Original Research Article

Comparative Study of the Effects of the Commonly-consumed Aqueous Extracts of *Hibiscus sabdariffa* (Zobo Drinks) on Hepatic and Renal Indices of Normal Wistar Albino Rats.

ABSTRACT

Aim: Investigating and comparing the effect of administration of different preparations of the commonly-consumed *Hibiscus sabdariffa* (Zobo) drinks on hepatic and renal indices.

Study design: Animal models (Wistar Albino Rats) with daily administration of the same concentration of different zobo drink samples.

Place and Duration of Study: University of Port Harcourt, Choba, Rivers State, Nigeria and its environs between November 2014 and February 2015.

Methodology: Thirty (30) Wistar albino rats were grouped into six (6) groups of five rats each. Group A served as the control and group B was administered an unblended zobo drink. Groups C – E were administered locally-produced zobo samples and group F was a National Agency for Food and Drug Administration and Control (NAFDAC)-branded zobo drink. A concentration of 200 mg/kg body weight of the samples was administered orally to the groups B – F for 21 days. Hepatic marker enzymes (ALT, AST and ALP), bilirubin (total and conjugated), albumin, total protein and renal indices were analyzed and compared

Results: The results indicate that the activities of ALT, ALP, Albumin and Unconjugated bilirubin were significantly lower *($P < 0.05$)*, while Total protein and Total bilirubin levels were significantly higher ($P < 0.05$) than the control (Group A). AST and Conjugated bilirubin showed no significant difference *($P > 0.05$)* in all the groups. The levels of Urea, Creatinine, Sodium (in group B), Potassium (K^+), and Chloride (Cl^-) were significantly higher *($P < 0.05$)* than the control. The Sodium (Na^+) level was significantly lower *($P < 0.05$)* than the control in groups C, D and E.

Conclusion: The results of this study suggest that all the samples were not hepatotoxic. However, the aqueous extract of Zobo may have nephrotoxic and hepatotoxic effects if the concentration is increased over time.

Comment [K1]: Authors should compare with normal value for rats. They could conclude nephrotoxic If the values are out of normal range.

Keywords: [Hibiscus sabdariffa; Zobo; Liver enzymes; Renal Indices; Hepatotoxic; Hepatoprotective; Nephrotoxic]

1. INTRODUCTION

Hibiscus sabdariffa belongs to the order of Malvales and the family of Malvaceae. It is believed to originate from East Africa [1]. *H. sabdariffa* plants are cultivated and consumed as vegetable and tea, whereas other *Hibiscus* varieties are planted for the fibres they produce. It is called different names like Roselle and Sorrel in English and it is locally called *zobo* and *Isapa* in Nigeria [2]. Various types of highly valued food and medicinal products are produced from parts of the *Hibiscus sabdariffa* including the seeds, leaves, fruits and roots. Among them, the fleshy red calyces are the most popular [3] as they are commonly used to prepare non-alcoholic drinks in form of beverage or juice.

The non-anthocyanin portions of the *Hibiscus sabdariffa* calyces also have hepatoprotective effects, as evidenced by a pigment free extract that is able to protect from acetominophen induced hepatotoxicity [4]. A study in persons with metabolic syndrome noted that 31 days of 100 mg Roselle extract (19 mg Anthocyanin Sambubiosides) was able to reduce serum ALT and AST, suggesting Roselle attenuates hepatic damage [5].

In Nigeria, the dried calyces of this plant are processed into a refreshing non-alcoholic local beverage commonly called *zobo, zoborodo* or Isapa (pronounced Isakpa) [6].

Zobo beverage is made from different varieties of calyces of the flower *Hibiscus sabdariffa* by boiling and filtration [7, 8]. *Zobo* drink, which is usually prepared with the calyces of *Hibiscus sabdariffa* is

believed to be highly nutritive and has many medical potentials including reduction in blood pressure, anti-diabetic, reduction in weight, antihyperlipidemic, hepatoprotective, anti-cancer, as well as an antioxidant e.t.c. [9, 10, 11, 12, 13].

Following increased religious and health awareness against consumption of alcoholic beverages in Nigeria and the consequent decrease in the consumption of alcoholic beverages in certain areas, Zobo drink has great potential as a local alternative to imported red wines in particular and alcoholic beverages in general [14]. Moreover, production of this and similar local beverages has become the main source of income in many homes in the rural communities and more recently in the urban areas where these have grown to cottage business proportions following governmental interventions through the poverty alleviation schemes, thereby alleviating poverty among the people [15].
Zobo is mostly consumed by low and middle class people due to its relative low cost, because the *Hibiscus sabdariffa* calyces and the ingredients are cheap and easy to get. It has recently gained wider acceptance, being consumed by several millions of people from different socio-economic classes and background in the West Africa sub-region and in Nigeria particularly.

Within the University of Port Harcourt and Choba community, the rate of consumption of Zobo drink by students and staff alike is enormous. This is reflective of the perception by many, that, zobo drink is highly nutritious, medicinal and of course, cheaper (following the prevailing economic downturn in Nigeria) than other non-alcoholic beverages sold around and within the environment.
However, many of the local producers of the drinks prepare the drinks under poor hygienic conditions, using different preparation methods and variants of synthetic flavours. Some of these synthetic flavours have been suspected of being toxic or carcinogenic and many have been banned whenever possible [16]. Some also believe that the consumption of zobo drink supplemented with flavour increased its antioxidant potentials and has no severe effect on the liver and kidney and thus, supplementation of *zobo* drinks with flavour additive maybe nutritionally beneficial [17].

Therefore, this study will be of importance in evaluating the effect of zobo drinks on hepatic and renal using normal Wistar albino rats. It will seek to find out and/or add to existing literature, whether or not, consumption of zobo drinks supplemented with synthetic flavours is beneficial to the body.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All chemicals and reagents used were of analytical grade. Alanine Transaminase (ALT) and Aspartate Transaminase (AST), Bilirubin, Total Protein and Albumin Test Kits by Randox Laboratories Ltd, Crumlin, England, UK were used.

2.2 Plant Material

Dried calyces of *Hibiscus sabdariffa* were bought from Choba market, Port Harcourt on 11th November, 2014. They were identified and authenticated in the Herbarium of the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt where voucher specimens with code number 2435 were deposited.

2.3 Samples and Preparations

The unblended zobo drink sample was prepared using the method described by [17]. The dry calyces of *Hibiscus sabdariffa* (HS) were carefully sorted to remove dirt and other unwanted materials. Sixty grams (60 g) of the dry HS calyces were washed with cold water and added to two litres of boiling distilled water. It was allowed to boil for 15 minutes and then cooled. After cooling, the mixture was sieved with muslin cloth and filtered with Whattman No. 1 filter paper. The clear filtrate was covered with aluminium foil and stored in the refrigerator at 4 ^{0}C until use.
The ZOBO COLA was also used for comparison. This is a commercially sold branded 400 mL Zobo drink produced by Zobo Cola Company Ltd, Nigeria, certified by National Agency for Food and Drug Administration and Control (NAFDAC) and sold in shops within the community. The ingredients used to prepare the Zobo Cola include: Purified water, HS extracts, Aspartame, Sugar, Cola flavour, Ginger and Citric acid. Other drink samples were obtained from shops around the community.

2.4 Experimental Animals

Thirty (30) Wistar Albino rats weighing between 110 – 195 g were used for the study. The animals were obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt, Choba, Rivers state. All the animals were housed in the animal house, University of Port Harcourt, Choba Campus – using plastic cages covered with wire gauze and given standard food pellets (Top Feeds' grower's mash) and water *ad libitum*. They were acclimatized for 2 weeks and marked for easy identification and monitoring, after their baseline weights were taken. All procedures and techniques in handling the animals were according to standard methods and complied with the guidelines of the National Institutes of Health of the United States [18].

2.5 Experimental Design and Administration of Samples

The acclimatized albino rats were sorted according to their weights into five groups of six rats each. Group A was fed the normal rat feed with water and served as the control. Groups B – F served as experimental groups and were administered 200 mg/kg body weight of the respective samples via oral intubation for a period of 21 days. Group B was administered the unblended zobo drink. Group F was administered the Zobo Cola, while groups C, D and E were administered with other drink samples obtained from shops around the community. All animals were allowed access to water and food for the 21 days.

2.6 Sacrificing of Animals and Collection of Blood Samples

All the animals from the groups were sacrificed at the end of the administration period. The animals were incapacitated with chloroform in a desiccator. Under this condition, the rats were dissected using dissecting tools and the blood was collected and put into lithium heparin and EDTA anticoagulant tubes (to prevent blood clotting) for biochemical analyses.

2.7 Determination of Biochemical Parameters

The plasma ALT and AST activities were determined by monitoring the concentration of pyruvate and oxaloacetate hydrazine respectively, formed with 2,4-dinitrophenylhydrazine according to [19] using Randox test kits. The plasma ALP activity was determined using the colorimetric end-point method according to Rec [20]. The plasma total and conjugated bilirubin concentration were determined using Randox Test Kits according to the Jendrassik and Grof [21] method. Total protein concentration was determined by Biuret Method using Randox Test Kits according to Tietz [22]. Albumin concentration was determined by the Bromocresol Green (BCG) Dye Binding Method by [23], using Randox Test Kits.

2.8 Determination of Renal Indices

The Urease-Berthelot method according to Fawcett and Scott [24] as provided by Randox diagnostics Ltd. UK was used. Creatinine was determined by the direct end method of [25]. Sodium concentration was determined using the method of Maruna [26] and Trinder [27]. Potassium and Chloride ions were determined using the Colorimetric method.

2.9 Statistical Analysis

All data obtained in this study were subjected to statistical analyses using One-way Analysis of Variance (ANOVA). Tukey's Multiple Range Test was used to test for differences between the administration groups. All analyses were done using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Statistics, UK). All the values were reported as means ± standard error of mean (SEM) and the results were considered significant at P-values of less than 0.05 ($P < 0.05$) i.e. at 95 % confidence level.

3. RESULTS AND DISCUSSION

Results of the comparative effect of different aqueous extracts of *Hibiscus sabdariffa* (Zobo drinks) on activities of liver marker enzymes (ALT, AST and ALP), Total protein (TP), Albumin (ALB), Total bilirubin, conjugated bilirubin and unconjugated bilirubin (TB, CB, UC respectively) are summarized in "Table 1". From the results, the plasma ALT level in groups B and C were lower than the control, though only group B was significantly lower *($P < 0.05$)* when compared with the control (Group A). Meanwhile, the ALT level in groups D, E and F was slightly higher but not significantly *($P > 0.05$)*

when compared with the control. Although the AST level was lower in groups B, C, E and F, they were not significantly different when compared with the control. The ALP level of groups B, C, D and E was significantly lower *(P < 0.05)* than the control, while there was no significant difference *(P > 0.05)* between group F and the control (Group A). The Total protein level of groups B, E and F was significantly higher *(P < 0.05)* than the control, while there was no significant difference *(P > 0.05)* in groups C and D when compared to the control (Group A). The Albumin level in groups C and D was significantly lower *(P < 0.05)* than the control and there was no significant difference *(P > 0.05)* in Groups B, E and F when compared with the control (Group A). The Total bilirubin level in groups C, D, E and F was significantly higher *(P < 0.05)* than the control, while it was almost significantly higher *(P > 0.05)* in group B when compared with the control (Group A). The level of Unconjugated bilirubin in groups D and F was significantly higher *(P < 0.05)* than the control, while it was also higher in groups B, C and E but not significantly *(P > 0.05)*. The levels of AST and conjugated bilirubin showed no significant difference *(P > 0.05)* when compared with the control (Group A).

Comment [K2]: The authors should not duplicate in writing the statistical result. They have presented in the table. Please compare with result from other research or explain the mechanism.

Table 1. Effect of 21 days administration of different zobo drinks on biochemical parameters of wistar albino rats

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/L)	ALB (g/L)	TB (µmol/L)	CB (µmol/L)	UB* (µmol/L)
A (CNTRL)	14.6 ± 0.87^{a}	23.0 ± 0.32^{a}	67.6 ± 0.68^{a}	69.6 ± 1.50^{a}	36.0 ± 0.63^{a}	5.66 ± 0.36^{a}	2.40 ± 0.63^{a}	3.26 ± 0.32^{a}
B (ZSTD)	8.0 ± 0.63^{b}	17.8 ± 0.58^{b}	56.0 ± 1.48^{a}	81.0 ± 1.00^{b}	37.2 ± 0.37^{a}	6.74 ± 0.05^{a}	2.24 ± 0.07^{a}	4.50 ± 0.10^{a}
C (ZAP1)	10.0 ± 0.89^{ab}	19.6 ± 1.57^{a}	43.0 ± 2.55^{a}	65.6 ± 1.47^{a}	31.8 ± 1.02^{b}	7.40 ± 0.07^{b}	3.80 ± 0.47^{a}	3.60 ± 0.43^{a}
D (ZAP2)	18.6 ± 2.27^{b}	25.2 ± 0.66^{b}	58.6 ± 2.16^{a}	73.6 ± 2.06^{a}	32.0 ± 1.41^{b}	9.00 ± 0.40^{b}	2.40 ± 0.03^{a}	5.60 ± 0.14^{b}
E (ZCHO)	14.8 ± 1.16^{a}	19.6 ± 1.21^{b}	30.6 ± 0.40^{a}	79.0 ± 1.00^{b}	36.8 ± 0.73^{a}	6.76 ± 1.02^{b}	4.86 ± 0.85^{a}	5.10 ± 0.70^{a}
F (ZCOLA)	15.4 ± 0.93^{a}	22.4 ± 2.79^{a}	61.0 ± 0.71^{b}	77.0 ± 0.37^{b}	34.2 ± 0.37^{a}	9.46 ± 0.57^{b}	2.36 ± 0.05^{a}	7.10 ± 0.54^{b}

Values in the table are means ± Standard error of mean (SEM) and n = 5. At (P < 0.05), means with different superscripts in a column are significantly different. ALT = Alanine transaminase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatise; TP = Total protein; ALB = Albumin; TC = Total bilirubin; CB = Conjugated Bilirubin; UC = Unconjugated bilirubin. * = UB values were calculated. **ZSTD** = Unblended Zobo drink; **ZAP1** = Zobo drink sold within Abuja Campus Park

(UNIPORT); **ZAP2** = Zobo drink sold outside Abuja Campus Park; **ZCHO** = Zobo drink sold in Choba; **ZCOLA** = Branded Zobo drink (Zobo Cola®)

To ascertain the state of living cells, AST, ALT and ALP are basic biochemical markers that are usually measured. These cytosolic enzymes are usually more concentrated in some organs like the liver, heart and in other parts of the body. These enzymes usually leak out of damaged tissues into the plasma, leading to their high level in the plasma. Such elevation of the enzymes in the blood is therefore an indication of tissue damage and altered membrane permeability [28]. Therefore, the results suggest that all the samples were not hepatotoxic. This is in line with Tseng *et al* [29], which reported a hepatoprotective effect of extracts of *Hibiscus sabdariffa*. It is worthy of note that group D showed higher levels of ALT and AST, though not significant. This is suggestive that if the concentration of the sample is increased over time, it may cause a physiological dysfunction or damage to the hepatocytes. The non-significant changes in the levels of ALT, AST and ALP suggest that the liver functions were not impaired in those groups. Total protein is a rough measure of all the proteins found in the fluid portion of the blood, specifically looking at the total albumin and globulin [30]. Total protein may be required to get an overview information about nutritional status like when someone has undergone a recent weight loss and also to provide information (alongside other tests) if there is any symptom that suggests a liver disorder. An increase in total protein is usually the result of tissue damage [31]. Therefore, the significant higher levels of total protein in groups B, E and F suggests that those drink samples may be hepatotoxic and also, the excretion of protein by the kidney may be impaired. Meanwhile, the albumin (a major component of total protein) level in groups C and D were significantly lower than – suggesting liver function was not impaired in those groups. The elevated levels of total bilirubin (in groups C, D, E and F) and unconjugated bilirubin (in groups D and F) is an indication that there may hemolytic anemia, internal hemorrhage, reduced hepatocyte uptake, impaired conjugation of bilirubin, reduced hepatocyte secretion of bilirubin or obstruction of the bile ducts in those groups.

Results of the Renal Function Test (Urea, Creatinine, Potassium, Sodium, Bicarbonate and Chloride) of groups A – F are summarized in "Table 2". From the results, the level of Urea in groups B, D and E was significantly higher *($P < 0.05$)* than the control. The level of Urea in group F was significantly lower *($P < 0.05$)* than the control, while there was no significant difference *($P > 0.05$)* in group C when compared with the control (Group A). The Creatinine level in groups B, D, E and F was significantly higher *($P < 0.05$)* than the control, while the increase in group C was not significant *($P > 0.05$)* when compared with the control. The Potassium level in group F was significantly higher *($P < 0.05$)*, while there was no significant difference *($P > 0.05$)* in the groups B, C, D and E when compared with the control. The Sodium level in group B was significantly higher *($P < 0.05$)*, while the level in groups C, D and F was significantly lower than the control. There was no significant difference *($P > 0.05$)* in group E when compared with the control. The chloride level was higher in all the groups (B – F), but this was only significant *($P < 0.05$)* in Groups E and F when compared with the control. There was no significant difference *($P > 0.05$)* in bicarbonate (HCO^-_3) level of all the groups.

Comment [K3]: It should be deleted. It doesn't need to duplicate. The result already presented in the table. Please compare with normal value of rat to conclude the toxicity

Table 2: Effect of 21 days administration of different zobo drinks on renal indices of wistar albino rats

GROUP	Urea	Creatinine	K^+ (mmol/L)	Na^+	HCO_3^-	Cl^-
A (CNTRL)	6.04 ± 0.70 [a]	330.00 ± 0.71 [a]	4.22 ± 0.23 [a]	60.0 ± 0.71 [a]	25.00 ± 1.48 [a]	73.60 ± 7.58 [a]
B (ZSTD)	6.70 ± 0.08 [b]	397.00 ± 5.64 [b]	3.95 ± 0.14 [a]	75.60 ± 3.37 [b]	26.00 ± 0.32 [a]	87.00 ± 1.10 [a]
C (ZAP1)	6.30 ± 0.17 [a]	336.60 ± 11.42 [a]	4.45 ± 0.09 [a]	10.00 ± 0.32 [c]	28.00 ± 0.71 [a]	87.20 ± 1.20 [a]

D (ZAP2)	6.75 ± 0.11 [b]	418.60 ± 11.03[b]	4.15 ± 0.06 [a]	10.00 ± 0.32 [c]	27.00 ± 1.00 [a]	87.80 ± 1.39 [a]
E (ZCHO)	7.65 ± 0.13 [b]	434.60 ± 5.45 [b]	4.10 ± 0.11 [a]	56.00 ± 10.07 [a]	27.80 ± 0.20 [a]	92.60 ± 2.73 [b]
F (ZCOLA)	4.80 ± 0.04[c]	382.60 ± 2.60 [b]	4.80 ± 0.03 [b]	10.20 ± 0.20 [c]	24.40 ± 0.24 [a]	91.20 ± 0.58[b]

Values in the table are means ± Standard error of mean (SEM) and n = 5. At ($P < 0.05$), means with different superscripts in a column are significantly different.

K^+ = Potassium ion; Na^+ = Sodium ion; HCO_3^- = Bicarbonate ion

The higher levels of renal indices in the groups (B, D, E and F) suggest that the zobo drinks may have nephrotoxic effects (kidney impairment) on the experimental animals. The higher levels of sodium, potassium and creatinine, and no significant difference in bicarbonate, are in line with [32], which obtained the same trend in those renal indices. The likely kidney impairment is in line with Ogundapo [17], which implied that nephrotoxic effects of zobo drinks supplemented with commercial flavour additives may have been due to the presence of sodium cyclamate present in the flavour, which causes kidney damage and other effects [33]. The high levels of sodium in group B, chloride in groups A – F and potassium in group F is in line the study carried out by [34] on the diuretic property of *Hibiscus sabdariffa*. The study observed that rats that consumed aqueous *Hibiscus sabdariffa* extract had elevated plasma sodium, chloride and potassium. Aqueous extract of *Hibiscus sabdariffa* had also been shown in another study to be rich in Na^+ [35]. By implication, the consumption of *Hibiscus sabdariffa* by the rats may have caused an increase in the oral Na^+ load which consequently increased plasma Na^+. A toxic insult to the kidney therefore, could have profound effect on total body metabolism [36]. This is because; the proper functioning of the kidney ensures proper excretion of metabolic wastes, maintenance of acid-base balance, electrolyte composition and regulation of intracellular fluid volume [37].

4. CONCLUSION

This study aimed at investigating and comparing the effects of 21 days administration of different preparations of *Hibiscus Sabdariffa* (Zobo) drink consumed around the University of Port Harcourt community hepatic and renal indices using normal albino rats. All the samples of zobo drink were not hepatotoxic. The non-significant changes in the levels of ALT, AST and ALP suggest that the liver functions were not impaired in those groups.
The higher levels of renal suggest that the zobo drinks had nephrotoxic effects (kidney impairment) on the experimental animals.
Despite the enormous nutritive, health and medical potentials of the aqueous extract of *Hibiscus sabdariffa* (Zobo drink), this study showed that the drink may have has nephrotoxic properties. Dose and time-dependent studies of the effects of different samples of zobo drink on biochemical and renal indices is strongly recommended. Also, histological analysis should be done on the vital organs of animals administered with Zobo drinks.

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ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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