicated that the photomicrograph of the liver in the control group showed normal histological features. The section indicated normal hexagonal boundary of the hepatic lobules being defined by portal tract and sparse collagen fibres (Plate 1 & 3). The microanatomy (H & E) indicated

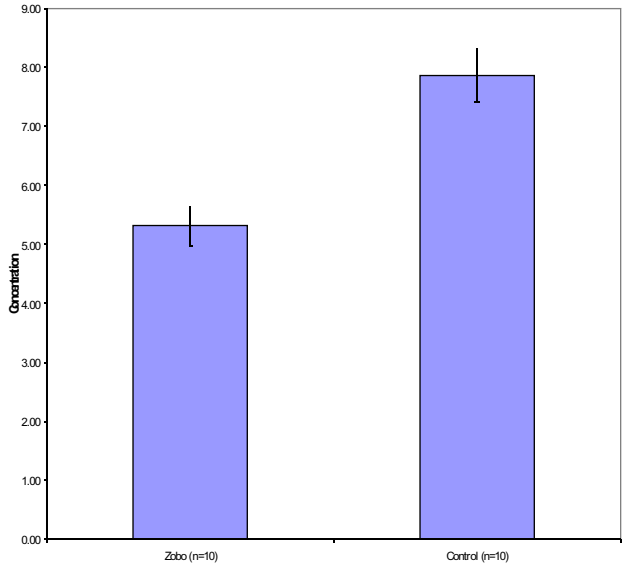
Table 1: the Mean Weight (g) of the Liver of the Animals

Parameters	Groups of Animals Group A Control (n=10)	Group B Test (n=10)
Body Wt. (G)	*244 9.39	*203 4.78
Liver Weight (g)	*7.87 0.46	*5.31 0.35

*significant ($P < 0.05$)
Values represent mean SEM



Values represent **mean** SEM
Fig. 1: Bar Chart Showing the Mean Body Weight (G) of the Animals



Values represent **mean** SEM
Fig. 2: Bar Chart Showing the Mean Liver Weight (G) of the Animals

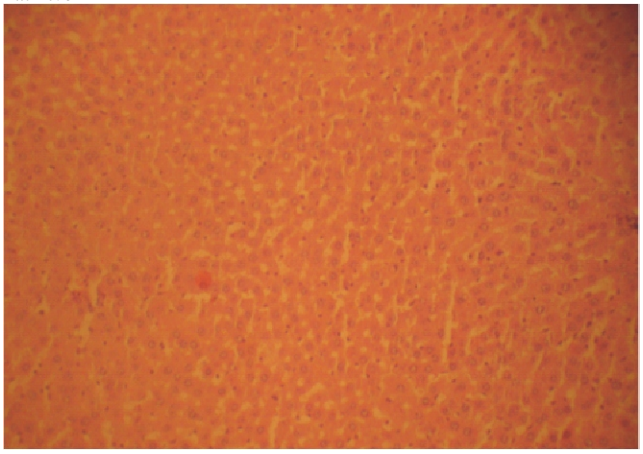


Figure 1: Control liver section x100

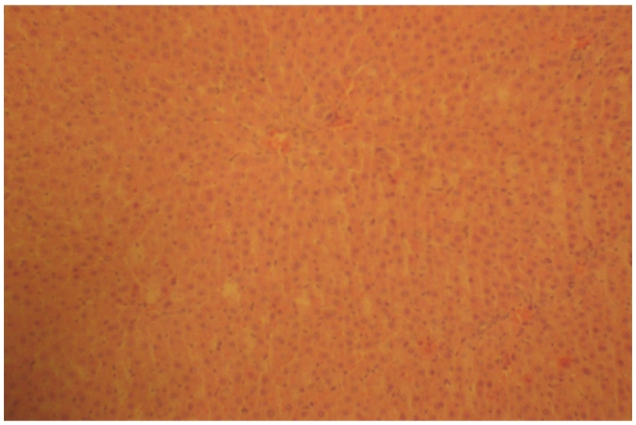


Figure 2: Tested liver section with Zobo drinks x100

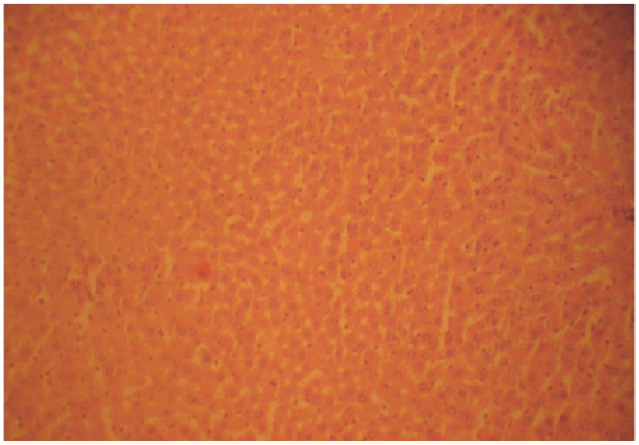


Figure 3: Control liver section x400

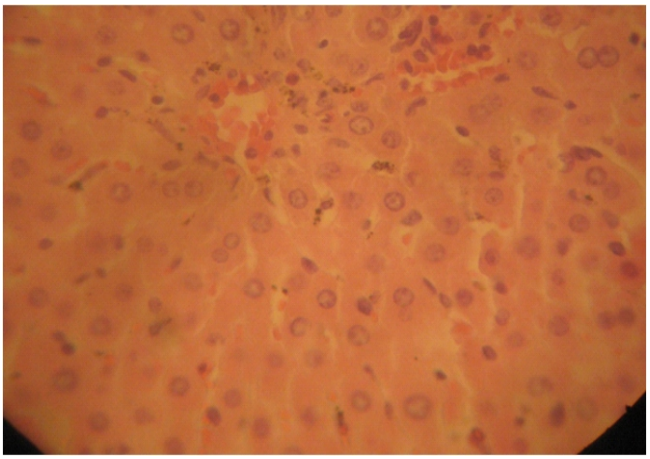


Figure 4: Tested liver section with Zobo drinks x400

that the liver in the test group showed some level of distortion and disruption of the cytoarchitecture with some marked congestion of blood at the central vein of the treated liver. There was an obvious reduction in the size of the hepatocytes and distortion of the collagen fibres of the treated liver as compared to the control group (Plate 1, 2, 3 & 4). The findings also indicated that there was a significant ($P < 0.05$) decrease in weights (g) of the test liver (group B) as compared to the control group (group A) (Table 1 Fig.1&2.).

DISCUSSION

The findings indicated that there was a significant ($P < 0.05$) decrease in weight (g) of the test liver as compared to the control group. The microanatomy (H & E) indicated that the liver in the test group showed some level of distortion and disruption of the cytoarchitectural structure with some marked congestion of blood at the central vein of the treated liver. There was an obvious reduction in the size of the hepatocytes of the treated liver 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 liver. It appeared that chronic consumption of zobo drinks is not as harmless as generally believed. The distortion and disruption of the cytoarchitecture of the liver 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 liver might be due to the cytotoxic effect of zobo drinks on the liver. 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 hyponatramia or hypernatremia (Johanson 1995). Ischemia or pharmacologic disruption of cellular transporters can cause swelling of parenchyma of any organ. The pharmacologic disruption of the liver weights caused by zobo drinks was 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 of the liver in this experiment usually involves intracellular swellings or shrinkage of the endothelia (Johanson 1995).

The obvious signs of distortion and disruption of the cytoarchitecture with some congestion of blood at the central vein of the tested liver observed in this experiment may have been due to the cytotoxic effects of zobo drinks on the liver. These findings implicated zobo drinks as a possible precipitant of liver disease by causing congestion of blood in the microanatomy of the liver. 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 liver. The result obtained in this experiment is in consonance with the work carried out by Enaibe *et al* (2007) where It was reported that administration of damiana (*Turnera diffusa*) to a matured wistar rats resulted in the distortion and disruption 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. In this experiment, zobo drinks may have acted as toxin to the cells of the liver thus resulting in the distortion and disruption of the cytoarchitecture of the liver with some marked congestion of blood at the central vein of the liver.

CONCLUSION

In conclusion, our findings indicated that chronic consumption of zobo drinks resulted in some level of distortion and disruption of the cytoarchitecture of the liver with some marked congestion of blood at the central vein of the treated liver. There is an obvious reduction in size of the hepatocytes and distortion of the collagen tissue of the treated liver as compared to the control group. The findings also indicated that there was a significant ($P < 0.05$) decrease in weights (g) of the test liver as compared to the control group. Chronic consumption of zobo drinks may therefore have an adverse effect on the liver of adult wistar rats. It is recommended that further studies aimed at corroborating these observations be carried out.

REFERENCES

Adjene JO**,** Arukwe FI (2009): Effects of chronic administration of efavirenz on the Brain and Inferior colliculus weights of adult wistar rats. *Rev Electron Biomed/ Electron J Biomed* **3**: 36-40.

Amin A, Buratovich M (2007): The Anti-cancer charm of flavonoids: A cup of-tea will do. Recent pat. Anti-cancer Drug Discovery. **2**(2): 109-117.

Burkitt HG, Health JW (1993): Wheater's Functional Histology(A Text and Colour Atlas),3rd Edition Churchill Livingstone (Publishers) London. 271-4

Drury RAB**,** Wallington EA, Cameron R (1976): Carleton's Histological Techniques: 4th ed., Oxford University Press NY. U.S.A. 279-280.

Duke J (1985): *Handbook of Medicinal Herbs*. Seventh edition, Livingstone Group Ltd , Edinburgh. Pp 228-229.

Enaibe BU, Adjene, JO, Eweka AO (2007): Histological studies of the effects of oral administration of Damiana (*Turnera diffusa*) on the Kidney of matured wistar Rats. *Int. J. Biomed. Hlth. Sci*. **3**(1):43-48

Farber JL**,** Chein KR, Mittnacht S (1981): The pathogenesis of irreversible cell Injury in ischemia; *American Journal of Pathology*. **102**:271-281

Gaxiane JM, Mansion JE, Hennekens CH (1994): Natural Antioxidants and cardiovascular disease observational epidemiologic studies and randomized trials. In B. frel (ed). Natural Antioxidants in Humans Heath and Disease. Academic press, San Diego. pp.387-409.

Gill LS (1992): Ethnomedical uses of plants in Nigeria. University of Benin Press, Benin City, Nigeria. Page132.

Harman D (1984): Free radical theory of ageing: The free radical disease, Age **7**: 111-131.

Ito U, Sparts M, Walker JR, Warzo I (1975): Experimental Cerebral Ischemia in Magolian Gerbils(1). Light microscope observations. *Acta. Neuropathology.* USA. **32**:209-223.

Jackson RL, Ku G, Thomas CE (1993): Antioxidants, a biological Defence mechanism for prevention of atherosclerosis. *Med. Res. Rev*. **13**: 161 162.

Johanson, C. E (1995): Effects of Fluid in Balances. Neurosciene in Medicine. P. Michael conn, J.B. Lippincott Company, Philadelphia. 187 189.

Junqueira L, Jose C, Kelly R (1998): Basic Histology. 9th edition Lange Medical Books,Mc Graw Hill Medical Publishing Division. USA, pp 309-22.

Katzung BG (1998): Basic and Clinical Pharmacology. 7th edition, Appleton and Lange, Stamford CT. pp 372-375

Martins LJ, Al-Abdulla NA, Kirsh JR, Sieber FE, Portera-Cailliau C (1978): Neurodegeneration in excitotoxicity, global cerebral ischaemia and target deprivation: A perspective on the contributions of apoptosis and necrosis. *Brain Res. Bull.* **46**(4): 281-309.

Morton JF (1987): Rosella in: Fruits of Warm Climates. Darling, GF (TD) Media Inc, Greenshore. pp 281-286.

Pietta PG (2000): Flavonoids as antioxidants. *J. Nat, Prod* **63** (7):1035,1042

Robinson T (1975): The Organic Constituents of Higher Plants. Their Chemistry and Interrelationship. 3rd edition. Cordus press, North Amherst. pp.190-220.

Stanner SA, Hughes J, Kelly CN, Butriss J (2004): A review of the epidemiological evidence for the antioxidant hypothesis. *Pub Health Nutr*. **7**(3): 407-422

Tseng TH, Kao ES, Chu FP, Lin Wa HW, Wang CJ (1997): Production effect of dried flower extracts of *Hibiscus sabdanffa linn* against oxidative stress in rat primary hepatocytes. *Food and Chem. Toxicol*. **35**(12):1159-1164.

Wang CJ, Wang J M, Lin W L, Chu CY, Chou F P, Tseng T H (2000): protective effect of Hibiscus anthocyanins against tort-butyl. Hydroporoxides induced hepatic toxicity in rats. *Food and Chem. Toxicol*. **38**:411-416.

Wolff SP, Garner RT, Dean RT (1986): Free Radicals, lipids and protein degradation. *Trends in Biochemical Sciences (TIBS)*. **11**:27-31.