ISSN 0974 – 5211

Journal of Natural Products

Volume 7 (2014)

www.JournalofNaturalProducts.Com

Research Paper

Alteration in biochemical parameters of albino Rats treated with aqueous extract of *Hibiscus sabdariffa* calyces (*zobo*) supplemented with commercial flavor additive

S. S. Ogundapo*, J. C. Onuoha, C. N. Olekanma, A. B. Okon, O. T. Soniran, D. A. Omoboyowa, D. A. Okoro

Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Uwana Afikpo Ebonyi State, Nigeria.

*Corresponding Author

(Received 16 February 2014; Revised 22 February - 12 June 2014; Accepted 20 June 2014)

ABSTRACT

Hepatic, renal, non-enzymatic and enzymatic antioxidant parameters were evaluated in rats treated with aqueous extract of *Hibiscus sabdarifa* calyces (*Zobo*) supplemented with a commercial flavor additive. Thirty two (32) albino rats randomized into eight groups of four (4) rats each were treated with aqueous extract of *Hibiscus sabdariffa* and distilled water supplemented with Joccy® flavor additive for seven (7) days. Groups B, C and D were treated with aqueous extracts containing 16.67, 33.34 and 50.01mg/kg body weight additive respectively while groups E, F, and G when treated with similar graded concentration of flavor dissolve in distilled water. Groups A and H treated with distilled water and aqueous extract only serve as negative and positive controls respectively. Results showed that serum ALT activity was elevated but not significantly (P>0.05) in the rats treated with *zobo* and Joccy (50.01mg/kg b.w) compared with the positive and negative controls. AST activity dose dependently decreased in rats treated with *zobo* and Joccy (16.67, 33.34 and 50.01mg/kg b.w) compared with rats administered water only while plasma urea was significantly (P<0.05) reduced in rats administered with *zobo* and Joccy (16.67, 33.34 and 50.01mg/kg b.w) compared with rats treated with *zobo* only. Plasma creatinine and glutathione decreased though not significantly (P>0.05) in the treatment group compared with the controls. Mean plasma vitamin C and β-carotene were elevated though not significantly in rats treated with *zobo* and Joccy (50.01mg/kg b.w) compared with the controls. Mean plasma Glutathione peroxidase, SOD and catalase activities were decreased though not significantly (P>0.05) in *zobo* and Joccy groups (16.67, 33.34 and 50.01mg/kg b.w) when compared with the controls. The findings of this study suggest that supplementation of aqueous extracts of *Hibiscus sabdariffa* calyces (*zobo*) with commercial additive Joccy® potentiates its antioxidant and nephro-protective properties.

Keywords: *Zobo*; Kidney; Liver; Antioxidants; Flavour.

INTRODUCTION

Abject poverty in some parts of the world particularly the developing nations has led to dependence on available plants whose many importance to man cannot be overemphasized.

The consumption of alcoholic beverages could also be on the decrease in certain areas due to increased religious and health campaigns against such beverages. This has made *zobo* drink a potential, ready local alternative to alcoholic beverages in particular and imported red wines in general (Mohammed, 2009).

According to Bola and Aboaba, (2004) *zobo* is the aqueous extract of the dried reddish-brown petals (calyces) of *Hibiscus sabdariffa*. The plant which is commonly known as roselle is native to India and Malaysia where it is commonly cultivated and is now found in many tropical countries of both hemispheres. It is a dicotyledonous plant belonging to the family, malveceae (Onoja, 1996; Bola, and Aboaba, 2004). In Nigeria, it is grown commonly in the middle belt regions like Plateau, Nasarawa and Benue states and south western states like Ondo and Osun. Phytochemical analysis of extracts of roselle showed that it contains anthraquinones, glycosides, alkaloids, tannins, polyphenols and saponins (Onoja, 1996; Bola and Abaoba, 2004). The plant has been claimed to posses antihypertensive, antiseptic, astringent diuretic and puragative activities, remedy for cancer, abscesses, cough, debility, dysuria, laxative, scurvy and fever (Onoja, 1996). Depending on individual preferences, the extract is normally sweetened to taste with sugar and sometimes flavoured with spices like ginger hot pepper alongside natural flavourings such as pineapple juice, lime juice or artificial flavourings like strawberry vanilla etc (Bola and Abaoba, 2004).

Artificial flavours are synthetic products that are added to food in order to change or to augment the natural flavour of the food (Zeolite, 2008). The great bulk of artificial flavourings used in non-alcoholic beverages are synthetic dyes. For decades, synthetic food dyes have been suspected of being toxic or carcinogenic and many have been banned whenever possible (Hirschbrush and Toores, 1998). Artificial flavourings can be found in most processed and packaged food. Even the aqueous extract of the dried calyces of *Hibiscus sabdariffa* (*zobo*) consumed by millions of people from different socio-economic classes and background in West African sub region is sweetened to taste with artificial flavours. These are now so common in our environment that we are not always aware just how much we depend on them. However, information is rife in the literature on effects of commercially branded flavours on biochemical parameters in animal models. Thus information on the possible effects of artificial flavours especially those commercially available as combination of several additives like Joccy flavour® on kidney, liver, enzymatic and non-enzymatic antioxidants parameters will shed some light on the safety or otherwise of the use of commercial flavouring. Findings from this study may also stimulate further scientific enquiries aimed at investigating biochemical effects of different combinations of additives with a view of reducing the side effects or toxicity of these flavoring agents when used to flavour beverages.

MATERIALS AND METHODS

The chemicals used for this study were of analytical grade in addition to the RANDOX, UK commercial assay kits which were used for the determination of ALT, AST, Urea, Creatinine, GPX, SOD and CAT assays.

Plant material: The dried calyces of *Hibiscus sabdariffa* were randomly purchased from traders in Eke market Afikpo North Local Government area in Ebonyi State of Nigeria and identified in the botany unit Department of Science Laboratory Technology (SLT), Akanu Ibiam Federal Polytecthnic, Unwana, Afikpo Nigeria.

***Animals*:** Thirty-Two (32) in-bred male Swiss albino rats weighing between 150g and 200g obtained from animal house of veterinary medicine, University of Nigeria, Nsukka were used for this study. The animals were kept in well ventilated rodent cubicles under 12 hours light/dark cycles and fed with animal mash (Top feeds, Nigeria) and water *ad libitum.* The caring and experimental uses of the mice were according to the guidelines of national institute of health guidelines for care of laboratory animals (Pub No. 85-23 revised 1985). The animals were acclimatized for 14 days before they were randomized into 8 experimental groups consisting of four (4) rats each.

***Commercial flavour additive*:** Joccy® (Kaadan, Nigeria) the pineapple flavour additive commercially available in most cities of Nigeria was the flavour considered for this study. The ingredients from the pack include: citric acid, sweeteners, Aspartate, Sodium cyclamate, Sugar, Tatrazine E102, Sunset yellow E110, approved flavouring agents, Vitamin C, Anti-caking Agent, (Tricalcium Phosphate) and phenylalanine.

***Experimental design*:** A total of 32 albino rats used for this study were divided into four groups of four animals each (n = 4):

- **GROUP A**: Treated with water only (control).
- **GROUPS B, C,** and **D**: Treated with graded concentrations of Joccy® pineapple artificial flavour dissolved in aqueous *H. sabdariffa e*xtract (*zobo).*
- **GROUPS E, F** and **G**: Treated with graded concentrations of Joccy® pineapple artificial flavour dissolved in water.
- **GROUP H**: Treated with *H. sabdariffa* extract only.

***Extraction of the plant material*:** Thirty grams (30g) of the dried calyces was added to 1L of boiling water and allowed to boil for 15 minutes. After cooling, the mixture was sieved with sieve cloth and filtered with Whatman No1 filter paper. The clear filtrate was covered with aluminum foil and stored in the refrigerator at 4^{o}C until use.

***Preparation and administration of extract*:** Similar concentrations of aqueous Roselle calyx extract were maintained from GROUP B to GROUP D and PCTNRL with varying concentrations of the Joccy pineapple flavour. The concentrations of the flavouring were administered at 16.67mg/kg, 33.34mg/kg and 50.01mg/kg body weight to groups B, C and D in combination with aqueous extract of the *Hibiscus sabdariffa.* The flavor concentration at 16.67mg/kg is the equivalent concentration estimated to be in 500ml of the *zobo* which is consumed by 75 kg adult according to manufacturer's instructions. The same graded doses were also administered dissolved in distilled water to groups E, F and G. Control groups 1 and 8 received distilled water and aqueous calyx extract respectively. These mixtures were administered about the same time each day for a period of seven (7) days.

Determination of biochemical parameters: Liver function parameters (serum Alanine aminotransferase ALT and Aspatate aminotransferase AST), Kidney function parameters (plasma Urea and Creatinine) and activities of anti-oxidant enzymes (Glutathione peroxidase, Superoxide Dismutase and Catalase) where determined using standard methods (Randox commercial kits, U.K.). Plasma antioxidants

(glutathione, β- carotene and Vitamin C) were determined using standard Spectrophotometric methods (Ellman, 1959; Tietz, 1970).

RESULTS AND DISCUSSION

Table 1 show the mean plasma urea and creatinine levels in the groups. Supplementation of *zobo* with graded doses of Joccy® flavour has no dose dependent effect in the groups B, C and D rats. The activity of the mean plasma urea was increased though not significant (P>0.05) in group D rats treated with *zobo* drink supplemented with highest dose (50.01mg/kg) of the Joccy® flavour compared with the negative control and showed a significant (P<0.05) decrease compared with the positive control implying that *zobo* drink alone has nephrotoxic effect on the experimental animals. The kidney impairment may have resulted due to the presence of sodium cyclamate present in the Joccy® flavour. It has been reported that prolonged consumption of cyclamate causes kidney damage and other effects (Gosselin, et al., 1976). Similar result was obtained for the mean plasma urea levels of group E, F and G rats treated with distilled water containing graded doses of Joccy® flavour. Supplementation of *zobo* with graded doses of Joccy® flavour had no dose dependent effect in the group B, C and D rats. The activity of the mean plasma creatinine was decreased though not significant (P>0.05) in the group D rats treated with *zobo* drink supplemented with highest dose (50.01mg/kg) of the Joccy® flavour compared with the positive and negative controls. This seems to suggest that the *zobo*-flavour combination exhibited synergistic nephroprotective effect. Similar result was obtained for the mean plasma creatinine levels of groups E, F, and G rats treated with distilled water containing graded doses of Joccy® flavour. The decrease in the plasma creatinine level of the groups treated with *zobo* drink supplemented with Joccy® flavour and the groups treated with distilled water combination suggests that the flavour showed nephroprotective activity alone and in combination with *zobo.* Uricosuric activity of aqueous calyx extracts have been reported elswhere (Vitoon, et al., 2008)

Table 2 shows the mean ALT and AST activities of the groups. Supplementation of *zobo* with graded doses of Joccy® flavour has no dose dependent effect in the groups B, C and D rats. The mean activity ALT was increased though not significantly (P>0.05) in the group D rats treated with *zobo* drink supplemented with highest dose (50.01mg/kg) of the Joccy® flavour compared with the positive and negative controls suggesting that the flavor-*zobo* combination had hepatotoxic effect on the experimental animals. There was no dose dependent effect in the mean ALT levels of groups E, F, and G rats treated with distilled water containing graded doses of Joccy® flavour. The AST activity reveals that supplementation of *zobo* with graded doses of Joccy® flavour has dose dependent reduction in the groups B, C and D rats. The mean AST activity was decreased though not significant (P>0.05) in the group D rats treated with *zobo* drink supplemented with highest dose (50.01mg/kg) of the Joccy® flavour compared with the positive and negative controls. This suggest that supplementation of *zobo* with this flavour has a hepatoprotective effect on the experimental animals. The dose dependent increase obtained for the mean AST levels of groups E, F, and G rats treated with distilled water containing graded doses of Joccy® flavour suggest hepato toxic activity of constituents of the additive as hepatoprotective effects of *zobo* alone has been reported (Tseng, et al., 1997).

Table 3 shows the activities of glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) for the different groups. The mean GPX activity reveals that supplementation of *zobo* with graded doses of Joccy® flavour has no dose dependent effect in the groups treated with *H.sabdariffa* aqueous extract supplemented with graded doses of the flavour. The activity of GPX was decreased though not significant ($P>0.05$) in group D treated with *zobo* drink supplemented with the highest doses of Joccy® flavour compared with the negative and positive control groups, suggesting that Joccy® flavour combination yielded a synergistic antioxidant effect. This is corroborated by the dose dependent increase in the mean GPX levels of groups E, F and G treated with graded doses of Joccy® dissolved in distilled water containing graded doses of Joccy® flavor as anti oxidant and free radical scavenging activity of aqueous extract of calyces of *Hibiscus sabdariffa* alone have been reported (Christian, et al., 2006; Yang, et al., 2012). The mean SOD activity reveals that supplementation of *zobo* with graded doses of Joccy® flavour has no dose dependent effect in groups B, C and D. The activity of SOD decreased though not significant ($P>0.05$) in group D treated with *zobo* drink supplemented with graded doses of Joccy® flavour compared with the SOD levels in the negative and positive control groups, suggesting that the *zobo* drink supplementated with graded doses of Joccy® flavour have antioxidant effect. There was a dose dependent increase in the mean SOD levels of groups E, F and G treated with graded doses of Joccy® dissolved in distilled water. The decreased expression of SOD may be due to decreased dismutation of superoxide to hydrogen peroxide as a result of the synergistic free radical scavenging activity of the rich array of phytochemicals in the extract of the calyx and the vitamin C of the flavour additive. The mean catalase activity was increased in both the *zobo*-flavour combination groups (B, C and D) and the flavour additive solution groups (E, F and G) but not dose dependently. The group H animals had a significant ($P<0.05$) increased mean CAT activity when compared with the positive control. This suggests that *zobo* and the flavor separately/combined produced a pro-oxidant activity, an effect contrary to that observed for SOD activity. High dose of vitamin c has been reported to have pro-oxidant activity (Markovic, et al., 2010).

Table 4 show mean plasma non-enzymic antioxidants vitamin C, β-carotene and reduced glutathione concentration for the different groups The supplementation of *zobo* with graded concentration of joccy artificial flavour in the groups B, C and D has no dose dependent effect on vitamin C. Mean plasma vitamin C increased significantly ($P<0.05$) in group D which has the high dose of the joccy flavour (50.01mg/kg) compared with the negative control and increased though not significantly ($P>0.05$) compared with the positive control. Also similar result was observed in the groups E, F and G treated with graded concentration of the flavour additive dissolved in distilled water. Vitamin C acts as a chain breaking antioxidant which participates in the detoxification of various ROS (Padh, 1990). Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness (Baillie, et al., 2009). However, being a good electron donor, excess ascorbate in the presence of free metal ions can not only promote but also initiate free radical reactions, thus making it a potentially dangerous pro-oxidative compound in certain metabolic contexts (Davis, et al., 1991). The mean β-carotene concentration for the different groups in Table 4 reveals that the supplementation of *zobo* drink with graded

concentration of joccy artificial flavour in the groups B, C and D has no dose dependent effects. The concentration of β-carotene is significantly increased (P>0.05) in group D compare with the negative control (treated with distilled water only) and positive control (treated with *zobo* drink only). In the groups E, F and G treated with graded concentration of joccy artificial flavour in distilled water has a dose dependent increase of β-carotene and group G is increased significantly (P<0.05) compared with the negative control and positive control. This observation is in agreement with the anti oxidant potentials of zobo (Yang, et al., 2012). The mean plasma glutathione concentration dose dependently decreased significantly (P<0.05) in the flavour-*zobo* treated groups compared with the controls. The significant (P<0.05) non dose dependent decrease in mean plasma glutathione in the flavour-distilled water treated groups compared with the two controls implies that the metabolic intermediates of the constituents of the favour additive probably caused a depletion of the antioxidant glutathione since the group H group had significant (P<0.05) higher glutathione level. Glutathione a non enzyme antioxidant is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms (Scholz, et al., 1994).

CONCLUSION

The consumption of the plant extract supplemented with the joccy® flavor increased its antioxidant potentials and has no severe effect on the liver and kidney. Thus supplementation of *zobo* drinks with flavor additive maybe nutritionally beneficial.

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Table - 1: Effect of *H. sabdarifa* and joccy flavour on kidney function parameters.

Group	Treatment	Urea (µmol/l)	Creatinine (µmol/l)
A	Water only	2.35 ± 0.53*	274.20 ± 54.66
B	*Zobo* + Joccy (16.67)	4.51 ± 1.96	129.99 ± 9.99
C	*Zobo* + Joccy (33.34)	1.60 ± 0.85*	299.99 ± 91.65
D	*Zobo* + Joccy (50.01)	4.11 ± 1.78*	210.00 ± 0.00
E	Water + Joccy (16.67)	5.52 ± 0.93	270.32 ± 62.77
F	Water + Joccy (33.34)	4.93 ± 1.29	193.33 ± 68.39
G	Water + Joccy (50.01)	10.51 ± 0.99	146.66 ± 21.86
H	*Zobo* only	8.84 ± 2.84	253.99 ± 35.29

- n = 4; $P<0.05$ compared with the controls (one way ANOVA; LSD post hoc test)
- Values in parenthesis represent concentration of joccy flavour (mg/kg b.w) in the *zobo*

Table - 2: Effect of *H. sabdarifa* and joccy flavour on Liver function parameters.

Group	Treatment	ALT (U/l)	AST (U/l)
A	Water only	21.70 ± 2.53	19.90 ± 0.78
B	*Zobo* + Joccy (16.67)	19.17 ± 1.20	21.00 ± 0.00
C	*Zobo* + Joccy (33.34)	32.33 ± 9.39	19.66 ± 1.34
D	*Zobo* + Joccy (50.01)	25.67 ± 6.23	16.83 ± 3.45
E	Water + Joccy (16.67)	17.83 ± 2.32	16.66 ± 3.85
F	Water + Joccy (33.34)	14.50 ± 0.76	17.33 ± 3.67
G	Water + Joccy (50.01)	18.83 ± 2.17	18.16 ± 0.17
H	*Zobo* only	16.60 ± 4.38	17.60 ± 1.97

- Foot notes are same as shown in table-1

Table - 3: Effect of *H. sabdarifa* and joccy flavour on enzyme antioxidants.

Group	Treatment	GLPX (U/gHb)	SOD (*U*/ml)	Catalase (KU/l)
A	Water only	35.68 ± 6.24	556.40 ± 190.69	186.89 ± 57.91
B	*Zobo* + Joccy (16.67)	33.13 ± 7.65	323.00 ± 163.00	617.22 ± 51.93
C	*Zobo* + Joccy (33.34)	37.40 ± 19.56	195.00 ± 0.00	332.86 ± 147.24
D	*Zobo* + Joccy (50.01)	27.19 ± 11.89	470.00 ± 30.00	388.16 ± 273.27
E	Water + Joccy (16.67)	25.49 ± 12.75	276.33 ± 81.33	239.68 ± 153.46
F	Water + Joccy (33.34)	33.98 ± 21.24	174.33 ± 82.33	348.56 ± 133.33
G	Water + Joccy (50.01)	76.47 ± 0.00	153.00 ± 21.00	216.15 ± 68.87
H	*Zobo* only	39.76 ± 12.94	1322.20 ± 961.14	528.65 ± 91.08*

- Foot notes are same as shown in table-1

Table - 4: Effect of *H. sabdarifa* and joccy flavour on non-enzyme antioxidants.

Group	Treatment	Vitamin C (mg/dl)	β – Carotene (µg/ml)	Glutathione (µM)
A	Water only	2.65 ± 0.44	4.83 ± 0.92	2.62 ± 0.59
B	*Zobo* + Joccy (16.67)	4.15 ± 0.79	7.59 ± 1.17	3.30 ± 0.42*
C	*Zobo* + Joccy (33.34)	3.06 ± 0.78	2.71 ± 0.43	1.73 ± 0.95*
D	*Zobo* + Joccy (50.01)	4.53 ± 0.38	9.60 ± 0.10	0.78 ± 0.084*
E	Water + Joccy (16.67)	4.53 ±0.27	2.77 ± 0.59	1.13 ± 0.21*
F	Water + Joccy (33.34)	4.04 ± 0.87	3.94 ± 0.30	1.49 ± 0.53*
G	Water + Joccy (50.01)	4.24 ± 0.67	5.90 ± 0.82	1.11 ± 0.35*
H	*Zobo* only	4.37 ± 0.25	3.11 ± 0.26	3.54 ± 0.25

- Foot notes are same as shown in table-1

