Coffee and Vitamin-C Consumption on Selected Biochemical Indices and Antioxidant Activities in Adult Wistar Rats

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ABSTRACT

Coffee and vitamin C are staples in our daily lives. This study aimed to investigate the effects of sole and combined usage of coffee and vitamin C on antioxidant enzyme activities and biochemical parameters in selected organs in adult rats. 20 adult male Wistar rats were divided into four groups: Group A (distilled water), Group B (9 mg/kg b/w vit.C), Group C (33.4mg/kg b/w coffee) and Group D (9 mg/kg b/w vit.C + 33.4mg/kg b/w coffee 2 hours after). After 21 days, the rats were sacrificed, and selected organs were excised, weighed and analyzed for antioxidant enzyme activity. Blood samples were collected for biochemical evaluation. Vitamin C intake caused an increase in chloride levels and antioxidant enzyme levels. Coffee intake resulted in the highest increase in most biochemical parameters and antioxidant enzymes studied, and the intake of both gave significant increases in some and decreases in other parameters considered, as compared to the control group. This study indicates that vitamin C consumption causes a decrease while coffee consumption causes significant changes in antioxidant enzymes and biochemical parameters. Their combined administration increased total protein, sodium, chloride, and antioxidant enzymes in the organs of Wistar rats.

Keywords: Coffee, Vitamin C, Antioxidant, Consumption.

INTRODUCTION

Coffee is one of the most commonly and frequently consumed beverages in the world and is assumed to have protective effects against metabolic syndrome. Its beneficial effects on human health have become the subject matter of several scientific studies (Cornelis, 2019; Feyisa *et al.,* 2019). It is the most popular nonalcoholic beverage in the world and is sometimes classified as a functional food (Feyera, 2020), which by nature or design, can deliver benefits beyond that of a basic diet (Dórea and da Costa, 2005). Coffee's bioactive profile contains many of the

most important constituents known to exist within functional foods, ranging from flavonoids (catechins, anthocyanins), to acids (chlorogenic, caffeic), and rutin (Tylewicz*et al.,* 2018). Even though the main physiological effects of its consumption are usually ascribed to the presence of caffeine, coffee is also extremely enriched with chlorogenic acids (CGA), melanoidins and diterpenes (Moeenfard*et al.,* 2014). These contents has been said to reduce the risk factors of cardiovascular disease, diabetes, obesity, cancer, Alzheimer's, and Parkinson's (Higdon and Frei, 2006).

On the other hand, vitamin C, also known as ascorbic acid, is necessary for the growth, development, and repair of all body tissues (Feyisa *et al*., 2019). It is one of many antioxidants that can protect against damage caused by free radicals, as well as toxic chemicals and pollutants like cigarette smoke, these toxic compounds can build up and contribute to the development of health conditions such as cancer, heart disease, and arthritis (Massey and Opryszek, 1990). Vitamin C is needed for protection against immune system deficiencies, stroke, prenatal health problems, eye disease, and even skin wrinkling. Its deficiency can lead to scurvy, characterized by weakness, anemia, bruising, and loose teeth (Engelbregt *et al*., 2001). Caffeine in coffee has a mild diuretic effect, which leads to an increase in urination. As a result, water-soluble vitamins like vitamin C may be depleted due to fluid loss (Klag*et al*., 1994). To maintain healthy levels, this vitamin must be consumed on a regular basis. Therefore, the study was aimed at investigating the effects of coffee and vitamin C on organ weights (duodenum, liver, kidney, stomach, heart, and spleen), biochemical parameters (blood glucose, albumin, cholesterol, sodium, and potassium) and antioxidant enzyme activities (sodium dismutase (SOD), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA)) in male Wistar rats.

MATERIALS AND METHODS

Experimental animals

Twenty (20) adult male Wistar rats weighing between 150-200g were obtained from a reputable annual house in Ibadan, Oyo State, Nigeria. They were kept in the animal house of the Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ago-Iwoye, under standard laboratory conditions and fed a standardized pellet diet with free access to water. The care and handling of the animals were in accordance with the internationally accepted standard guidelines for animal use (Hiruma-Lima et al., 2006).

Grouping of animals

The rats were randomly divided into four groups of five (5) rats each. Group A was kept as the control group while Group B, C and D were the test groups.

Group A received only distilled water and normal feed.

Group B received 9mg/kg body weight of vitamin C.

Group C received 33.4mg/kg body weight of coffee.

Group D received 9mg/kg vitamin C + 33.4mg/kg coffee at two hours interval.

The administered dose is in accordance with the normal daily intake for coffee (Lara, 2010) and vitamin C (Lykkesfeldt et al., 2014)

Ethical approval

The Ethical Committee for Research of the Department of Physiology, Faculty of Basic Medical Science (FBMS), Olabisi Onabanjo University, Sagamu, Ogun state, Nigeria, ensured ethical considerations in compliance with the guiding principles and regulations of approval for the use and care of animals

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Preparation and administrationof coffee dose/solution

Coffee (100% *Coffea arabica*) samples were purchased from Notable Super Store, Sagamu, Ogun State and used for this experiment. 0.5 ml of distilled water that contained 3.34 mg of coffee and was administered to rats weighing 100 g (33.4 mg/kg body weight of rats).

Preparation and administrationof vitamin C

100mg commercial grade vitamin C tablet (Kunimed pharmachem ltd) was crushed and 0.9mg dissolved in 0.5ml of distilled water and was administered to 100 g of rats (9mg/kg body weight of rat).

Blood sample collection

At the end of 21 days of administration, the animals were anesthetized with sodium pentobarbital at a dose of 50 mg/kg body weight and blood samples for glucose determination and other biochemical parameters were collected via cardiac puncture into ethylene diamine tetra-acetate (EDTA) bottles as described by Hoff (2000) and reported by Oruganti and Gaidhani (2011) after an overnight fast.

Determination of blood glucose concentration

The concentration of glucose in the blood was determined after enzymatic oxidation in the presence of glucose oxidase, the hydrogen peroxide formed reacted under catalysis of peroxidase with phenol and 4-aminophenazone forming a red violet quinine-imine dye as indicator (Barham and Trinder, 1972).

Determination of total cholesterol

The total cholesterol concentration of the sample was estimated according to the enzymatic method (PAPS Protocol) of Agappe Diagnostics Kit, India.

Tissue collection and preparation for antioxidant enzyme determination

The tissue (duodenum, liver, stomach heart, kidney, and spleen) for antioxidant enzymes were carefully removed after the animals were opened up, weighed and transferred to the ice-cooled testtubes for homogenization. The homogenate was then centrifuged at 12,000 rpm at 4°C for 10 min. Supernatant aliquot was decanted and stored in a freezer for determination of Total Protein (TP), Catalase (CAT), Superoxide Dismutase (SOD) , Glutathione (GSH) and Malondialdehyde (MDA).

Total protein and albumin content determination

The total protein content of the tissue was estimated by the method of Lowry *et al*., (1951) using bovine serum albumin as a standard.

Catalase activity determination

Catalase activity was determined from the tissue according to the procedure of Goth(1991) by following the absorbance of hydrogen peroxideat 230 nm at pH 7.0.

Superoxide dismutase activity determination

The activity of SOD was determined bymeasuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30ºC by the method of Misraand Fridovich (1972). One unit of SOD activity represents the amount of SOD necessary to cause 50% inhibition of adrenaline auto-oxidation

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Malondialdehyde level determination

Lipid peroxidation of the blood and the tissue weredetermined spectrophotochemically at 533 nm and MDA concentration was quantified by usingthe molar extinction coefficient, 1.56 x 105mol-1Ecm-1 (Buege and Aust, 1978)

Glutathione activity determination

The spectrophotometric method for glutathione activity involves the oxidation of Ellman's reagent, 5, 5'-dithiobis-2-nitrobenzoic acid to form yellow derivative, 5'-thio-2-nitrobenzoic acid (TNB) which is measurable at 412nm(Beutler*et al.,* 1963).

Organ weight determination

The weight of liver, kidney, duodenum, spleen, heart and stomach were determined per 100gramm body weight using a weighing scale (kerro Bl20001)

Electrolyte level determination

Serial sample from the centrifuge blood was used for the analysis according to the method described by Abubakar and Sule (2010). Serum sodium, potassium, and chloride concentrations were determined using an automated biochemical analyzer.

Statistical analysis

Data was expressed mean ± standard deviation (m ± SD) and all results were analyzed by one-way ANOVA using SPSS software version 16.0. Differences was considered significant at p value < 0.05.

RESULTS

Biochemical indices

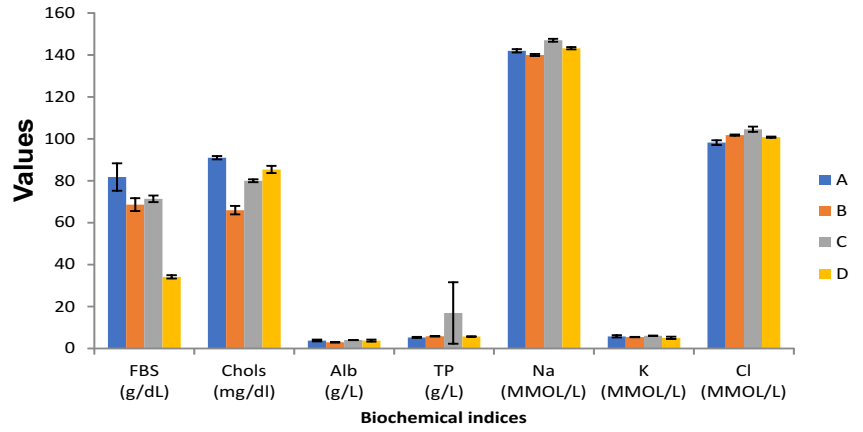
BMI, blood glucose, and cholesterol levels were highest in group A, indicating that the intake of vitamin C and/or coffee causes a reduction in these biochemical parameters, as shown in Figure 1. The intake of coffee caused an increase in albumin and potassium levels, while the intake of vitamin C solely and/or followed by coffee caused a decrease. The total protein level increased in all the test groups, with a remarkable increase in group C. Sodium levels decreased in group B but increased in group C, and D. Intake of coffee and vitamin C, whether solely or together, caused a significant increase in chloride level

In Figure 2, results showed that SOD levels were lower in all test groups, except in those fed with vitamin C alone, when compared to those of the control group. The intake of only coffee and vitamin C + coffee caused an increase in CAT with a decrease in those fed with only vitamin C. GSH levels increased with the intake of vitamin C or coffee but decreased in rats fed with the two. The MDA level was higher in all test groups except in group B, which showed an extremely low value.

Results in Figure 3 showed that SOD levels were raised in all test groups when compared to those of the control group, but extremely high in group B. CAT levels were raised in rats fed with only vitamin C, but lower in other test groups when compared to those in the control group. All test rats showed a decrease in GSH levels, except those that received only coffee, which showed a significant increase when compared to those of the control group. The MDA level was slightly lower in rats that received vitamin C only, but higher in other test groups as compared to those of the control group.

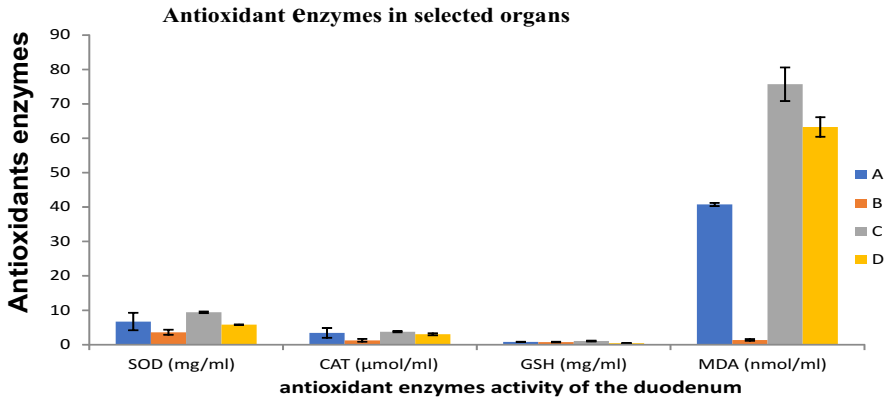
In Figure 4, SOD and CAT levels were significantly higher in all test groups as compared to those of the control group. The GSH level was lower in all test groups as compared to those of the control group. The MDA level was higher in all test groups, except those administered only vitamin C, when compared to those of the control group.

Figure 5 showed that the SOD level was higher in rats administered vitamin C but lower in other test groups when compared to those of the control group. The CAT level was higher in all test groups except in those fed with vitamin C followed by coffee, when compared to those in the control group. Rats' GSH levels were higher in rats administered only coffee, but lower in rats given



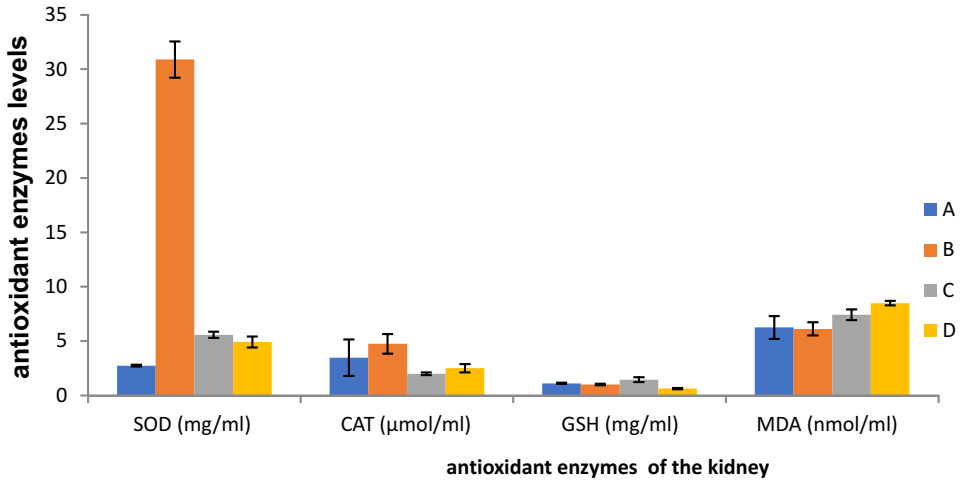
A; control group, B; 9mg/kg body weight of vitamin C, C; 33.4mg/kg body weight of coffee, D; 9mg/kg

Fig 1: Chart showing the biochemical indices in rats administered with coffee and



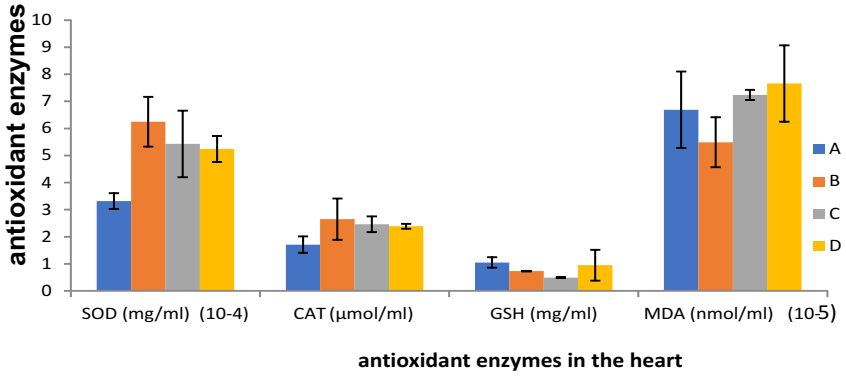
A; control group, B; 9mg/kg body weight of vitamin C, C;33.4mg/kg body weight of coffee, D; 9mg/kg vitamin C + 33.4mg/kg coffee

Figure 2:Chart showing antioxidant enzyme levels in the duodenum of rats administered with coffee and vitamin C



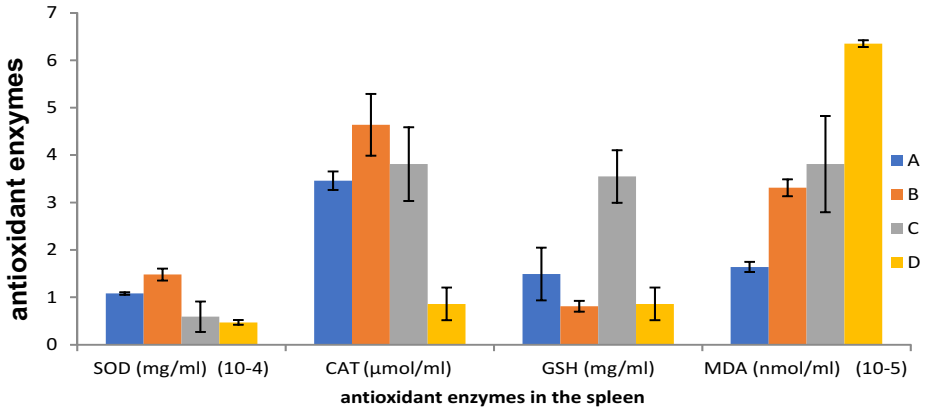
A; control group, B; 9mg/kg body weight of vitamin C, C; 33.4mg/kg body weight of coffee, D; 9mg/kg vitamin C + 33.4mg/kg coffee

Figure 3: Chart showing antioxidant enzyme levels in the kidney of rats administered with coffee and vitamin C



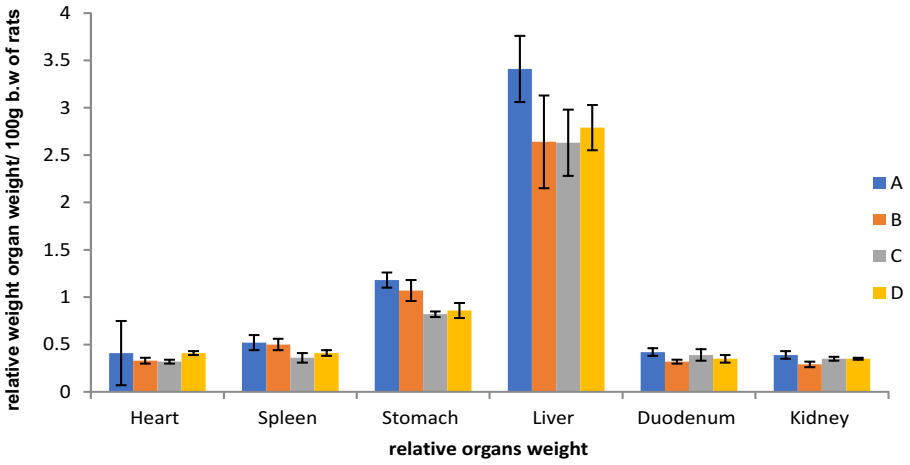
A; control group, B; 9mg/kg body weight of vitamin C, C; 33.4mg/kg body weight of coffee, D; 9mg/kg vitamin C + 33.4mg/kg coffee

Figure 4: Chart showing antioxidant enzyme levels in the heart of rats administered with coffee and vitamin C



A; control group, B; 9mg/kg body weight of vitamin C, C; 33.4mg/kg body weight of coffee, D; 9mg/kg vitamin C + 33.4mg/kg coffee

Figure 5:Chart showing antioxidant enzyme levels in the spleen of rats administered with coffee and vitamin C



A; control group, B; 9mg/kg body weight of vitamin C, C; 33.4mg/kg body weight of coffee, D; 9mg/kg vitamin C + 33.4mg/kg coffee

Figure 6: Chart showing weight changes in heart, spleen, stomach, liver, duodenum and kidney in rats administered with coffee and vitamin C

either only vitamin C or vitamin C followed by coffee, when compared to those of the control group. The MDA level was higher in all the test groups when compared to those of the control group.

Organ weight results in Figure 6 showed that heart weight was lower in rats fed with either vitamin C or coffee, but indifferent in those given both vitamin C and coffee, as compared to the control group. Also, spleen, stomach, liver, duodenum, and kidney weights were lower in all the test groups when compared to those of the control group.

DISCUSSION

This study has corroborated the fact that vitamin C and coffee are inversely related to BMI (Johnston et al., 2007), beneficial in decreasing blood glucose levels (Kotb and Azzam, 2015) and cholesterol levels (McRae, 2018), although biologically active compounds in coffee, such as chlorogenic acid, caffeine, trigonelline, and magnesium, have been shown to be associated with anti-obesity benefits (Higdon and Frei, 2006). While there was an increase in albumin and potassium levels due to coffee consumption, consumption of coffee followed by vitamin C caused a decrease, showing the antagonistic effect of vitamin C on coffee. The increase in total protein in all the test rats is indicative of the positive effects of the antioxidant components of both coffee and vitamin C. Consumption of only vitamin C resulted in a decrease in sodium level but an increase with coffee, and coffee followed by vitamin C resulted in an increase in sodium level. Coffee and vitamin C consumption, whether solely or in combination, caused a significant increase in chloride levels across the test groups.

The interplay between the trio of free radicals, antioxidants, and diseases is important in maintaining health, aging, and age-related diseases (Rahman, 2007). Antioxidant enzyme results for all the vital organs examined showed a sole and combined effect of coffee-dependent and vitamin C-dependent changes. The increase in SOD levels due to coffee consumption is indicative of reactive oxygen species and the

coresponding decrease in CAT levels shows the presence of free radicals. Vitamin C intake resulted in an increase in SOD levels in relation to its antioxidant capacity. Also, the consumption of coffee followed by vitamin C revealed an imbalance in the levels of antioxidant enzymes, which might be caused by the interplay between the ROS levels and antioxidant capacity in the studied organs. The significant organ weight changes are indicative of the general health status and drug-related toxicity in animals.

CONCLUSION

In light of the results observed from this study, it could be concluded that vitamin C and coffee has antioxidant properties with effects based on the sole or combined consumption.

RECOMMENDATIONS

Our recommendation is that vitamin C and coffee dosage and administration should be modified so as to decipher whether the varied timeliness and dosage of the intake of sole or combined dosage is a causative factor to be considered for increase or decrease in hematological and antioxidant parameters' evaluation or not. More so, the effect of coffee and vitamin C metabolism would be better assessed, respectively. The result will guide in the better dosage and intake of both substances which are widely considered non-derogatrory to health.

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