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CAFFEINATED ENERGY DRINK INDUCES OXIDATIVE STRESS, LIPID PEROXIDATION AND MILD DISTORTION OF CELLS IN THE RENAL CORTEX OF ADULT WISTAR RATS

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ABSTRACT

Over the years, there has been an alarming increase in the production of different brands of caffeinated energy drink (ED) with different flavours that attracts the consumers with the aim of providing energy boost in their day to day activities. This study was conducted to effect of low and high doses ED on the renal histology system. Twenty-five adult male Wistar rats weighing 150-200 g were divided into five (5) experimental groups; Groups A: control, B treated with 2 ml ED, C treated with 2 ml energy drink and discontinued for 7 days, D treated with 4 ml ED, and E was treated with 4 ml energy drink and discontinued for 7 days. Histological analysis using haematoxylin and eosin, and PAS was conducted to ascertain the histoarchitectural integrity of the renal tissue. It was deduced from the study that caffeinated ED caused a significant increase in serum superoxide dismutase (SOD) level as compared with the control while MDA (malondialdehyde) serum activity increased as compared with control and withdrawal groups; increased in Bowman's space, space, decreases the inner epithelial layer of the glomerulus as well as the epithelium of the proximal convoluted tubule. It was shown that ED consumption for a long time could affect the kidney by inducing oxidative stress and altering the histoarchitecture of the renal tissue. However, discontinuation of ED for 7 days shows positive potential of restoring the integrity of the renal cortex.

1. INTRODUCTION

Energy drinks belong to a class of products, in liquid form, that typically contains caffeine, with or without other added dietary supplements.[1] such as taurine, l-carnitine, carbohydrates, glucuronolactone, vitamins, and herbal supplements like ginseng, guarana, yerba mate, cocoa, and kola nut which may increase the caffeine content of energy drinks.[2] Since the increase in consumption of energy drink, ED drink market has grown dramatically, with various brands released worldwide, with a major constituent caffeine ranging from 50mg to 550mg per can or bottle.[3]

Caffeine in ED is one of the most commonly consumed alkaloids worldwide, which can be found in other forms like coffee, tea. However, consumption of caffeinated ED in high dose has been shown to cause abnormal stimulation of the nervous system.[4] as well as adverse effects in the cardiovascular, hematologic, and gastrointestinal systems.[2] With ED becoming a worldwide phenomenon, the short- and long-term effects of these beverages must be evaluated more closely in order to fully comprehend the psychological impact of these products.[5] It was reported the that caffeinated ED can potentiate neuronal oxidative stress through stimulation of glia cells anti-inflammatory response mechanism in a high dose and long term duration of consumption.[6]. Stimulation of anti-inflammatory response of glia cells in a high dose and long term duration of consumption.[6] Energy drink is widely consumed by adolescents and adults because of its ability to improved physical and cognitive performance after its consumption[7] and this report also observed side effects associated with mixing Caffeinated energy drink with theophylline leading to blockage of renal reabsorption resulting in diuresis and natriuresis health conditions.

This study was carried out evaluate changes in the serum activity of SOD, lipid peroxidation enzyme marker MDA and histology of the renal cortex following oral ingestion of low dose (2ml) and high dose (4 ml) of energy drink.

2. MATERIALS AND METHODS

2.1: Experimental Animal Procurement and Breeding

Twenty–Five (25) Adult Male Wistar rats with weights ranging between 150-200 g were obtained from National Veterinary Research Institute (NVRI), Vom-Jos, Plateau State Nigeria. The animals were kept in well ventilated cages and housed in the Animal House of Bingham University in standard laboratory conditions; room temperature [37°C], the humidity of 50-60% and a 12 h dark/light cycle and access to pelleted rat feed and water

ad libitum. All experiments were performed according to guidelines for the Care and Use of Animals in Research as documented in the National Research Council Guide (NRC 2011). Animals were allowed to acclimatize for two weeks before experimentation commenced.

2.3 Experimental Drink

2.3.1 Experimental Drink Constituent

Energy drink used for this study was manufactured by Power Horse Company, Austria. Each can of ED measure 250ml which is made up of the following chemical constituents; Caffeine (32mg/100ml), B-group vitamins, glucuronolactone, Sucrose and Glucose, Taurine, Inositol, Niacin, and Pyridoxine HCL. LD_{50} of energy drink is five (5) cans per day equivalent to ingestion of 400mg of caffeine per day.[8]

2.3.2 Mode of Administration of Energy Drink

Animal studies revealed that ED consumption has been evaluate to have detrimental effect on the kidney,[9] with several effects showing renal failure, oxidative damage to kidneys of mice pups, liver and kidney alteration in rats.[10-12] ED used for this study (Power Horse) was obtained from a drink factory in Karu, Nassarawa State Nigeria. The LD_{50} for ED given orally is 2-10 mg/kg. This study made use of LD_{50} of 2-4 ml/kg which was similar to,[11] whose study made use of 2.5 ml/kg of ED for low dose and 5ml for high dose. The purpose of using different volume of ED was to investigate the effect of low and high consumption of ED on the kidney, bearing in mind the variation to which ED is been consumed. Mode of administration of ED was done according to method described by,[12] modified as follows; Power horse ED was emptied into an oral calibrated gavage for easy measurement of the required dose of 2ml for low dose and 4ml for high dose administered. The dosage was in accordance to,[5] experimental procedures were 2ml and 4ml ED consumption was found to cause alterations in the astrocytes of the prefrontal cortex.

2.3.3: Experimental Design

Experimental animals were divided into six (6) groups of five animals per group.

Group A: Control feed with rat pellets and distilled water for 28 days

Group B: Treated with 2ml of Energy drink and feed plus water ad libitum for 21 days.

Group C: Treated with 2ml of Energy drink and feed plus water *ad libitum* for 21 days and discontinued for 7days

Group D: Treated with 4ml of Energy drink and feed plus water ad libitum for 21 days

Group E: Treated with 4ml of Energy drink and feed plus water *ad libitum* for 21 days and discontinued for 7 days

2.4 Animal Euthanasia, Tissue Collection, and Tissue Processing

Twenty-four hours after the last administration of ED to the experimental animals, the animals were euthanized, using cervical dislocation under mild anesthesia. An incision was made on the midline of the abdomen; both kidneys (right and left) were excised. The kidneys were washed under a slow running tap water and preserved in a 10% formal solution for histological evaluation, using automatic tissue processor (Leica TP 1020) for histological processing, the following steps were taken: fixation, dehydration, clearing and filtration,[13,14] Tissue blocks were section on rotatory microtrome set at 5µm thickness. Haematoxylin and Eosin stain for histological stain to show the general cytoarchitecture of the renal cortex while Periodic Acid Schiffs' reaction (PAS) was carried out to demonstrate mucin granules and cell membrane.[13]

2.4.1 Histological Staining Procedure Using H and E for Renal cortex Cytoarchitecture

The well labeled renal cortex (RC) slide sections were arranged in a staining slide racks with handles, which was immersed in two changes of xylene for five minutes each to de-wax and was then hydrated by immersing the slides in descending grades of alcohol: 100%, 90%, and 70% for two minutes each respectively. They were rinsed in running tap water to wash off the alcohol for three minutes before taking the sections for staining with Haematoxylin for five minutes; stain was differentiated in 1% acid alcohol two-three seconds and further rinse in running tap water for about three minutes to allow bluing. The slides were counterstained using Eosin for about three minutes, rinse in water and dehydrated through ascending grades of alcohol: 50%, 70%, 90%, and 100% for a minute each. The slide sections were then cleared briefly in xylene set to dry in an oven at 80°C for sixty seconds and then covered with microscopic cover glass (22 mm X 50mm) with the aid of DPX (Distrene Plasticizer Xylene) mountant.[15-17]

2.4.2 Histological Staining Procedure Using Periodic Acid Schiff Demonstrate Mucin

Labeled block of RC tissue were fixed and oxidized in 0.1%, 0.5%, 1.0% or 2.0% periodic acid in 10% formalin for 48 hr, then washed thoroughly under a slow running tap water for 2 to 4 hr. the blocks were stained in Schiff reagent for 3 hr with excess stain removal by washing under a running tap water for 2 hr after which they were placed in 2 changes of sulfite, rinse for 1 hr each. The tissue blocks were then washed in tap water for 1 hr, dehydrate in ascending grades of absolute alcohol; 70%, 85%, and 95% for 3 hr each and clear in xylene for 4 hr. RC tissue were embedded in 2 changes of paraffin for 2 hr and 1 hr respectively and the RC tissue were sectioned at 5µm. the RC sections were deparaffinized and hydrated through graded alcohols to distilled water and dehydrated through graded alcohols to xylene. RC sections were mounted using synthetic resin.[18]

2.5 Statistical analysis and Tissue Photomicrography:

Statistical analysis was performed using one-way ANOVA to plot the (mean ± S.E.M.) for each group. Values were compared with the mean value of the

control group, and differences were considered significant when the *p* value was less than 0.05. Thus, $p<0.05$(*). Tissue photomicrography were captured using an Olympus (Tokyo, Japan) binocular LLight microscope connected to a 5.0-megapixel Amscope camera (Amscope Inc., Irvine, CA, USA) at 100x.

2.6 *SOD* (*superoxide dismutase*)

The tissue homogenates were diluted in carbonate buffer (pH 10.2) in preparation for the SOD assay (1:10). The colorimetric reaction utilizes the ability of SOD to inhibit free radical production form epinephrine following the effect of xanthine oxidase. 0.1 ml of microsome was diluted in 0.9 ml of distilled water to make a 1 in 10 dilution of microsome.

An aliquot of 0.2 ml of the carbonate buffer pH 10.2 to equilibrate in a cuvette and the reaction started by the addition of 0.3 ml of 0.3 M adrenaline. The reference cuvette contained 2.5 ml of carbonate buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of distilled water. The increase in absorbance at 480 nm was monitored every 30 s for 150 s and using a spectrophotometer.

2.7 Enzyme indicating Lipid peroxidation (MDA)

This method is based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde: an end product of lipid peroxide during peroxidation. On heating in acidic pH, the product is a pink complex which absorbs maximally at 532 nm and is extractable into organic solvents such as butanol. An aliquot of 0.4 ml of the sample was mixed with 1.6 ml of Tris–KCl buffer to which0.5 ml of 30% TCA was added. Then 0.5 ml of 0.75% TBA was added and placed in a water bath for 45 min at 80°C.This was then cooled in ice and centrifuged at 3000 × *g*. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. The MDA level was calculated. Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 105\ m{-}1cm{-}1$.*2.3.*

3.0: RESULTS

3.1: Energy Drink consumption increased body weight

This study shows that the oral administration of energy drink caused a significant increase in body weight. As shown in Figure 1 below, there was an overall increase in body weight gain. However, there was a statistical significance increase in body weight in Group B&E given 2ml&4ml of Energy drink respectively as compared to the Control group (A). Groups C&D had a significant decrease in body weight as compared to B&D treated energy drink group at P<0.05.

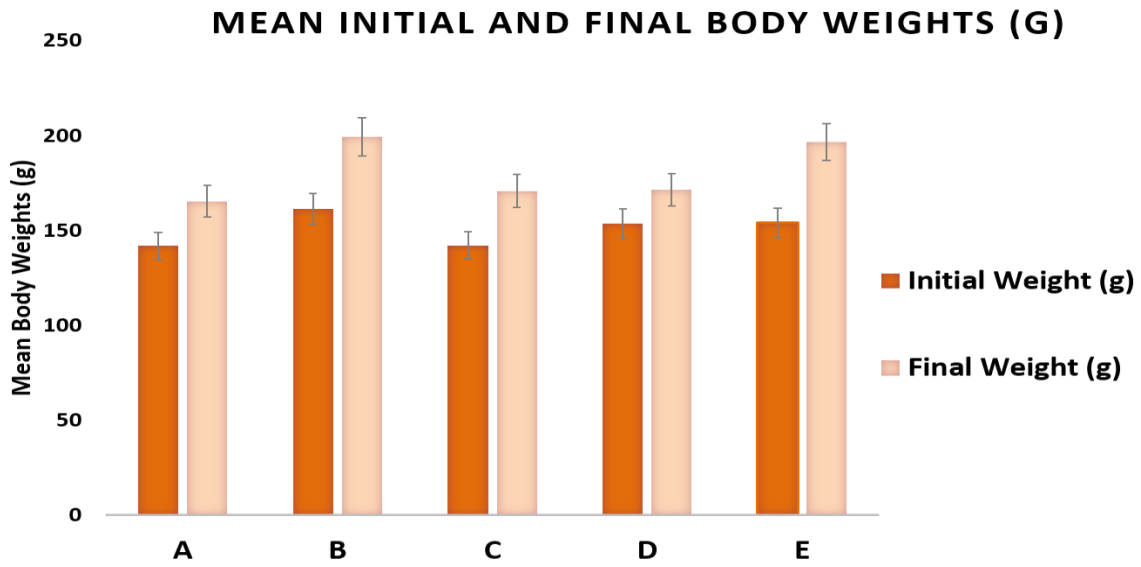


Fig. 1: Graphical representation of the mean body weight of experimental animals following Energy drink oral administration in Adult Male Wistar rat. Data analyzed using ANOVA (*) statistical Significance taken at P<0.05. Legend: A= Control group; B= Low dose group of 2ml ED, C= Low dose of 2ml ED with Withdrawal, D= High dose group of 4ml ED, and E= High Dose group of 4 ml ED with withdrawal. Data Expresses as Mean ± SEM. *P<0.05: *B vs A, C and D; *E vs D, A.

3.2: 3.2: Energy drink consumption mediates activation of Lipid Peroxidation in renal cortex

This results is to showcase the correlation between energy drink and lipid peroxidation profile, using MDA as marker. Activity of MDA. As shown in Figure 2. There was a statistically significance increase in MDA activity in 2ml (B) and 4ml (D) energy drink treated animals as compared to the control group (A) at P<0.05. However their respective withdrawal C and E had a statistically significance reduction in lipid peroxidation as compared to treatment groups B and D respectively at P<0.05.

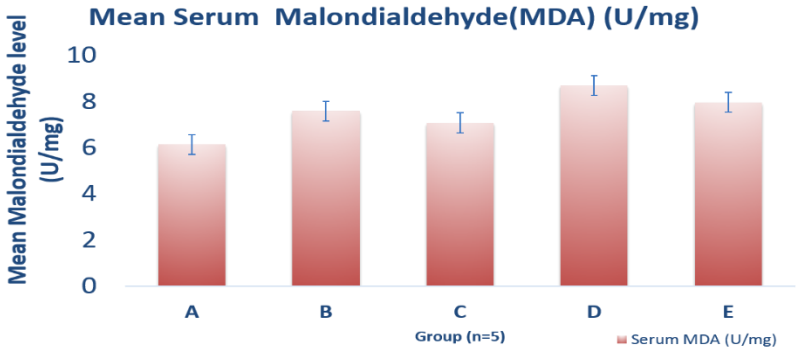


Fig 2: Graphical representation of the Mean Malondialdehyde (MDA) activity in experimental animals following Energy drink oral administration in Adult Male Wistar. Data analyzed using ANOVA. (*) statistical Significance taken at P<0.05. Legend: A= Control; B= Low dose (2ml) C= Low dose (2ml) Withdrawal Group; D= High dose (4mls) and E= High Dose Withdrawal Group. Data Expresses as Mean ± SEM. *P<0.05: *B vs A, C and D; *D vs A and E.

Energy drink consumption increase superoxide dismutase (SOD) antioxidant enzyme activity: Superoxide dismutase (SOD) is an antioxidant enzyme marker, used to determine antioxidant activity in cells. In this present study, energy drinks activity dose dependent increase antioxidant enzyme activity. 2ml of energy drink (B) shows a statistically significant (P<0.05) increase in SOD as compared to the control (A) and their withdrawal group; which shows a statistically significance reduction as compared to their Control group A. Group D given 4ml energy drink shows had a statistical significance increase in SOD as compared to the 2 ml (B) treated group. Both recovery groups C and E showed a statistically significance decrease as compared to the Control and respective treatment groups B and D.

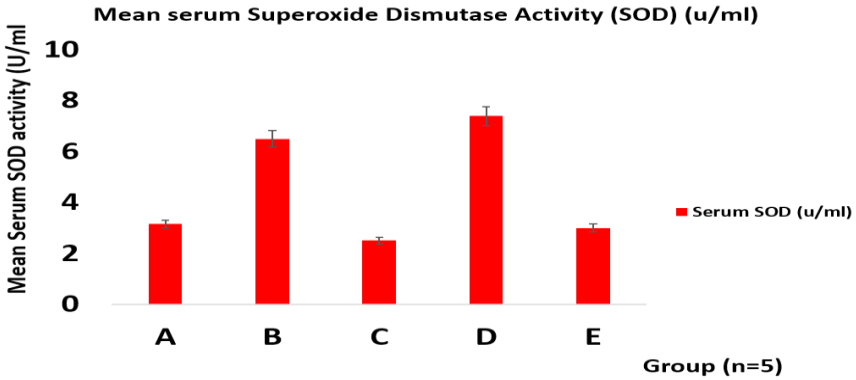


Fig. 3: Graphical representation of the Mean Superoxide Dismutase (SOD) activity in experimental animals following Energy drink oral administration in Adult Male Wistar. Data analyzed using ANOVA. (*) statistical Significance taken at P<0.05. Legend: A= Control; B= Low dose (2ml) C= Low dose (2ml) Withdrawal Group; D= High dose (4mls) and E= High Dose Withdrawal Group. Data Expresses as Mean ± SEM. *P<0.05: *B vs A and C; *D vs A and E.

3.4: Energy drink consumption results in loss of renal cortical cells integrity as shown in Histological stain H&E and PAS

As a principal stain, H&E staining technique is used to diagnose and study disease conditions, with the aim of determining morphologic changes in tissues. Deduced from this study, histological appearance of the renal cortex of Control (4A) shows well stained renal cortical cells, glomerulus (GL), normal Bowman's capsular space (BS) and proximal tubule epithelium undistorted. 4B-2ml treated and 4D–4ml treated group both shows dose renal cortex disruption characterized by palely stained renal cortical cells, increase Bowman's Capsular space with loss of glomerular and proximal epithelium cellular integrity {Figure 4B & 4D} as compared to their respective control group {Figure 4A}. The recovery group/withdrawal groups (4C & 4E) are characterized by presence of red blood cells and regenerating renal cortical cells {Figures 4C & 4E}.

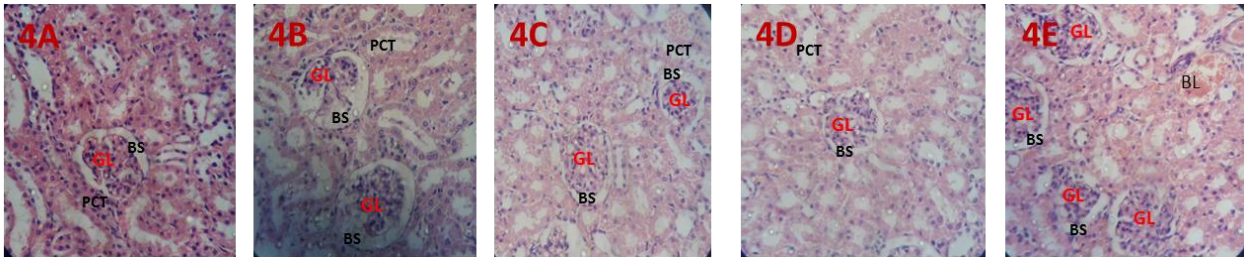


Fig. 4: Micrograph showing section of renal cortex of the kidney of experimental animals following Energy drink oral administration in Adult Male Wistar. Stained with Haematoxylin and Eosin Stain (H and E). Magnification: 400x. Legend: A= Control; B= Low dose (2ml) C= Low dose (2ml) Withdrawal Group; D= High dose (4mls) and E= High Dose Withdrawal Group. Figure legend: PCT: Proximal convoluted tubule, GL: Glomerulus, BS: Bowman's space

3.4: Energy drink consumption results in loss of renal cortical cells membrane integrity as shown in periodic Acid Schiffs' (PAS) stain

The control group PAS stain demonstrates intact cell membrane and presence of Mucin granules, as shown in Figure 5A. Those treated with 2ml and 4ml of Energy drink (5B & 5D) shows severe loss of cell membrane, palely stained cells and loss of mucin granules as shown in Figure 5B and 5D. The withdrawal groups 5C & 5D show regeneration of the cell membrane and few mucin granules as shown in Figures 5C and 5E.

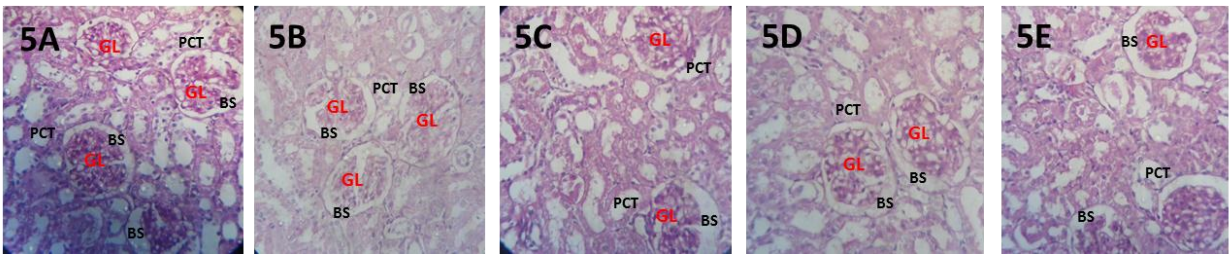
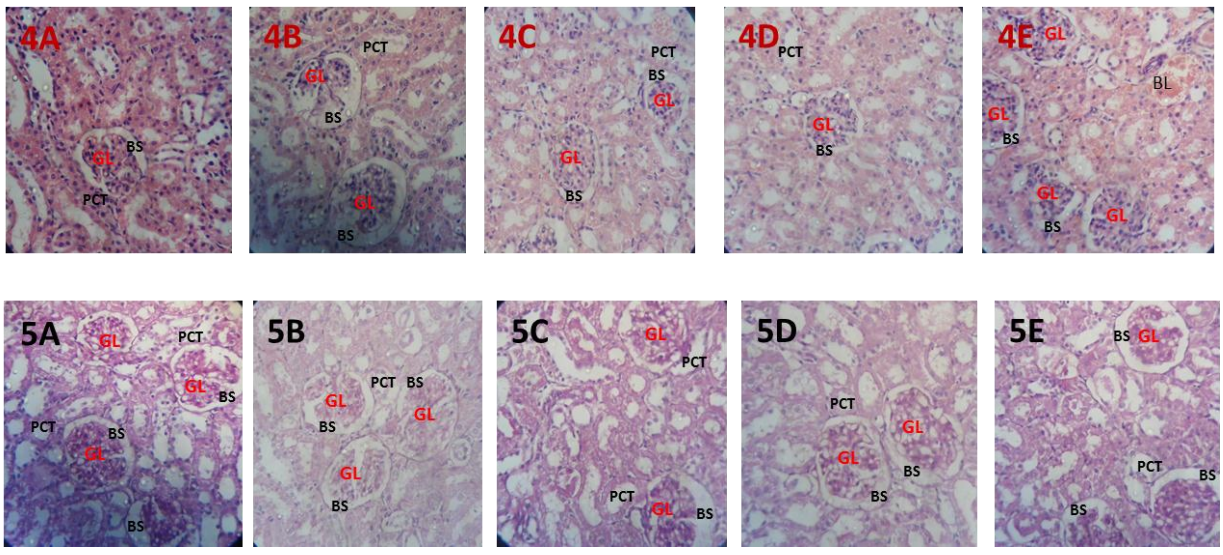


Fig. 5: Micrograph showing section of renal cortex of the kidney of experimental animals following Energy drink oral administration in Adult Male Wistar. Stained with Periodic Acid Schiffs' (PAS) Stain Magnification: 400x. Legend: A= Control; B= Low dose (2ml) C= Low dose (2ml) Withdrawal Group; D= High dose (4mls) and E= High Dose Withdrawal Group.



Comparing the structural changes of kidney cells stained with H&E and PAS, the control groups 4A&5A shows well defined Bowman's space, glomerulus and proximal convoluted tubules with distinct cuboidal epithelium that could be observed. 4B&5B fed with low dose (2ml) ED revealed slight distortion in histoarchitecture of the kidney were 4B shows a significant increase in the Bowman's space, as compared to 5B, reduction in glomerular cytoarchitecture was observed in 4B as compared to 5B. However, group 4B&5B shows a significant decline in the cuboidal epithelium as compared to the control. Withdrawal groups 4C&5C showed significant restoration of the lost cytoarchitecture, while a significant increase in

Bowman's space observed in 5C was something remarkable. Photomicrograph of 4D&5D fed with high dose ED (4ml) showed similar distortion in kidney histoarchitecture, reduced proximal convoluted tubule integrity and surrounding epithelium with congested glomerulus observed in 4D. The recovery group 4E revealed significant restoration of the kidney's histoarchitecture with the presence of blood vessels (BL), showing that the function of the kidney is been restored.

DISCUSSIONS

The present study provides significant evidence that low and high consumption of ED for a long time is associated with changes in the histoarchitecture of the Kidney. The kidney is involved in the removal of metabolic waste and could be affected by a continuous or daily routine that includes high consumption of energy drinks which could alter its function and histological integrity.[19] High consumption of energy drinks has been on the increase due to the presence of sweeteners and strong stimulants in form of caffeine that tend to make its consumers addicted. Figure 1 shows a significant increase in body weight of Group B which received a low dose of ED as compared with Group A. The significant increase in body weight of Group B over Group C could be attested to the presence of large amounts of sugar in form of sucrose, glucose or fructose which are by-products of carbohydrate metabolism of which constant consumption for a long time could induce weight gain and other associated disease condition like diabetes which was in agreement with.[20] Group C&E weight loss in comparison with Group B&D could be traced to the discontinuation of ED. It is a general practice that people consume ED with unhealthy foods like junks that contain high calories and are rich in carbohydrates which could cause increased body weight as observed by.[21]

This study recorded a significant increase in serum SOD levels in Group B&D which could be attributed to certain ingredients found in ED that are responsible for causing oxidative damage to the kidney. It has been observed that for ED to cause oxidative damge to an organ, the ED must contain several mixture of different ingredients with high antioxidant ability or the ED has a high synergistic oxidative consequence.[22,23] Serum SOD is an antioxidant enzyme whose antioxidant role are to protect the renal tissues from free radical attack.[25] However, high levels of serum SOD in the renal tissues show a high deposit of superoxide radical produced in the tissue during metabolism with a corresponding production of hydrogen peroxide and oxygen through a catalytic reaction. In high serum SOD levels, hydrogen peroxide deposited in the renal tissue has the potential of producing a toxic effect that could obstruct the normal functioning of the renal tissue through an increase in oxidative stress in the tissue. The presence of high levels of oxidative stress in the renal tissue inhibits the protective mechanism of the kidney against oxidative stress which could induce impairment in the renal tissue.

This study reveals a significant decrease at $P<0.05$ in serum SOD levels in the withdrawal group (Group C&E) as compared with Group B&D revealing a decline in oxidative stress after withdrawal from ED. Elevated MDA levels in Group B&D ED exposed groups could be linked to a decrease in the cellular defense mechanisms,[22] of the kidney, inducing oxidative stress and renal impairment. The increased lipid peroxidation levels observed in this study indicate an increase in ROS production and oxidative stress,[12,22]

Deduced from the study, high exposure without withdrawal (Group 4D&5D) from ED was found to be toxic to the renal tissue by increasing the Bowman's space, decrease the inner epithelial layer of the glomerulus. Group 5D stained with PAS was able to indicate that high consumption of ED generally reduced the histological structure of the kidney, reduction of the cuboidal epithelium of PCT. However, this study also showed that withdrawal from ED for 7 days has the possibility of restoring the histoarchitecture of the kidney as shown in Figure 4E&5E. Analyses from this study are quite similar to,[22] whose research observed several detrimental effects on the renal tissues such as; glomerular degeneration, alteration, and dilation of the renal tubules, congestion of blood vessels after exposure to energy drink. The histological distortion following ED consumption shows the diuretic effect associated with a consistent intake of ED either in low or high doses for a long time. This aligned with,[27] whose study reveals that for ED to have a hypokalemia effect, the diuretic effect would have occurred as it also causes high creatinine kinase levels as well as renal impairment. However, comparing the result from the study with that of,[12] their result showed no histological associated with consumption of ED on the kidney. However, they observed elevated excretion in N-acetly – β- d-glucosaminidase (NAG) activity, understanding it as a lysosomal enzyme abundantly present in the proximal tubular cell; the authors observed renal injury due to elevated levels of the enzyme in the urine.

CONCLUSION

This study demonstrated that caffeinated energy drink could induce oxidative stress following elevated level of MDA which further obstruct the normal functioning of the kidney. The low and high dose ED consumption lead to several alteration in the histoarchitecture of the renal tissue that includes increase in Bowman's space, reduction in the cuboidal epithelium indicating that ED could alter the normal functioning of the renal system.

Recommendation: Following the effect observed from this study, it is recommended that consumption of ED should not be done on a periodic basis to limit the detrimental effect. Further study should be carried out on each ingredient found on ED to know the exact effect of each and how it could be avoided.

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Authors contribution: Memudu A.E and Olarenwaju E designed the experimental methodology while Osahon I.R was involved in the manuscripit write up and data analysis. Each authors contributed equally to the manuscript write up.

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Consent for publication: Not applicable.

Competing interests: Authors declare that they have no competing interest.

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