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A COMPARATIVE STUDY OF ANTIOXIDANT POTENTIALS OF HONEY AND SOME SELECTED BEVERAGES IN MALE WISTAR RATS

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Abstract
This study compared the antioxidant potentials of honey and some selected beverages in male wistar rats. Twenty five male wistar rats (220-240g) were assigned into 5 groups of 5 animals each, such that the rats in groups I, II, III, IV and V received orally 1mL distilled water, 0.2mg/kg body weight (BW) of honey, 0.2mg/kg BW of vitamin C, 0.3mg/kg body weight (BW) of zobo and 0.2mg/kg BW of cocoa power, respectively. Catalase (WBC), superoxide dismutase (SOD) and malondialdehyde (MDA) were measured using standard methods. There was significant ($p<0.05$) increase in catalase and superoxide dismutase (SOD) levels for all the treatment groups when compared with the control. The order of significant ($p<0.05$) increased in these two parameters among the treatment groups were: cocoa powder < vitamin c < zobo < honey respectively. However, there was significant ($p<0.05$) decrease in malondihydehyde (MDA) levels for all the treatment groups when compared with the control. The order of significant ($p<0.05$) decreased in MDA levels among the treatment groups were: honey < zobo < vitamin c < cocoa powder respectively. This study showed that honey, zobo, vitamin c and cocoa powder could stimulate the activities of antioxidant enzymes by preventing the formation of lipid peroxidation. The consumption of honey, zobo, vitamin c and cocoa powder are therefore recommended in order to potentiate the activities of antioxidant enzymes. In addition, honey has the highest antioxidant potential and could consume more than cocoa powder which has the lowest.
Keywords: Antioxidant enzymes; Honey; Zobo; Cocoa powder; Vitamin C

Introduction
Antioxidants are chemical substances that donate an electron to the free radical and convert it to a harmless molecule. Antioxidants intercept free radical and protect cells from the oxidative damage that leads to aging and disease. Antioxidants prevent injury to blood vessel membranes, helping to optimize blood flow to the heart and brain, defend against cancer-causing deoxyribonucleic acid (DNA) damage, and help lower the risk of cardiovascular disease. Natural honey has both medicinal and nutritional values (Abubakar, Abdullah, Sulaiman and Suen, 2012; Othman, 2012). Studies have shown that Honey has both enzymatic and non enzymatic antioxidants which include: catalase, flavonoids and other polyphenols, as well as vitamins such as thiamine, riboflavin, pyridoxine, pantothenic acid, ascorbic acid, and nicotinic acid (Kishore, Halim, Syazana and Sirajudeen, 2011; Abubakar *et al.*, 2012).

The leaf, fleshy calyx, seed and fibre of *Hibiscus sabdariffa*, are very important herbs belonging to the family Malvaceae and is usually cultivated in all parts of the world (Dalziel, 1973). It is mostly planted in the northeast and middle belt regions in Nigeria. It has two major species which are well known in Nigeria, *H. sabdariffa* with red calyces as well as *Hibiscus rosasinesis* with green calyces (Babalola, 2000). The dried calyces of H. sabdariffa are used as medicinal herb and raw material for the production of a local soft drink commonly known as ‘zobo’ in Nigeria (Usoh, Akpan, EEtim and Farombi, 2005).

Vitamin C (Ascorbate) is a cofactor in at least eight enzymatic reactions, including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy. In animals, these reactions are especially important in wound-healing and in preventing bleeding from capillaries. Ascorbate may also act as an antioxidant against oxidative stress. Food sources such as Grape fruit juice, Strawberries, Tomato, Sweet red pepper, Broccoli, Orange Orange, Straw berries, Tomato, Sweet red pepper, juice and vegetables are the major sources of vitamin C.

Cocoa powder contains several minerals such as calcium, copper, magnesium, phosphorus, potassinm, sodium and zinc (Steinberg, Bearden and Keen, 2003). Study has shown that cocoa powder is mostly used as anti-oxidants because it contains flavonoids (Gressner, 2012). This study therefore compared the antioxidant potentials of honey, zobo, cocoa powder and vitamin c in male wistar rats. .

Methodology

Animals

Twenty five male rats (220-240g) were used for this experiment. They were obtained from the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria, were housed at room temperature with unrestricted access to diet and water and maintained on a daily light/dark cycle. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed.

Collection and Preparation of Hibiscus sabdariffa (zobo) Calyx Extract.

Fresh red flowers of H. Sabdariffa (with the calyces) were bought directly from Kalaah farm at Mubi, Adamawa State of Nigeria. The calyces were identified using the identification key of Morton (1987). The plant materials were then air-dried for four weeks under room temperature after which the dried plant materials were weighed again to determine the appropriate moisture content and ground into powder using a laboratory milling machine (Thomas Willey model 4, USA). The method of extraction followed that of Carvajal-Zarrabal *et al*., 2009. 200g of the powdered plant material was introduced into 1000 mls flat bottom flask and 250mls of distilled water was added. The content was mixed thoroughly and left for 24 hours with an occasional shaking to increase the extraction capacity. Thereafter, the soaked substance was filtered with Whatman filter paper (grade 1: 11 µm) and the resulting filtrate dried into powder using a rotary evaporator (Stuart, model RE-300, UK). The solid extract was weighed and re-dissolved in distilled water according to the body weights of the animals for oral administration.

Vitamin C (250mls) used for the study was obtained from, Momrota Pharmacy, Ilorin, kwara State and was administered orally depending on the weight of the animals. Cocoa powder (150g) was bought from Ile-Oluji, Ondo State, Nigeria. 300mls of distilled water was added to it and shaked vigorously until it formed solution. The solution was administered orally depending on the weight of the animals. Honey (300mls) was purchased from University of Ilorin, Ilorin, Nigeria and was administered orally depending on the weight of the animals.

After 2 weeks of acclimatization, animals were randomized into five groups (I–V) of five animals each. Animals in Groups I, II, III, IV and V were given orally 1mL of distilled water, 0.2mg/kg body weight (BW) of honey, 0.2mg/kg BW of vitamin C, 0.3mg/kg body weight (BW) of zobo and 0.2mg/kg BW of cocoa power, respectively. The doses were administered twice daily (morning and evening) for 30 days. The Animals were sacrificed under ketamine anesthesia after the last treatment.

The male rats were sacrificed under ketamine anesthesia and blood was collected from the heart puncture into sample bottles. The blood was left for 30 min to clot and thereafter centrifuged at 625×g for 10 min using a Uniscope Laboratory Centrifuge (Model SM800B, Surgifield Medicals, Essex, England). The serum was collected into plain bottles with the aid of a Pasteur pipette. Sera were stored in a freezer maintained at -5 °C and used within 12 hours of preparation.

The determination of serum superoxide dismutase (SOD) concentration was done with SOD colorimetric assay kit (Fortress Diagnostics Ltd., Antrim, UK; Product code: BXC0531), following the manufacturer's protocols. The assay employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The SOD activity is then determined by the degree of inhibition of this reaction. Briefly, 1.7 ml of mixed substrate (consisting of xanthine and I.N.T.) was added to microplates containing 50 µL each of serum, SOD standards of known concentration, and sample diluent. After mixing, 250 µL of xanthine oxidase was added, mixed again, and incubated for 30 s at room temperature. Absorbances were read at 0, 1, 2, and 3 min with microplate reader (Spectramax Plus; Molecular Devices, Sunnyvale, CA, USA). The mean absorbance changes per minute (ΔA) were determined and used in the calculation.

% inhibition of the standards = 100 x $\Delta A_{standard}$/ $\Delta A_{sample\ diluent}$

% inhibition of the serum = 100 x ΔA_{serum}/ $\Delta A_{sample\ diluent}$

The percentage inhibition of each standard was plotted against the Log_{10} of its concentration.

Finally, the percentage inhibition of the serum was used to get its SOD concentration from the standard curve. The continuous catalase activity was determined through spectrophotometric reading (modification of the method used by Claiborne, 1985). The continuous spectrophotometric rate reduction determination (A_{480nm}, light path = 1cm) is based on the following reaction

$$H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$$

Absorbance is read at 480nm at interval of 30 seconds for the duration of 3 minutes.

The assay method of Hunter *et al.*, (1963), modified by Gutteridge and Wilkins (1982) will be adopted. Malondiadehyde (MDA), a product of lipid peroxidation, was estimated by taking 1ml of serum combined with 2ml of TCA-TBA-HCl reagent and mixed thoroughly. Stock solution of TCA-TBA-HCl composed of 15g of TCA, 0.375g of TBA and 0.25N of HCl were prepared. The solution was heated for 15min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm against a blank that contains all the reagents.

Results

Results were expressed as the mean ± standard error of mean. Data were analyzed using a one-way analysis of variance, followed by the LSD post-hoc test to determine significant differences in all the parameters with Students Package for Social Science, version 20.0. Differences with values of $p<0.05$ were considered statistically significant.

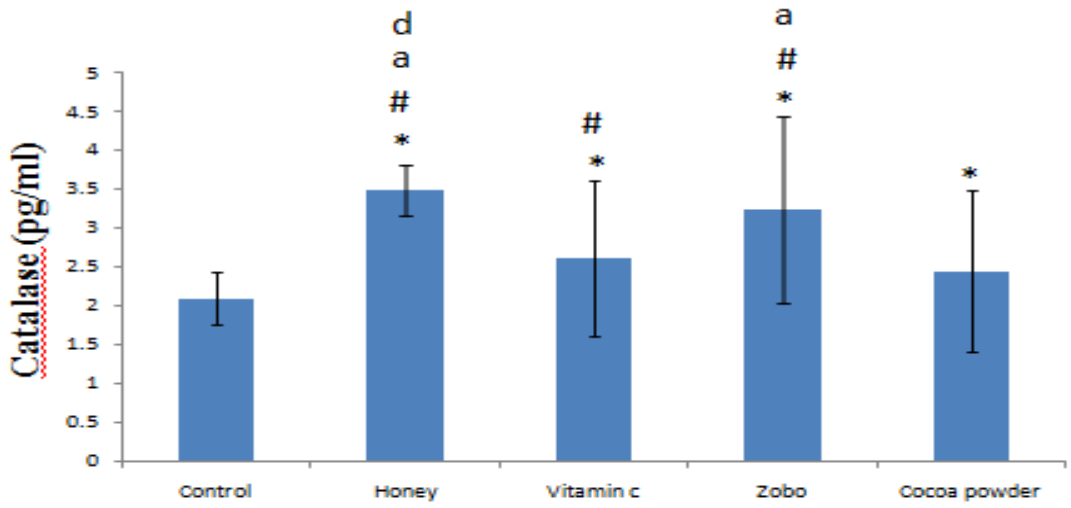

Fig. 1: Catalase activity of rats that received honey, vitamin c, zobo and cocoa powder respectively. Values are expressed as mean ± S.E.M. *p<0.05 vs Control; #p<0.05 vs cocoa powder c; ap<0.05 vitamin c; dp<0.05 vs zobo.

There was significant (p<0.05) increased in catalase activity for all the treatment groups compared to the control. However, honey showed highest significant (p<0.05) increased in catalase activity compared to other treatment groups.

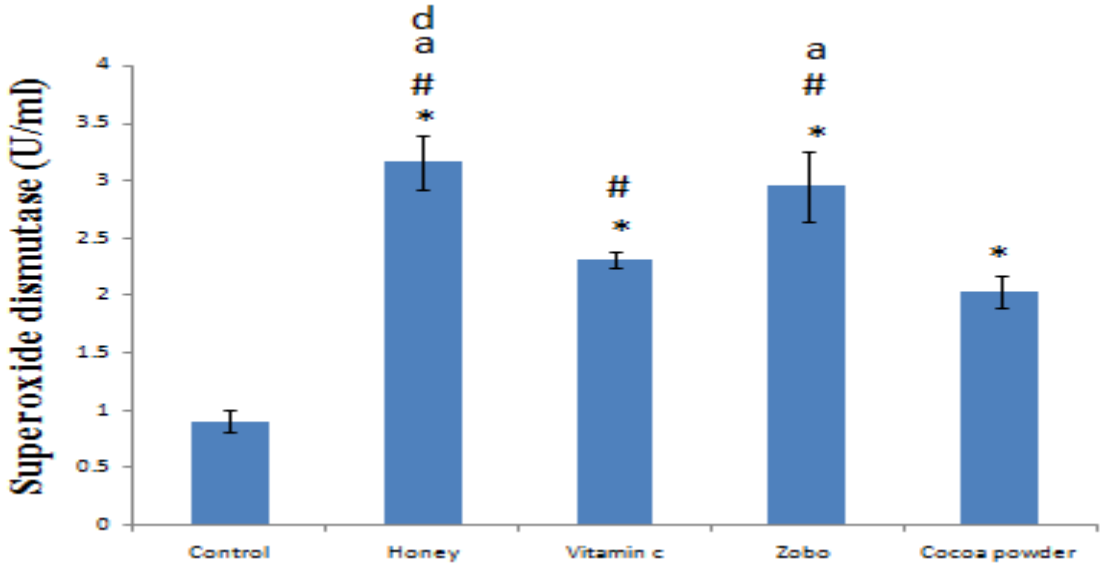

Fig. 2: SOD activity of rats that received honey, vitamin c, zobo and cocoa powder respectively. Values are expressed as mean ± S.E.M. *p<0.05 vs Control; #p<0.05 vs cocoa powder c; ap<0.05 vitamin c; dp<0.05 vs zobo.

There was significant (p<0.05) increased in SOD activity for all the treatment groups compared to the control. However, honey showed highest significant (p<0.05) increased in SOD activity compared to other treatment groups.

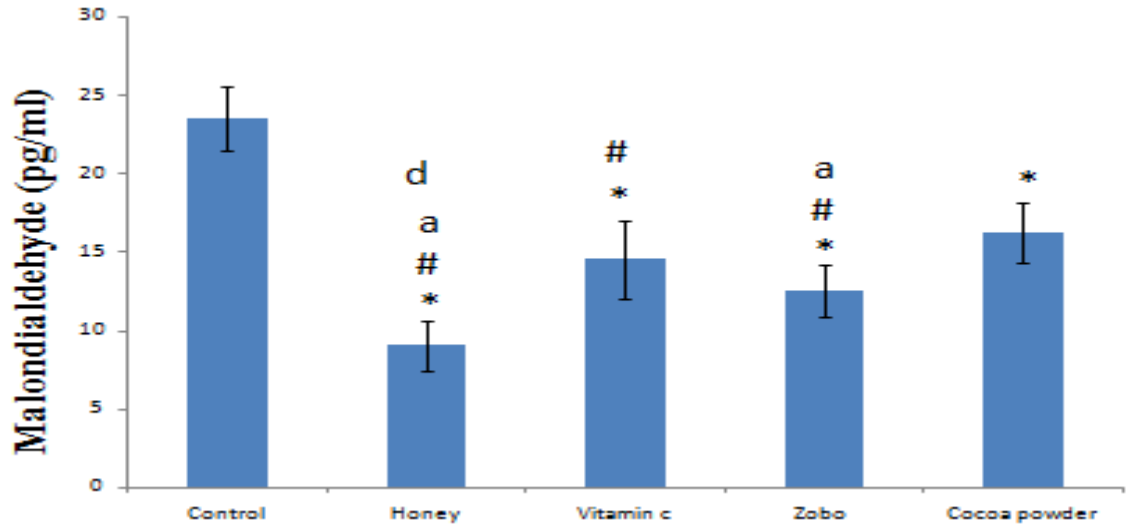

Fig. 3: MDA activity of rats that received honey, vitamin c, zobo and cocoa powder respectively. Values are expressed as mean ± S.E.M. *p<0.05 vs Control; #p<0.05 vs cocoa powder c; ap<0.05 vitamin c; dp<0.05 vs zobo.

There was significant ($p<0.05$) decreased in MDA activity for all the treatment groups compared to the control. However, honey has the lowest significant ($p<0.05$) decreased in MDA activity compared to other treatment groups.

Discussion

Oxidative stress occurs when the number of ROS is more than that of anti-oxidants that scavenge them, thus, resulting in pathological effects (Ruder, EHartman and Goldman, 2009). The results of this present study showed a significant ($p<0.05$) increased in catalase and SOD activities in rats treated with honey, zobo, vitamin c and cocoa powder compared with the control. The order of significant ($p<0.05$) increased of these two parameters among the treatment groups were: cocoa powder < vitamin c < zobo < honey respectively. This study showed that honey has the highest antioxidant potential which could be due to its antioxidant constituents such as phenolic acids and flavonoids (Andrade, Ferreres and Amaral, 1997), certain enzymes (glucose oxidase, catalase) (White, 1975), ascorbic acid (White, 1975), carotenoid-like substances (Tan, Wilkins, Holland and McGhie, 1989), organic acids (Cherchi, Spanedda, Tuberoso and Cabras, 1994), Maillard reaction products (White, 1975), amino acids and proteins (White and Rudyj, 1978), and catalase (Abubakar *et al.*, 2012), all these constituents could potentiate the activities of antioxidant enzymes. Next to honey is zobo which could also be due to the presence of ascorbic acid (Vitamin C) and tocopherol (Vitamin E) which are sources of antioxidant. Next to zobo is vitamin c which could be explained by its ability to capture and deactivate free radicals (Siess, Le Bon, Canivenc-Lavier and Susch, 2000) while cocoa powder has the lowest antioxidant potential which could be due to the presence of small amount of antioxidants such as procyanidins and flavanoids (Gressner, 2012). However, there was significant ($p<0.05$) decreased in the MDA activity for all the treatment groups when compared to the control. The order of significant ($p<0.05$) decreased in MDA activity among the treatment groups were: honey < zobo < vitamin c < cocoa powder respectively. This could be due to the variations in their antioxidant constituents.

Conclusion

This study concluded that honey, zobo, vitamin c and cocoa powder could be used to stimulate the activities of antioxidant enzymes by preventing the formation of lipid peroxidation which could result from increased activities of MDA. The consumption of honey, zobo, vitamin c and cocoa powder are therefore recommended in order to potentiate the activities of antioxidant enzymes. Also from this study, honey has the highest antioxidant potential which implies that it could be consumed more than the other beverages most especially cocoa powder with the lowest antioxidant potential.

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