

Sodium sesquicarbonate elicits oxidative stress in erythrocytes, liver and kidney tissues.

§,¹Nwaigwe Chioma Uchenna, ¹Udem Samuel Chukwuneke, ²Nwaigwe Chukwuemeka Onyekachi and ¹Madubunyi Ifeanyi Innocent.

¹Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

²Department of Animal Health and Production, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Corresponding author: Nwaigwe, Chioma Uchenna; Email: uchenna.nwaigwe@unn.edu.ng

Abstract

Sodium sesquicarbonate also known as Sodium sesquicarbonate dihydrate (SSD) has been used globally for centuries in food and traditional medical practices. There is paucity of scientific information on the safety of this common food additive. This study was designed to find out if the oral administration of SSD is capable of generating oxidative stress in erythrocytes, liver and kidney using albino rats as experimental models. The total number of animals used for this study was fifteen. The experimental animals were grouped into three. There were five animals in each group. The rats in the first group which was the control group, were dosed with 1 ml distilled water, while groups 2 and 3 were treated with 400 mg/kg and 800 mg/kg body weight (bw) of SSD, respectively, once daily *per os* for 28 days. After the duration of treatment, the erythrocytes, hepatic and kidney tissues were processed for the analysis. The biomarkers of oxidative stress, superoxide dismutase (SOD), catalase; and thiobarbituric acid reactive substances/ malondialdehyde (TBARS/MDA) were assayed. The result indicated that catalase enzyme activity was overexpressed in the red blood cells, liver and kidneys of the group that consumed the lower dose of SSD. The dose-dependent increase in the lipid peroxidation of the tissues as indicated by increased levels of MDA in the erythrocytes and TBARS in the tissues of the treated groups was significant ($P < 0.05$). The SOD enzyme activity in all the tissues assayed showed a dose-dependent decrease, which was significant at the probability level of 0.05. The consumption of SSD therefore caused lipid peroxidation and reduction in activity of the antioxidants present in the tissues studied.

Key words: Oxidative stress, Erythrocytes, Liver, Kidney, Lipid peroxidation

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INTRODUCTION

Oxidative stress can be described as a condition characterized by an imbalance between the rate of oxidation and efficacy of the antioxidant systems in ameliorating the effects of the oxidative process (Yoshikawa and Naito, 2002). It can be due to either reduction in production of antioxidants or increased generation of free radicals (Czerska *et al.*, 2015). Oxidative stress results in peroxidation and subsequent destruction of macromolecules such as lipids, nucleic acids, proteins, carbohydrates and bases; and has been reported to cause pathological conditions such as cardiovascular disorders, diabetes, hepatitis, renal diseases and cancers (D'Azy *et al.*, 2016; Kurian *et al.*, 2016; Zhang *et al.*, 2020). Conventional biochemical indicators of oxidative stress include thiobarbituric acid reactive substances (TBARS), catalase and superoxide dismutase. Catalase, an antioxidant cytosolic enzyme which can be found in both plant and mammalian tissues such as the liver, erythrocytes and renal cells facilitates the formation of molecular oxygen and water by dissociating hydrogen peroxide. It also neutralizes the anion radical O_2^- , hydroxyl radicals and the radicals of unsaturated lipids. (Boriskin *et al.*, 2019; Olson *et al.*, 2017). Superoxide dismutases (SODs) are ubiquitous metalloenzymes that provide front line protection to the organism in the event of oxidative stress (Younus, 2018), while TBARS are products of lipid peroxidation that are formed when free radicals attack carbon-carbon bonds in lipids (Tsikas, 2017).

Erythrocytes are prone to oxidative changes that often affect the cell membrane which possesses fatty acids, oxygen and haemoglobin. The structural and functional composition of the membranes are important factors that determine the longevity of red blood cells in plasma of circulating blood (Skrzep-Poloczek *et al.*, 2020). The deleterious effects of redox imbalance on erythrocytes include decrease in their ability to transport oxygen, early aging, irreversible damage and removal from circulation (Maurya *et al.*, 2015; Revin *et al.*, 2019). In kidney disorders, oxidative stress is known to cause the progression of both acute and chronic kidney disease by generating oxidants, hyperchlorous acid, and myeloperoxidase (MPO) (Gwozdziński *et al.*, 2021; Kisić *et al.*, 2016). Oxidative stress plays a major role in the development of hepatic disorders. Hepatic inflammation observed in fatty

liver disease and viral hepatitis is a consequence of mitochondrial dysfunction, stress on the endoplasmic reticulum and excess release of free radicals (Cichoż-Lach and Michalak, 2014).

Sodium sesquicarbonate dihydrate (SSD) also known as trisodium hydrogen dicarbonate ($Na_3(CO_3)(HCO_3) \cdot 2H_2O$) is an equimolar mixture of sodium bicarbonate and sodium carbonate which occurs in nature as the evaporite mineral Trona. SSD is utilized in several industrial processes such as leather tanning and in production of cosmetics, soaps and detergents (Lewis, 2007). In traditional medical practices, it is claimed to be an effective therapeutic agent against ailments such as toothache, stomach ache and constipation (Okoye *et al.*, 2016). However, the commonest use of the natural occurring form of SSD, Trona, across several cultures in Africa is in cuisines as a food additive. Trona is used as tenderizer in the cooking of legumes, or as component of different drinks and dishes in West, East and Central Africa such as Makande in Tanzania and Achu soup in Cameroon as well as *tuwo* and *abacha* in Nigeria (Ene-Obong *et al.*, 2013; Nielsen and Dahi, 2002). Trona had been documented to contain heavy metals especially iron, zinc and lead as well as caused loss of weight in experimental rats following oral administration (Imafidon *et al.*, 2016). Okoye *et al.*, (2016) and Ajiboye *et al.*, (2018) reported in their studies that SSD administration to albino rats resulted in hepatotoxicity. The toxic effect of a xenobiotic on an organ may be attributed to oxidative changes caused by expression of reactive oxygen species and other free radicals by the substance (Singh *et al.*, 2010). This study therefore evaluated the lipid peroxidation and antioxidant enzymes activity of SSD on erythrocytes, liver and renal tissues of albino rats.

MATERIALS AND METHODS

Ethical approval

The approval to carry out this research was given by the University of Ghana Animal Care and Use Committee (UG-IACUC) with ethical clearance number- UG-IACUC 008/18-19, according to the principles guiding the use of laboratory animals in research.

Experimental Animals

Fifteen male Albino rats were used for the study. They weighed between 100 and 150 g and were gotten from University of Ghana, Department of Animal Experimentation, Noguchi Memorial Institute of Medical Research (NMIMR). They were maintained in standard housing and environmental conditions and given pelleted feed and water *ad lib*. The experiment commenced after an acclimatization period of 7 days.

Sample Preparation

The SSD sample was procured from Borno State Nigeria where it is locally mined and identified in the Department of Earth Science, School of Physical and Mathematical Sciences, University of Ghana. It was finely pulverized and kept at ambient temperature in an air-tight bottle. Prior to use, the ground sample was reconstituted with appropriate concentration of distilled water and further dissolved using ultrasonic homogenizer to get a more uniform solution.

Experimental Design

The experimental animals were randomly selected into three groups comprising five rats per group. The sample was administered *per os* using a gastric tube. The animals in group one was administered distilled water, while those in groups two and three were given 400mg/kg and 800mg/kg of Trona respectively, once daily for 28 days. At the end of the treatment period, 3 ml of blood sample was collected from each animal after which they were humanely sacrificed. The hepatic and renal tissues were excised and sections kept for assay of oxidative markers.

Blood and Tissue Collection

The erythrocytes were prepared as described by Skrzep-Poloczek *et al.* (2020). Tubes treated with ethylene diamine tetra acetic acid (EDTA) were used to collect the blood samples. To separate the erythrocytes, the tubes containing the samples were centrifuged at a temperature of 4 °C for 10 minutes at 5000 revolutions per minute. The pellets were washed thrice with 0.01 M phosphate buffer solution (pH 7.4), containing 0.14 M NaCl and stored at a temperature of -80 °C. The excised tissues samples were dipped in 0.9% (*w/v*) solution of sodium chloride in order to eliminate external contaminants, weighed and kept at a temperature of -20°C until needed.

Determination of Markers of Oxidative Stress in Erythrocytes

Catalase-The spectrophotometric technique as described by Aebi (1982), was used to determine the activity of catalase enzyme in the erythrocytes. Summarily, 10µl of the erythrocytes was mixed with 2 ml of 50 mM phosphate buffer and 1 ml of 10mM hydrogen peroxide. At the intervals of 10 seconds, absorbance at 240 nm wavelength was read for 1 minute against water blank. The result of the catalase activity was expressed as mmol H₂O₂ utilized/min/mg of haemoglobin.

Malondialdehyde (MDA)-The colometric method for determining the activity of MDA in erythrocytes was used for the assay (Shafiq-Ur-Rehman, 1984). The erythrocytes were put in an incubator at a temperature of 37°C for 2 hours. After incubation, the erythrocytes were mixed at the ratio of 1:1 with 10% *w/v* trichloroacetic acid and then centrifuged for 10 minutes at 2000 rpm. To the supernatant was added Thiobarbituric acid (0.67%) and then boiled at 100°C for 10 minutes in a water bath. The absorbance was read at the wavelength of 535 nm after the addition of 1 ml of distilled water. The result was expressed as nmol MDA/ml of erythrocytes.

Superoxide Dismutase (SOD)- The spectrophotometric technique of Madesh and Balasubramanian (1998) was employed in the determination of the activity of SOD. A tube containing 10 µl of hemolysate, 0.65 ml Phosphate buffered saline at pH 7.4, 75 µl of 100 µM pyrogallol and 30 µl of 1.25 mM (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide) (MTT) solution was incubated for 5 minutes at 27°C. Dimethyl sulfoxide was added at the volume of 0.75 ml and the absorbance was taken at 570 nm. The activity of SOD was expressed as Unit/ml, with one unit defined as the quantity of hemoglobin in micrograms, capable of effecting an inhibition of 50% in the reduction of MTT.

Determination of Markers of Oxidative Stress in Tissues

The hepatic and kidney tissues were individually processed by blending with 1.15% (*w/v*) ice-cold potassium chloride solution. To the mixture was added 0.05 M phosphate buffer and then centrifuged at a temperature of 4 °C for 20 minutes at 10,000g. The supernatant was used for the analyses.

Catalase- The catalase enzyme activity in the tissues was determined spectrophotometrically using the method of Hadwan (2018). To test tubes marked test, was added 500 µl of sample while in standard and blank tubes was put 500 µl of distilled water. To the test and standard tubes were added 1000 µl of hydrogen peroxide. After mixing, the samples were incubated at a temperature of 37 °C for 2 minutes. A working solution of 1800 ml of sodium bicarbonate, 100 ml of cobalt (II) solution and 100 ml of Graham salt solution, at a volume of 6000 µl was prepared and put in all the test tubes. The tubes were vortexed for 5 seconds and kept away from light for 10 minutes. The absorbance reading was taken at 440 nm and the result expressed as kU/litre.

Superoxide Dismutase (SOD)- The spectrophotometric method used to analyze the activity of superoxide dismutase in the tissues was earlier described by Kakkar *et al.* (1984). A working solution containing 0.1 mL of 186 µM of phenazine methosulphate, 1.2 mL of 0.052 M of sodium pyrophosphate buffer (pH 8.3), 0.2 mL of 750 µM of nicotinamide adenine dinucleotide hydrogen (NADH) and 0.3 mL of 300 µM of nitroblue tetrazolium was mixed with 0.1 mL of the tissue. After incubation at a temperature of 30° C for 90 seconds, glacial acetic acid (0.1 mL) was added to the mixture. The tubes were vortexed for 10 minutes and centrifuged after the addition of 4.0 mL of n- butanol. Absorbance of the layer containing the butanol was read at an absorbance of 560 nm, and the activity of superoxide dismutase was expressed as Units/mg of sample.

Thiobarbituric acid reactive substances (TBARS)- A Spectrophotometric method was used in determining the level of lipid peroxidation in liver and kidneys of the experimental rats

(Ohkawa *et al.*, 1979). A mixture of 0.1 mL of sample aliquot, 0.2 mL of 8.1 % sodium dodecyl, 1.5 mL of 0.8% thiobarbituric acid and 1.5 mL of 20% acetic acid was prepared. Using a water bath, the mixture was kept at a temperature of 100°C for 60 minutes, and then cooled for 10 minutes. To the mixture was added 5 mL of n-butanol-pyridine and 1 mL of distilled water. The tubes were centrifuged at 4000 revolutions per minute for duration of 10 minutes. The absorbance reading was taken at a wavelength of 532 nm. The concentration of TBARS present was expressed as nM/g of tissue.

Statistical Analysis

One-way analysis of variance (ANOVA) was used in analyzing the data obtained from the study. The Statistical package for social sciences (SPSS) version 20.0 was used for the statistical analyses. The values were presented as mean ± standard error, and the probability level of less than 0.05 was accepted as significant.

RESULTS

Effect of SSD on Erythrocyte markers of oxidative stress

Catalase enzyme activity in the red blood cells was reduced to a significant level ($P < 0.05$) in the 800mg/kg bw group when compared to the untreated group (Table 1). However, the 400mg/kg bw group had the highest value (138.70 ± 1.63) compared to the other groups (132.22 ± 2.15 , 125.08 ± 1.15). The marker of lipid peroxidation was significantly elevated ($P < 0.05$) in the group administered the highest dose of SSD when compared to the control group. This trend was reversed in the values representing the activities of SOD in the erythrocytes.

Table 1: Oxidative marker parameters in erythrocytes

Oxidative Marker	Control	400mg/kg	800mg/kg
Catalase (mmol H ₂ O ₂ /min/mg hb)	132.22 ± 2.15ab	138.70 ± 1.63a	125.08 ± 1.15b
MDA (nmol MDA/ml)	5.92 ± 1.87a	6.13 ± 2.00a	7.05 ± 1.46b
SOD (Unit/ml)	6.39 ± 3.01a	5.40 ± 2.13b	4.48 ± 2.66c

Alphabetical superscripts a, b, c, in a row show significant difference in the means ($P < 0.05$)

Effect of SSD on Tissue markers of oxidative stress

The activity of catalase enzyme in the liver of treated animals did not significantly differ ($P >$

0.05) among the groups; however, in the kidney, the rats that were administered 800mg/kg of the test sample had significantly lower ($P < 0.05$) average catalase activity (18.15 ± 1.18)

compared to those in the control group (22.43 ± 1.17) (Fig. 1). The 400mg/kg group had higher catalase enzyme activity in both tissues when compared to the other groups. Figure 2 shows that in liver and kidney tissues, when SSD was given at the dose of 800mg/kg, there was significant reduction ($P < 0.05$) in the superoxide dismutase enzyme activity (21.55 ± 1.36 , 11.63 ± 0.49) compared to the control groups ($28.11 \pm$

0.99 , 19.93 ± 0.69). The TBARS value for the untreated group was significantly lesser ($P < 0.05$) in both organs (34.94 ± 0.78 , 25.05 ± 1.14) when compared to the treated groups for the liver (39.22 ± 1.00 , 48.37 ± 1.50) and kidney (40.31 ± 1.72 , 52.13 ± 1.43) (Fig 3). The downregulation of superoxide dismutase enzyme activity and the increase in TBARS concentration in both tissues were dose-dependent.

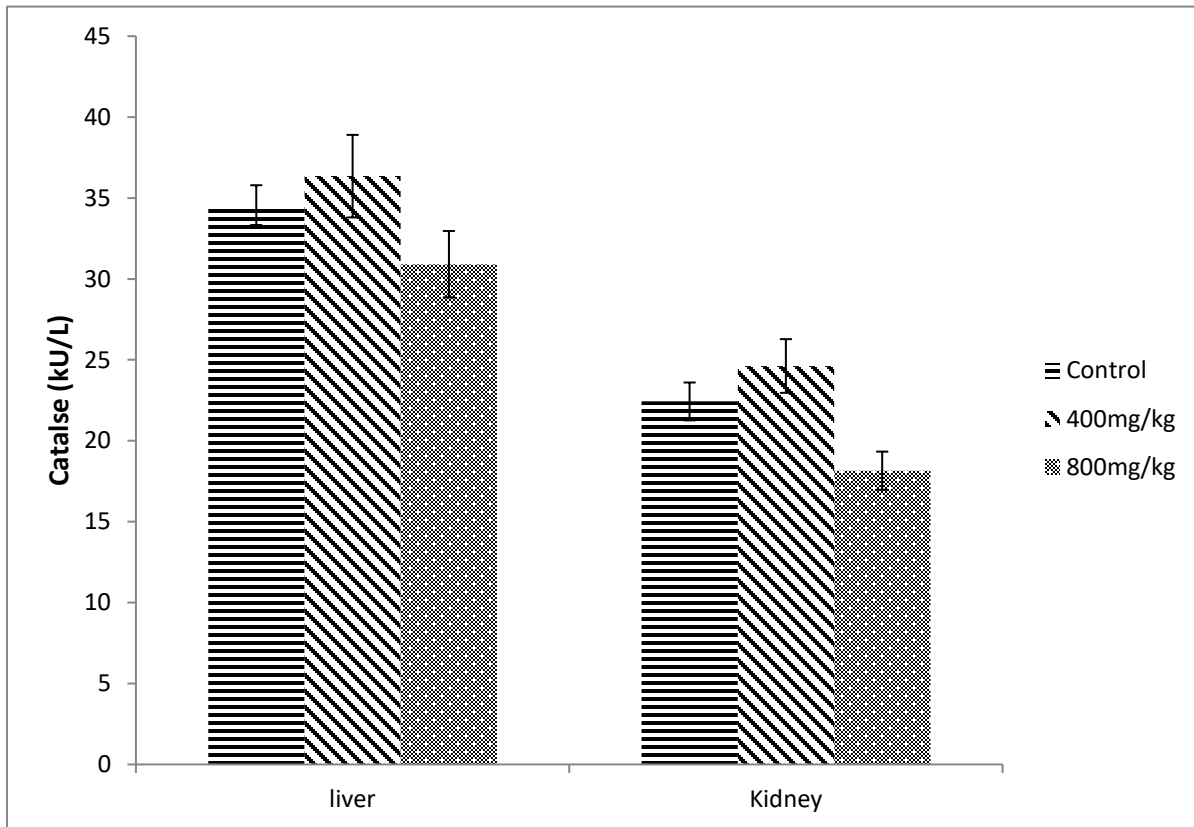


Figure 1: Catalase activity in tissues of control and SSD treated groups. Values are expressed as mean \pm standard error. Values with different superscripts vary significantly at $P < 0.05$.

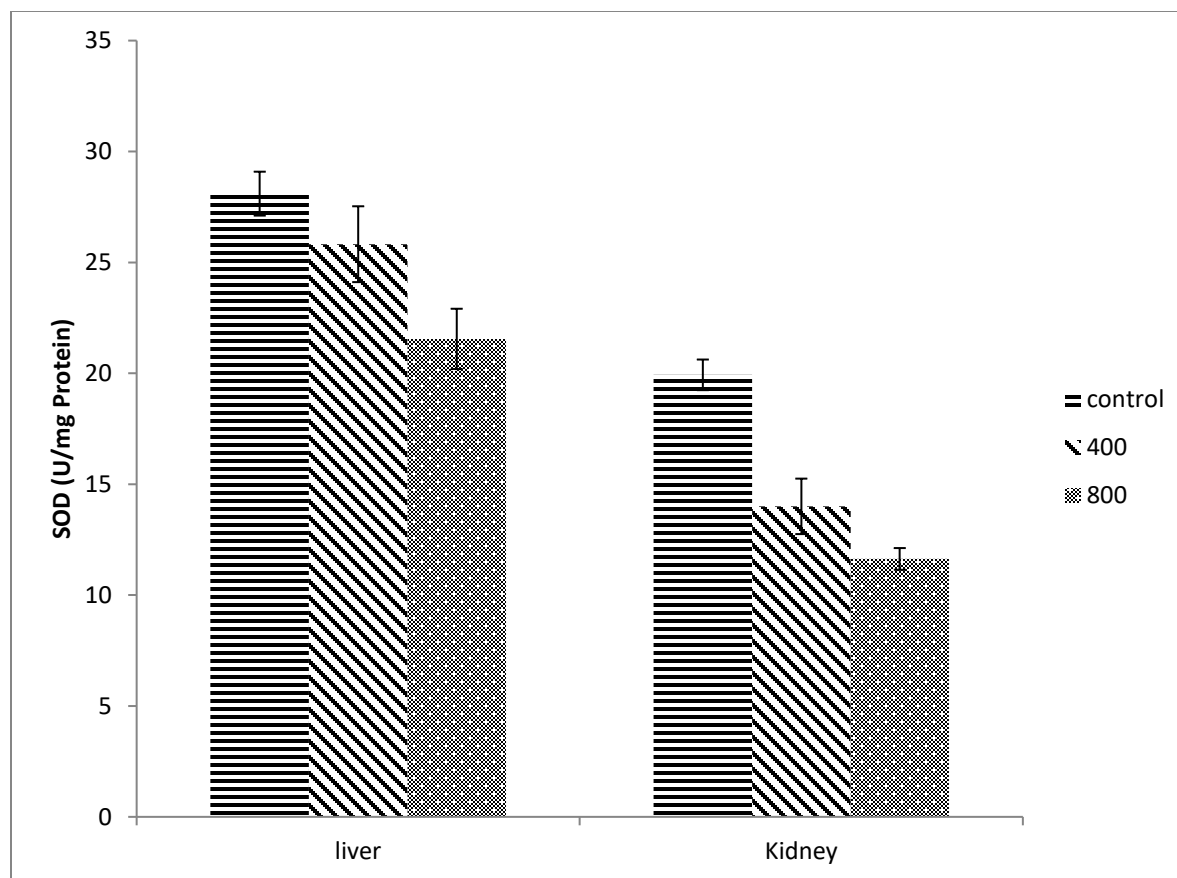


Figure 2: SOD activity in tissues of control and SSD treated groups. Values are expressed as mean \pm standard error. Values with different superscripts vary significantly at $P < 0.05$.

DISCUSSION

Oxidative stress is a major risk factor in the pathogenesis of several ailments and has been described as a loss of equilibrium between antioxidants and oxidants due to elevation of free radicals (Dhalla *et al.*, 2000). The results of this work show that consumption of SSD increased the activity of oxidative enzyme markers and enhanced lipid peroxidation in erythrocytes. Reduction in SOD and catalase enzyme activities

with higher doses of xenobiotics as observed in this research can be attributed to the depletion of enzymes due to oxidative stress (Jadhav *et al.*, 2007). Studies have shown certain substances can induce oxidative stress in erythrocytes. These include atrazine, cadmium, chromium, arsenic, iron, manganese, mercury and lead (Alburaidi *et al.*, 2022; Jadhav *et al.*, 2007; Singh *et al.*, 2010). The constant exposure of RBCs to the products of oxidation released by auto-oxidation of the oxygen carrier protein, haemoglobin, depletes capacity of the cells to fight against oxidation,

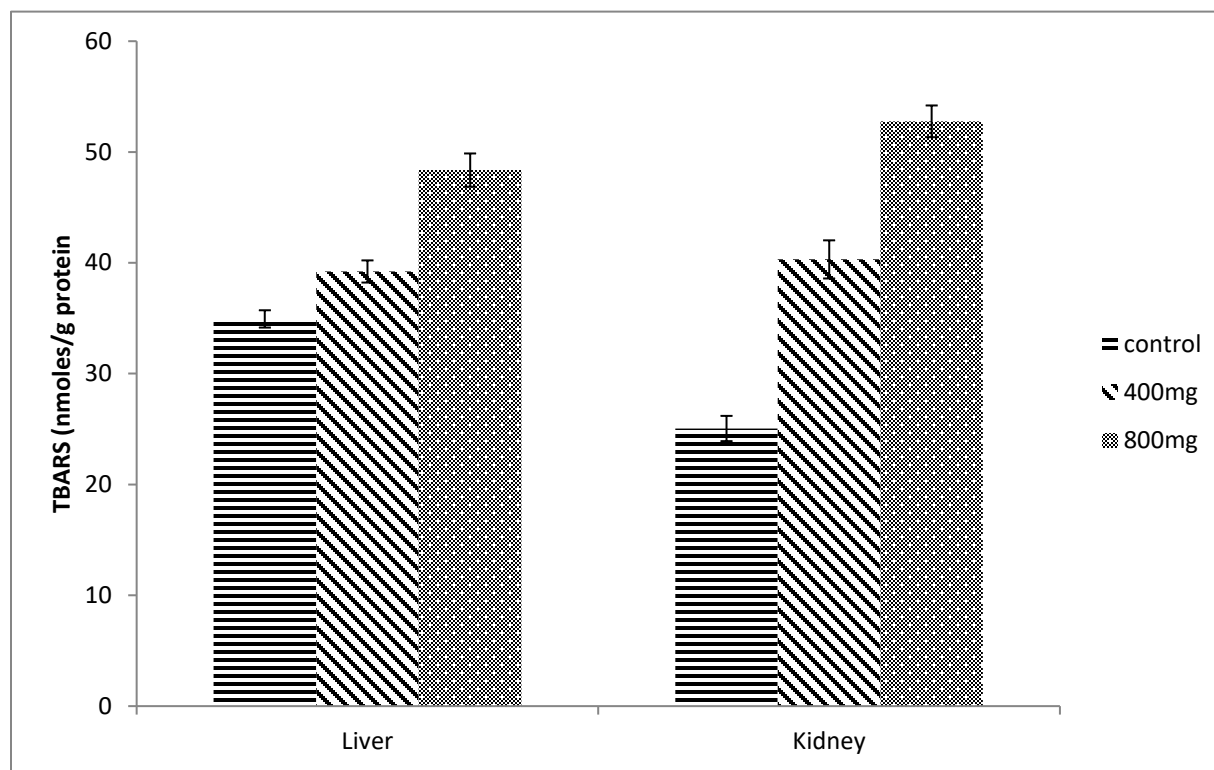


Figure 3: TBARS levels in tissues of control SSD treated groups. Values are expressed as mean \pm standard error. Values with different superscripts vary significantly at $P < 0.05$.

predisposing them to haemolysis (Maurya *et al.*, 2015). This implies that the consumption of SSD is capable of making an individual susceptible to the side effects of red blood cell haemolysis such as anaemia, reticulocytosis and hamoglobinaemia. The elevated antioxidant activity of catalase enzyme in the hepatic and renal tissues as observed in the group that was administered lower dose of the sample has earlier been reported by Ajiboye *et al.* (2018) in the intestine, kidney and liver of rats administered Trona. This increase has been described as a compensatory regulatory response to increase in oxidative stress (Li *et al.*, 2015). Sometimes referred to as catalase overexpression, this phenomenon has been documented to provide protection against oxidative stress in chemical agent toxicity (Gurgul *et al.*, 2004), cardiac disease and aging (Yao *et al.*, 2015), and hepatic carcinogenesis (Nilakantan *et al.*, 1998). A reduction in catalase activity in the renal and

hepatic tissues is an indicator of neuropathological conditions and oncological diseases (Glorieux *et al.*, 2015). Deficiency of catalase enzyme in the kidney has been attributed to contribute to chronic renal failure-induced stress caused by oxidative activities (Sindhu *et al.*, 2005). Researchers have also reported reduction in catalase enzyme activity in humans in advanced renal disease and in mice following nephrectomy (Inal *et al.*, 1999; Kobayashi *et al.*, 2005).

Free radicals include the reactive nitrogen species, hydroxyl radicals and the reactive oxygen species. Reactive oxygen free radicals are normal physiological by-products of aerobic respiration that only poses a danger when over-produced or when the antioxidative systems are compromised (Gwozdziński *et al.*, 2021). The enzyme superoxide dismutase functions primarily in dismutation of the superoxide ion, thereby defending the biological system against the

reactive oxygen species (Singh et al., 2010). The decrease in the SOD activity in the present study is an indication that the test substance caused oxidative changes that overwhelmed the antioxidative ability of the enzyme. The down regulation of superoxide dismutase activity has earlier been reported in the hepatic and renal tissues of rats with chronic renal failure (Vaziri *et al.*, 2003). Increase in TBARS level in organs of the treated rats as reported in this research work agrees with the findings of Ajayi & Akhigbe, (2017). Malondialdehyde is considered an accurate determinant of peroxidation of lipids, as it is an oxidative product of peroxidized poly unsaturated fatty acids. Thiobarbituric acid reactive substances are products of oxidative stress, principally Malondialdehyde (MDA) as well as other substances (Tsikas, 2017). When an organ is exposed to lipid peroxidation, there is oxidative damage leading to disintegration and deterioration of the membrane and damage to the organ. There had been earlier reports of injury to the liver following administration of SSD to rats (Ajiboye *et al.*, 2018). The increase in products of lipid peroxidation observed in this study therefore implicates oxidative stress as a possible mechanism of injury to the tissues by the test substance.

CONCLUSION

The oral administration of SSD decreased the antioxidant activity of catalase and SOD which are conventional biomarkers of oxidative changes in cells, organs and tissues. There was also increase in lipid peroxidation marked by overexpression of MDA and TBARS. The production of oxidative stress in the erythrocytes, hepatic and kidney tissues show that sodium sesquicarbonate dihydrate suppresses the antioxidant systems and can therefore be deleterious to the organs and erythrocytes. It is therefore recommended that the use of SSD as food additive and in ethnomedical practices be dissuaded. Further studies are needed to evaluate other potential toxicity mechanisms of this substance.

Authors Contribution

CUN and CON designed and carried out the research work. SCU and IIM supervised the work. All the authors contributed to writing, revising and approving the final draft of the manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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