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Comparative Investigation of the Effects of the Commonly-consumed Aqueous Extracts of *Hibiscus sabdariffa* (Zobo Drinks) on Body Weight, Glucose Level and Lipid Profile Using Normal Wistar Albino Rats

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Authors' contributions

This study was carried out in collaboration between all authors. Authors CCN and AJO designed the study. Authors CCN and ICC wrote the protocol. Authors AJO and ICC supervised the work. Author CCN carried out all laboratories work and performed the statistical analysis. Author CCN wrote the first draft of the manuscript and managed the literature searches. Author AJO edited the manuscript. All authors read and approved the final manuscript

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ABSTRACT

Aim: Investigating and comparing the effect of administration of different preparations of the commonly-consumed *Hibiscus sabdariffa* (Zobo) drinks on body weight, glucose level and lipid profile.
Study Design: Animal models (Wistar Albino Rats) with daily administration of the same concentration of different zobo drink samples.

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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 B was administered an unblended zobo drink. Groups C – E were administered locally produced zobo samples and 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 all the groups for 21 days. Changes in body weight, glucose level and lipid profile were analyzed and compared
Results: The percentage weight gains in groups B, C, E and F (4.93%, 6.68%, 0.55%, and 9.99%) were very low compared with the control (23.75%). The glucose level was significantly lower ($P < 0.05$) in groups B and C, significantly higher in group F and showed no significant difference in D and E when compared with the control. Total cholesterol level was significantly lower ($P < 0.05$) in groups C, E and F, while High density lipoprotein level was higher in all the groups but only significantly ($P < 0.05$) in group D when compared with the control. Triglyceride, Low density lipoprotein and Very low density lipoprotein levels showed no significant difference when compared with the control.
Conclusion: The results of this study suggest that the aqueous extract of different samples of *Hibiscus sabdariffa* (Zobo drink) possesses anti-obesity, hypoglycemic and hypolipidemic properties.

Keywords: Hibiscus sabdariffa; zobo; body weight; glucose level; lipid profile.

1. INTRODUCTION

Hibiscus sabdariffa Linn, a tropical plant, belongs to the super order 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 z*obo* 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].

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) [4].

Zobo beverage is made from different varieties of calyces of the flower *Hibiscus sabdariffa* by boiling and filtration [5,6]. Zobo drink being made with part of a plant 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. [7-11].

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 [12]. 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 [13].

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 [14]. 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 [15].

Therefore, this study will be of importance in elucidating the effect of zobo drinks on normal health indices and functions vis-à-vis body weight, glucose level and lipid profile, 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

Cholesterol Test Kits and High Density Lipoprotein (HDL) Test Kits by Randox Laboratories Ltd, Crumlin, England, UK were used.

2.2 Plant Material

Dried calyces of the plant were bought from Choba market, Port Harcourt. They were authenticated in the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt as *Hibiscus sabdariffa.*

2.3 Samples and Preparations

The unblended zobo drink sample was prepared using the method of Ogundapo et al*.* [15]. 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℃ until use.

The ZOBO COLA was also used for comparison. This is a commercially sold branded 40 cl 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. ZAP1 (Zobo drink sold within Abuja Campus Park). This was prepared using the following ingredients: Dried HS calyces, tap water, ginger, zobo pepper and flavourings. The flavouring used was Joccy® Pineapple flavour with NAFDAC No. A1-2269, manufactured by Kaadan Nigeria Ltd, Kano. ZAP2 (another Zobo drink sold around Abuja Park by another local producer) and ZCHO (Zobo drink sold in one of the shops in Choba campus) were also used.

The local producers use almost the same method and ingredients as stated above. The differences are either with the proportions of the ingredients used and the particular flavouring (particularly pineapple, orange and cola flavours).

Some Zobo drink products used the flavour with the following ingredients: citric acid, sweeteners (Aspartame, sodium cyclamate), sugar, tatrazine E102, sunset yellow E110, approved flavouring agents, Vitamin C, anti-caking agents (tricalcium phosphate) and phenylalanine. According to the manufacturer, 5g of the flavor should be used for 2 litres of the drink.

2.4 Determination of Concentration of Administered Samples

The concentrations of the Zobo extract in the drinks were determined by evaporating the drinks to dryness using hot air oven.

Five hundred (500) ml of each sample (unblended) was added to pre-weighed bottles and subjected to evaporation in the hot air oven. After evaporation, the samples were re-weighed and the differences reflected the concentration of the *Hibiscus sabdariffa* extracts in the drinks. The intended concentration to be administered was 200 mg/kg body weight.

2.5 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 [16] of the United States.

2.6 Experimental Design and Administration of Samples

The acclimatized albino rats were sorted according to their weights into six groups of five 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.

2.7 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 for analyses.

2.8 Determination of Body Weight

Prior to the administration of the samples after acclimatization, the animals were weighed to get the initial weights (day 0) using an electronic weighing balance. Subsequently, weekly weights were determined at days 7, 14 and 21 using Golden-Mettler electronic balance.

2.9 Determination of Blood Glucose Level

Glucose level was determined using an electronic glucometer (One touch Ultra easy Blood glucometer, Life scan, USA) with glucose strips.

2.10 Estimation of Plasma Lipid Profile

The Plasma triglyceride (TG) concentration was enzymatically assayed using spectrophotometric method as reported by Ochei and Kolhatkar, 2006 [17]. Plasma Total Cholesterol (TC) and High density lipoprotein cholesterol (HDL) were assayed enzymatically using RANDOX Cholesterol and High Density Lipoprotein commercial Test Kits [18]. Low density lipoprotein cholesterol (LDL) and Very low density lipoprotein (VLDL) were calculated using the Friedelwald equation [19].

2.11 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

The result in changes in body weight of the animals during administration of the different *zobo* samples showed a mixed trend (Table 1). At week 1, the control group showed the highest weight gain (6.9%) compared to other groups. Groups C, D and F showed increases in weight (3.6%, 5.4% and 1.5%), but these were lower than the control. Groups B and E showed decreases in weight (1.1% and 3.3% respectively). At week 2, there were increases in weight of all the groups, but the least increase was observed in group B (1.7%) compared to the control which had an 8.2% increase. In addition, groups D, E and F showed higher increases (11.4%, 10.7% and 9.4% respectively) when compared with the control. At week 3, 13.4% increase in weight was observed in the control group more than all the other groups. Meanwhile, there was a reduction in weight (6.0%) of group E and no change in weight in group F. After three weeks of administration, the control (group A) had a 23.75% increase in weight, while groups B, C, D, E and F increased in weight by 4.93%,

Table 1. Changes in body weight of the animals during administration of the different zobo samples after 1, 2 and 3 weeks

Week	Group/Day	Initial weight (g)	Final weight (g)	% Weight gain/loss
	Control	136.8 ± 2.82	146.2 ± 4.50	6.9%
	ZSTD	166.2 ± 2.87	164.4 ± 4.60	-1.1%
Week 1	ZAP1	150.8 ± 7.86	156.2 ± 8.05	3.6%
	ZAP2	129.6 ± 6.96	136.6 ± 7.59	5.4%
	ZCHO	180.2 ± 4.60	174.2 ± 5.33	-3.3
	ZCOLA	142.4 ± 4.57	144.6 ± 4.00	1.5
	Control	146.2 ± 4.50	158.2 ± 5.02	8.2
	ZSTD	164.4 ± 4.60	167.2 ± 6.04	1.7
Week 2	ZAP1	156.2 ± 8.05	166.4 ± 8.48	6.5
	ZAP2	136.6 ± 7.59	152.2 ± 10.8	11.4
	ZCHO	174.2 ± 5.33	192.8 ± 5.21	10.7
	ZCOLA	144.6 ± 4.00	158.2 ± 6.94	9.4
	Control	158.2 ± 5.02	179.4 ± 7.33	13.4
	ZSTD	167.2 ± 6.04	174.4 ± 7.51	4.3
Week 3	ZAP1	166.4 ± 8.48	161.6 ± 7.33	2.9
	ZAP2	152.2 ± 10.8	161.0 ± 12.6	5.7
	ZCHO	192.8 ± 5.21	181.2 ± 6.44	-6.0
	ZCOLA	158.2 ± 6.94	158.2 ±7.82	0
	Control	136.8 ± 2.82	179.4 ± 7.33	23.75
	ZSTD	166.2 ± 2.87	174.4 ± 7.51	4.93
After 3	ZAP1	150.8 ± 7.86	161.6 ± 7.33	6.68
weeks	ZAP2	129.6 ± 6.96	161.0 ± 12.6	19.50
	ZCHO	180.2 ± 4.60	181.2 ± 6.44	0.55
	ZCOLA	142.4 ± 4.57	158.2 ±7.82	9.99

Values in the table are means ± Standard error of mean (SEM) and n = 5. 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®)

6.68%, 19.50%, 0.55% and 9.99% respectively. The results therefore, show that the zobo drinks possess anti-obesity properties as reported by earlier studies [19,20], which observed a drastic loss of weight among animals treated with various concentrations of *H. sabdariffa* extracts. Carvajal-Zarrabai et al. [21] opined that the weight decreases might be as a result of dietary palatability problem when H. sabdariffa concentration was increased. Orisakwe et al. [22] reported loss of appetite in treated animal models due to daily administration of *H. sabdariffa* extracts as a cause for weight loss. However, from this study, groups B and E showed the least increases in weight. This is in line with the palatability problem reported by Carvajal-Zarrabal et al. [21]. The trend of the results suggests that the zobo samples possess anti-obesity properties, which may be of greater effect if the concentration is increased and/or the period of consumption prolonged.

The result of the blood glucose level is shown in (Table 2). The blood glucose in groups B and C (5.39±0.06 and 5.56±0.06) were significantly lower (P<0.05) than the control (6.57±0.03). The blood glucose in group F (7.29±0.28) was however, significantly higher (P < 0.05) than the control (Group A). The blood glucose level of groups D and E (6.01±0.16 and 6.77±0.28) was not significantly affected (P > 0.05). This result suggests that the zobo drink possesses hypoglycaemic property. This is in line with the report of Farombi and Ige [9] and Ajagbonna and Adebayo [23] that, HS extract reduces blood glucose level. In another report by Ademiluyi and Oboh [24], it was shown that HS appears to inhibit the alpha-glucosidase enzyme. This enzyme breaks down starch and disaccharides to glucose by acting on the 1, 4-alpha bonds. Inhibition of this enzyme would cause a subsequent decrease in blood glucose levels [25]. The significantly higher level (P < 0.05) of glucose in group F (Zobo Cola®) may be due to the sugar used for the preparation of the drink.

The result of the lipid profile is shown in (Table 3). From the results of the lipid profile, the Total cholesterol level in all the groups was lower than the control (2.00±0.14), showing significant

Table 2. Effect of administration of different zobo drinks on glucose levels of wistar albino rats

Group	GLU (mmol/L)
ZSTD	6.57 ± 0.03 [a]
ZAP1	5.39 ± 0.06 [b]
ZAP2	5.56 ± 0.06 [b]
ZCHO	6.01 ± 0.16 [a]
ZCOLA	6.77 ± 0.11 [a]
ZSTD	7.29 ± 0.28 [c]

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 when compared with controlled. GLU = Glucose

Table 3. Effect of administered different zobo drinks on the serum lipid profile of wistar albino rats for 21 days

Group	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)
ZSTD	2.00 ± 0.14[a]	1.40 ± 0.23 [a]	1.05 ± 0.08 [a]	0.32 ± 0.13 [a]	0.67 ± 0.12 [a]
ZAP1	1.70 ± 0.10 [a]	1.04 ± 0.10 [a]	1.20 ± 0.07 [a]	0.15 ± 0.10 [a]	0.35 ± 0.07 [a]
ZAP2	1.30 ± 0.08 [b]	1.15 ± 0.02 [a]	0.95 ± 0.05 [a]	0.04 ± 0.02 [a]	0.39 ± 0.09 [a]
ZCHO	1.90 ± 0.10 [a]	1.40 ± 0.06 [a]	1.45 ± 0.04 [b]	0.04 ± 0.02 [a]	0.41 ± 0.12 [a]
ZCOLA	1.31 ± 0.12 [b]	0.96 ± 0.05 [a]	1.20 ± 0.09 [a]	0.06± 0.04 [a]	0.27 ± 0.11 [a]
ZSTD	1.50 ± 0.07 [b]	1.00 ± 0.14 [a]	1.06 ± 0.10 [a]	0.09 ± 0.03 [a]	0.47 ± 0.07 [a]

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 when compared with the control. TC = Total Cholesterol; TG = Triglycerides; HDL = High density lipoprotein; LDL = Low density lipoprotein cholesterol; VLDL = Very low density lipoprotein cholesterol

difference (P < 0.05) in groups C, E and F (1.30±0.08, 1.31±0.12 and 1.50±0.07). The High density lipoprotein cholesterol (HDL-C) level in all groups A – F was higher than the control (1.05±0.08), showing statistical significance (P < 0.05) in group D (1.45±0.04). The Triglyceride, Low density lipoprotein cholesterol (LDL-C) and Very low density lipoprotein (VLDL) levels in all the groups were lower than the control (0.67 ± 0.12).

In this study, the lower level of total cholesterol, triglycerides, LDL-C and VLDL in all the groups agrees with several earlier reports that the aqueous extract of *Hibiscus sabdariffa* possesses a hypolipidemic effect. In vitro experiments attribute lipid-lowering action to inhibition of low-density lipoprotein (LDL) oxidation. Possibly, these effects are related to suppression of hepatic fatty acid synthesis and burning of fats [26-29,7-10]. This hypocholesterolemic effect has been attributed to the abundant antioxidant composition of Hibiscus sabdariffa especially anthocyanins. An elevated level of plasma total cholesterol is a risk factor of cardiovascular problems. Therefore, the ability of all the drink samples to reduce total cholesterol, triglyceride and LDL levels (bad cholesterol) and then increase high density lipoprotein HDL (good cholesterol) level is indicative of them being cardio-protective. According to different authors, it has been suggested that Zobo drink possess health and medical potentials. Therefore, it is pertinent that further studies be carried out to ascertain the safety and toxicity levels in order to serve as guide to producers and potential consumers.

4. CONCLUSION

The result shows that Zobo drink possesses anti-obesity properties. Group D had the least effect in terms of weight control, while group E had the highest effect. Unblended zobo drink has better anti-obese properties than those blended with flavours. Also, Zobo drink possesses hypoglycaemic property – causing a decrease in blood glucose levels. The flavours used in Zobo Cola may have increased the glucose level of animals. The lower levels of total cholesterol, triglycerides, LDL and VLDL in all the groups agree with several earlier reports that the aqueous extract of *Hibiscus sabdariffa* possesses a hypolipidemic effect.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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