



Effects of Administering Soft Drinks on Some Biochemical Parameters and Histopathology of Liver in Wistar Rats

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ABSTRACT:The ruling of the Lagos High Court at Igbosere, Nigeria on the 13th March, 2017 gave birth to this research work. The court ordered NAFDAC to enforce the Nigerian Bottling company (NBC) to write warnings on the potential dangers of taking soft drinks, especially with Vitamin C on their drinks. Twenty-five albino rats (weighing 194±9.5g) were randomly assigned to five groups (n=5). The soft drinks were labeled A, B, D, "A + Vitamin C" (400mg/20ml) and distilled water (control) were administered orally to groups 1, 2, 3, 4 and 5 respectively for 14 days. The administration was done in accordance with Pedro Montilla's method (400ml/70kg body weight). The rats were

sacrificed and subjected to biochemical and histological analysis. The levels of AST, ALT, TB and DB for soft drinks B, A, D and "A+ Vitamin C" were significantly higher ($P < 0.05$) than the control. This is a strong marker for potential liver dysfunction. Similarly, A and "A+ vitamin C" significantly increased ($P < 0.05$) bad cholesterol while weight and histological results of rats are normal. Moreover, the amount of soluble sugars found in D is more than double the level approved by the Standard Organization of Nigeria (SON). All the soft drinks are biological culprits as none is totally free of potential toxicity. This research supports the ruling of the High Court to include warnings of the potential dangers but consistently maintained that Vitamin C when added to soft drink A reduced its potential toxicity.

Keywords: lipid profile, liver, histology, LDL, A + vitamin C

I. INTRODUCTION

Everyone in Nigeria loves the thrill of carbonated drinks in their mouth. Soft drinks are sweetened, coloured and flavoured water-based non-alcoholic beverages, mostly with balanced acidity, which are always available to drink directly (Eyong et al., 2010). They are available in different forms, colours and tastes. In prolific quantities for young and old to relieve and satisfy thirst (Adepoju-Bello *et al.*, 2012). A prove of our love is the following statistics. The consumption of non-alcoholic beverages in Nigeria was rated at 159.85g/person/day in 2007 (FAO, 2011). We have also realized that the specific non-alcoholic beverage this statistic is referring to are soft drinks, many people consume soft drinks on a daily basis in Nigeria (Sodamade, 2014)

Soft drinks may look harmless and innocent because they provide pleasant flavoured minerals, antioxidants and fibers, which are essential vehicles for hydration. They are also absorbed more readily than water (because of their osmolality). They can replace lost salt and energy quickly (Jasmine, 2012). However, they are not completely harmless. All carbohydrates are important sources of energy but soft drinks generally contain soluble sugars which are easy to administer (Euromonitor, 2014). Health profile of a community is greatly influenced by its nutritional status and life style, that is the reason why we need to be sure if soft drink consumption is really necessary for the citizens of our dear country, Nigeria (Rakesh et al., 2013). To achieve its characteristic taste, sugar is one of the essential ingredients added to soft drinks. It was suggested that too much sugar could pose harmful health effects as people could develop diabetes, obesity, heart disease, and other complications from excess consumption (France, 2000). To ascertain how harmful or helpful a substance is to our biological system. It is also pertinent to determine the effect of that substance on the liver. The liver is one of the largest organs in the human body and the major site for intense metabolism and excretion (Ahsan, 2009). The liver helps in detoxification and excretion of many endogenous and exogenous compounds. Therefore any injury to the liver or impairment of its functions may lead to adverse implications on the health (Subramaniam, 2015)

A Lagos High Court in Igbosere has ordered the National Agency for Food and Drug Administration and Control (NAFDAC) to mandate the Nigerian Bottling Company PLC (NBC), to include a written warning that the content of the bottles cannot be taken with Vitamin C (Punch Newspaper, 2017). The court also declared that NAFDAC has failed Nigerians by its certification as satisfactory for human consumption, products which in the United Kingdom failed sample test for human consumption (Punch Newspaper, 2017). This court order gave birth to this research. Therefore, this study is aimed at determining the effects of soft drinks on the liver function, liver histology and weight of male wistar rats. Moreover, the amount of soluble sugar present in each brand of soft drinks will be ascertained

II. MATERIALS AND METHODS

2.1 Experimental Design

Seven weeks old, twenty-five adult male wistar rats weighing 93.0 ± 7.0 g were bought from the Animal house of NVRI (National Veterinary Research Institute) Vom, were used and randomly assigned to five groups. Each was allowed free access to standard pellet diet and drinking water for a period of 14 days. The soft drinks were labeled A, B, D, "A+ Vitamin C" (400mg/20ml) and distilled water. "A+ Vitamin C" (400mg/20ml) meant a solution of 400mg vitamin C was dissolved in 20ml soft drink brand labeled A to form a concentrated solution. This was administered orally using syringe and tourniquet to each rat according to the kg body weight (4000mL/70-kg body weight) (Pedro *et al.*, 2006). The animals were individually housed in wire cages in an animal house with 12h-light/dark cycle and the rats were weighed every day.

2.2 Preparation of Samples for Biochemical Analysis

The rats were sacrificed after 14 days treatment under anesthesia with chloroform. Blood samples were obtained approximately 24 hours after the last fluid consumption. The blood samples were collected in plain plastic tubes. The liver was carefully removed using dissecting scissors from the animal and blotted on filtered paper, weighed and stored in 10 percent formalin for further analysis (Geetha, 2011).

2.3 Determination of Parameters

Total Cholesterol (CHOL), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Alkaline phosphatase (ALP), Alanine amino transferase (ALT), Total Bilirubin (TB), Alkaline phosphatase (ALP) Direct Bilirubin (DB) and Aspartate amino transferase (AST) were determined by the kit method of Randox according to the principle explained by Geetha (Geetha, 2011; Ranjna, 2000)

2.4 Total Soluble Sugars

Four samples of each brand of soft drink were used for spectrophotometric analysis, to make a total of 16 samples analyzed. A, B, D, "A+ Vitamin C" were analyzed for total soluble sugars using spectrophotometric

technique which is compared with the specified standard of the standard organization of Nigeria (SON) 7.00 – 14.00 g/100ml according to the method described by Association Official Methods of Analysis (AOAC, 2012).

2.5.Histology

Tissues were harvested and fixed in 10% formalin for 3 days, cut into thin slices of 5mmX 2mm X 1mm thick and then processed using The SPIN tissue processor, according to the specification of Avwioro (Avwioro,2011) and Choji (Choji *et al*,2015).

2.6. Statistical Analysis

All the grouped data were statistically evaluated with SPSS software version 21. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SEM.

III. RESULTS

3.1 The Results of Serum Biochemical Parameters

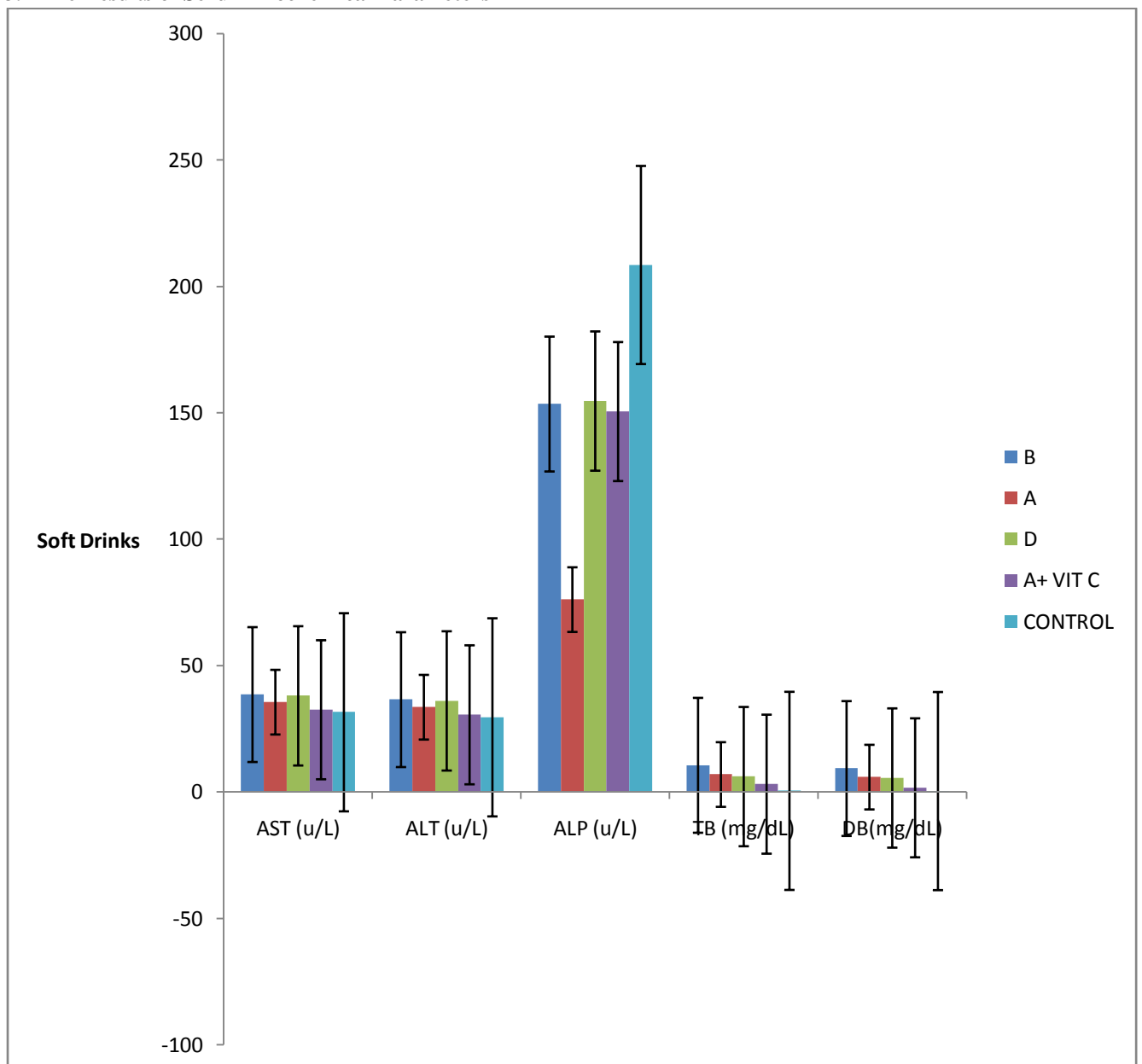


Fig.1: The sera results of AST,ALT,ALP, TB and DB

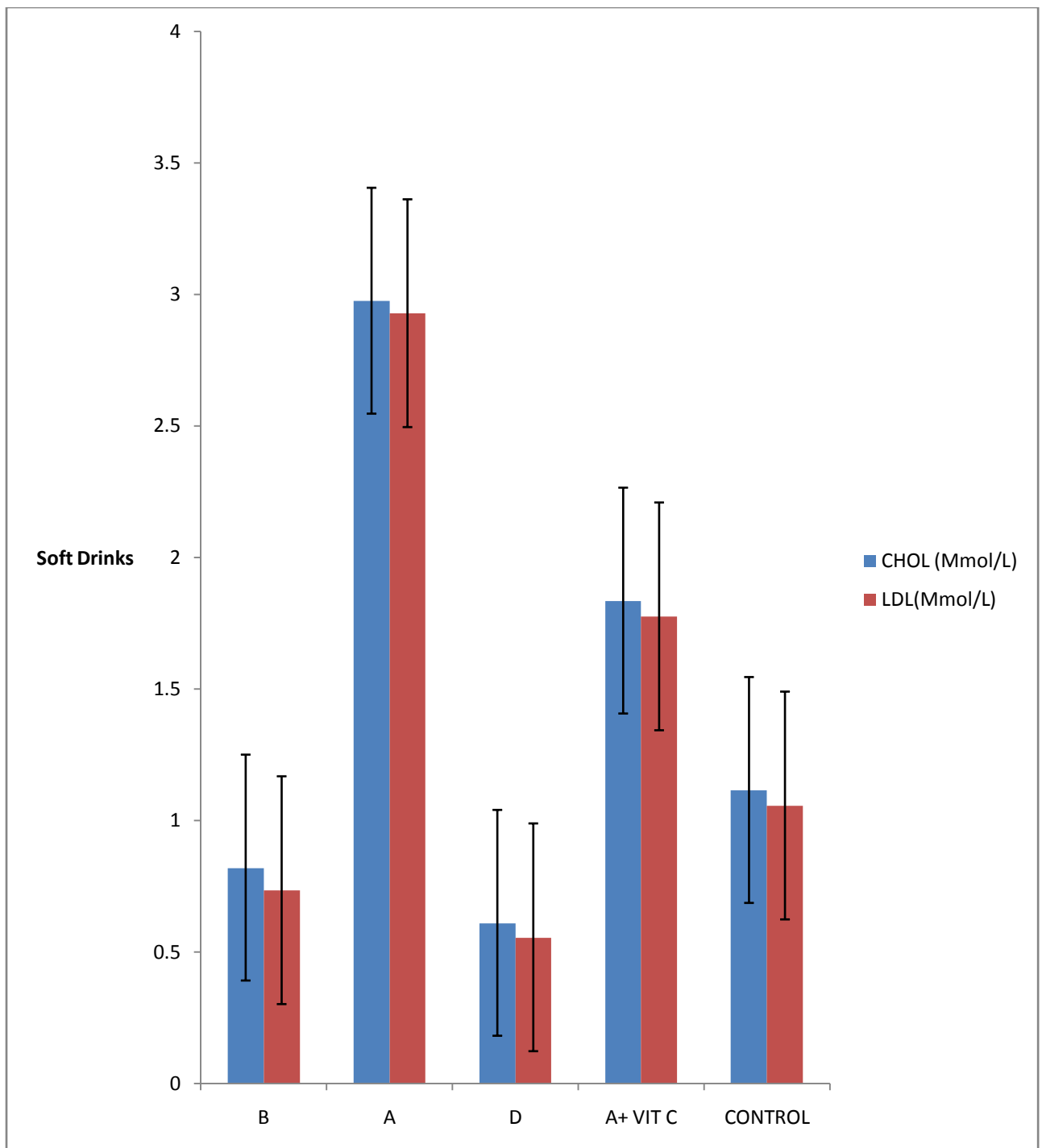


Fig. 2 The sera results of CHOL and LDL.

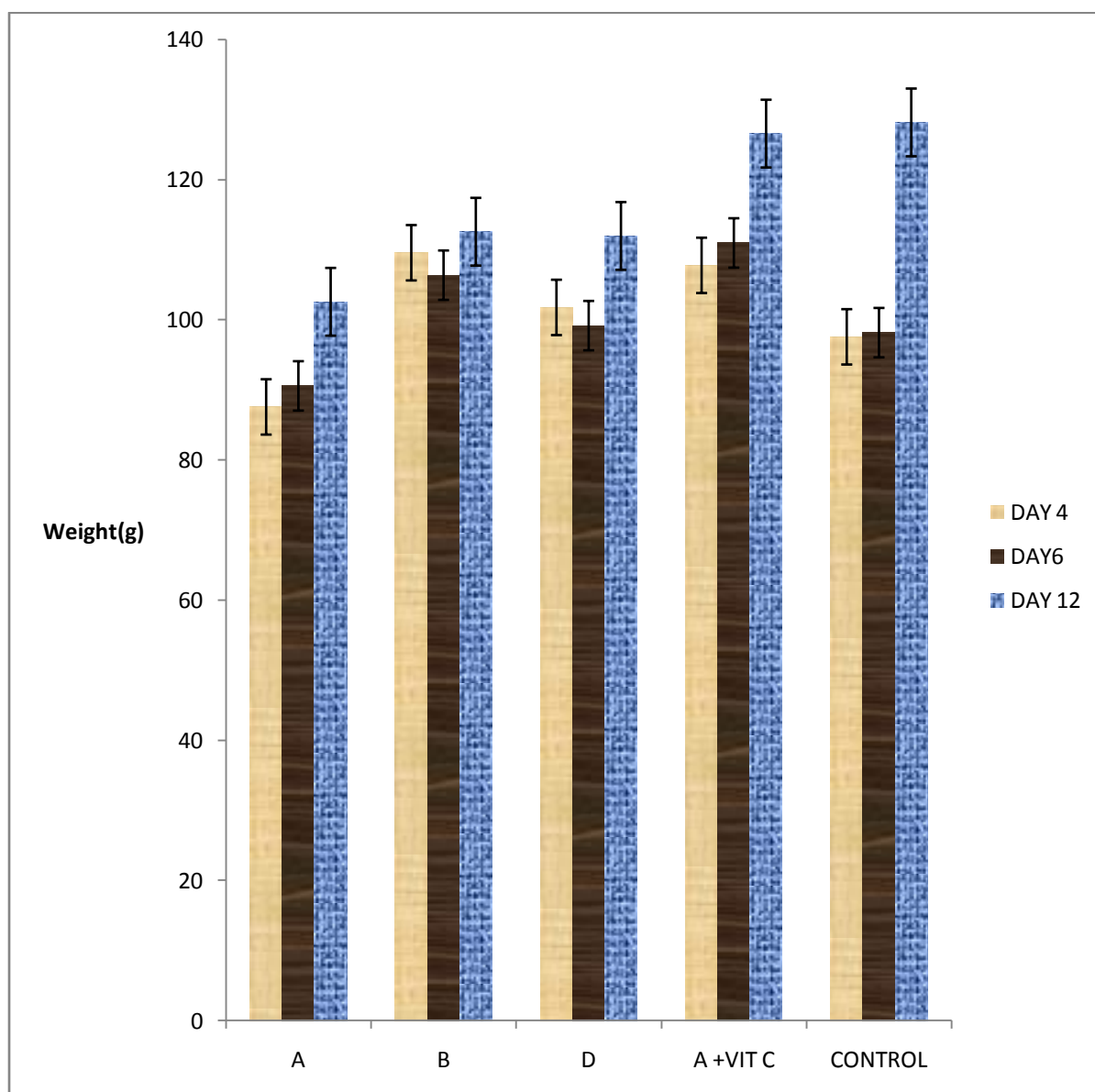


Fig. 3: Results showing weight of Rats for Days 4, 6 and 12

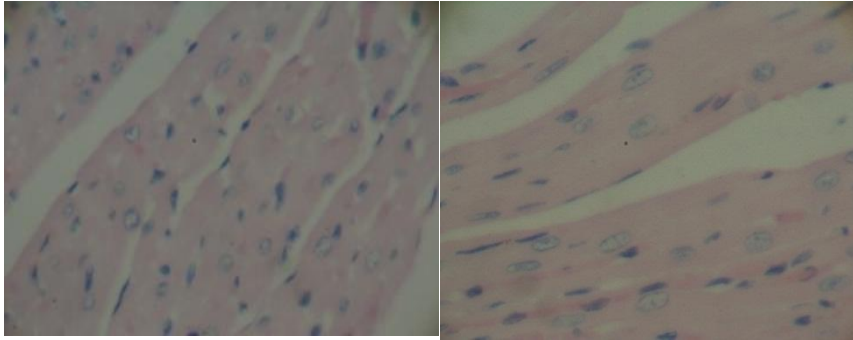
Values are expressed as mean ± SEM. Mean values are compared using One-Way ANOVA. Level of significance was evaluated using Duncan’s Multiple Range Test (DMRT) at $P \leq 0.05$.

3.2. Results Showing Total Soluble Sugar Analysis

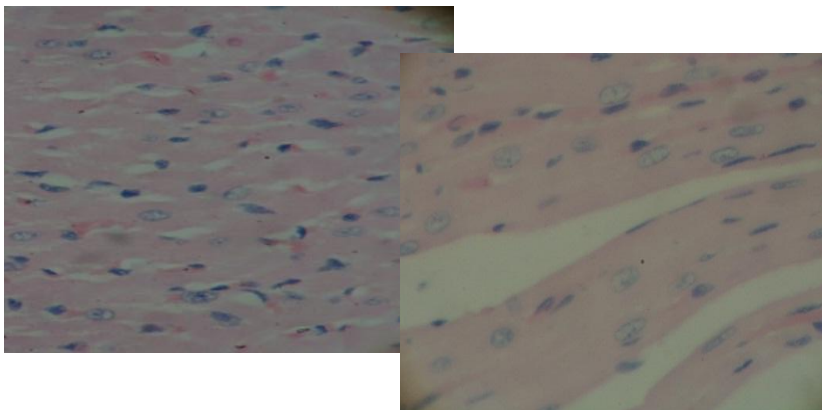
SOFT DRINKS	SOLUBLE SUGAR LEVEL
A	6.5439g/100ml
B	9.114g/100ml
D	38.691g/100ml
A+VITC (400mg/20ml)	0.6528g/100ml

Standard Organization Of Nigeria (SON) Stipulated Standard 7.00-14.00g/100ml.

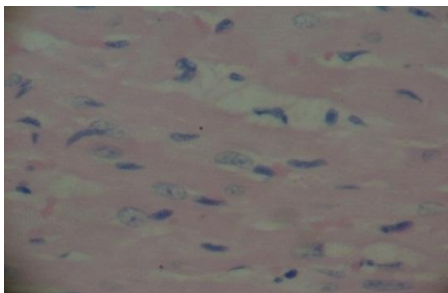
3.3. Histopathology of Liver Samples



B A



DVITC+A (400mg/20ml)



CONTROL

Fig.4 : Liver tissue of wistar rats administered with soft drinks labeled B, A, D and “A +Vitamin C” for 14 days. Intact nuclei are seen within the intact cytoplasm. The liver tissues seen are normal, with no visible lesion and the liver architecture is not from the control.

IV. DISCUSSION

There were no significant differences ($P > 0.05$) among all the soft drinks treatments for Total Protein, Albumin and HDL cholesterol determinations. The levels of AST and ALT were significantly higher ($P < 0.05$) for B, A, D and “A+ Vitamin C” treatments. In the same manner, the levels of Total Cholesterol and LDL Cholesterol were significantly higher ($P < 0.05$) for A and “A+Vitamin C” treatments than the control. Moreover, the levels of TB and DB were significantly higher ($P < 0.05$) for all the treatments than the control. Finally, the level of ALP was significantly lower ($P < 0.05$) for B, A, D and “A+Vitamin C” treatments. These significant differences are determined in comparison with the control.

The result of this study shows that there were significant increases ($P < 0.05$) in the levels of AST, ALT, TB and DB for B, A, D and "A+ Vitamin C" treatments. Moreover, it was A and "A+Vitamin C" that significantly increased ($P < 0.05$) the level of LDL cholesterol. According to a study carried out by Subramaniam, he discovered that levels of many biochemical markers like transaminases, alkaline phosphatase, bilirubin, triglycerides and cholesterol are elevated in liver diseases (Subramaniam *et al.*, 2015). Implying that all the soft drink treatments that triggered the rise of these biochemical markers are potentially dangerous. Increase in the level of LDL-Cholesterol is not encouraged in the system. LDL Cholesterol is also known as bad cholesterol, its presence is a sign for cardiovascular disease. This claim was supported by Alrawi in 2017 who discovered that LDL cholesterol and HDL cholesterol are key factors in pathogenesis of atherosclerosis and coronary artery diseases. Increased LDL cholesterol levels and decreased HDL cholesterol levels are associated with increased risk for coronary heart disease (Alrawi, 2017). The parameters of this research expressed identical behaviour with the findings of Goye, who detected that soft drinks showed high level of total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein (Goye *et al.*, 2014). It is noteworthy of mentioning that the level of ALP was significantly lower ($P < 0.05$) for all the treatments, this is the only instance when all the treatments fared well.

Adepoju-Bello *et al.* (2012) were the first to report on the presence of heavy metals in soft drink samples marketed in Lagos. They found out that 15% of the soft drink samples randomly selected at the market contained lead, 20% contained cadmium; nickel was detected in 30% of the samples, and 5% contained chromium. They concluded that Health Authorities and Soft drink producers should pay attention to this observation so that they can save the nation. The average value of the amount of soluble sugars of soft drink samples considered were compared to the standard value given by the Standard Organization of Nigeria (SON) (7.00-14.00g/100ml). The value of soft drink D was apparently far higher than the values recommended by SON. Excessive consumption of sugar has also been traced to heart disease, arteriosclerosis, mental illness, depression, senility, hypertension and cancer (Cynthia *et al.*, 2011). Sugars also have an extremely toxic effect on the endocrine system and glands such as the adrenal glands, pancreas and liver. (Agbazue *et al.*, 2014). Sugars also cause Insulin resistance which can eventually lead to diabetes, hyperglycemia and hypoglycemia. Sugar is the major cause of dental deterioration-cavities in the teeth, bleeding gums, failure of bone structure and loss of teeth (Sodamide, 2014).

The weight of the rats that received treatment were not significantly different ($P > 0.05$) from the control for most of the days of Soft drink administration, except for days 4, 6 and 12. On day 4, all the rats that received soft drinks B, A and "A+ Vitamin C" weighed higher significantly ($P < 0.05$) than the control. On day 6, it was only B and "A+ Vitamin C" treated rats that have significantly higher ($P < 0.05$) weight than control. Ultimately, on day 12 all the treated rats have weight significantly lower ($P < 0.05$) than the control. This means that the continuous administration of soft drinks does not lead to additional weight gain. Soft drinks are usually absorbed more readily than water (because of their osmolality). They can replace lost salt and energy quickly and are rapidly thirst quenching. (Jasmine, 2012). Since they are readily used up they may likely not elicit additional weight gain.

Finally, histological plates of the liver has no trace of inflammation or necrosis on the cells of the liver. The implication of this is that, systemic effects of acute inflammation like fever, malaise, and leukocytosis will not be a threat to soft drink consumers (Benjamin and Ugbogu, 2004).

V. CONCLUSION

The levels of AST, ALT, TB and DB for B, A, D and "A+ Vitamin C" treatments is a strong marker for liver dysfunction. On the other hand there was significant increase in the level of bad cholesterol for A and "A+Vitamin C" treated rats. However, D was significantly lower for LDL and total cholesterol. No soft drink was significantly different for HDL, TP and ALB treatments. Moreover, the amount of soluble sugars found in D is more than two folds of the upper limit approved by the Standard Organization of Nigeria (SON). Eventually, all the soft drinks treatment did not lead to additional weight gain.

All the soft drinks considered within the scope of this research are biological culprits. None of the soft drinks considered is free of potential toxicity to the liver and cardiovascular health.

This research supports the ruling of the Lagos High Court in Igbosere which ordered the National Agency For Food and Drug Administration and Control (NAFDAC) to henceforth, write warnings on the potential dangers of taking soft drinks, especially with Vitamin C (Punch Newspaper, 2017). This work is not totally agreeing with the presence of Vitamin C. This is because the presence of Vitamin C in soft drink A (A+ Vit C) in all the parameters considered has reduced the adverse effect of A. As far as we are concerned, regular consumption of soft drinks is a national burden that must be discouraged. In fact, the House of Representatives has asked the Nigerian Bottling Company (NBC) to as a matter of compulsion tell Nigerians that two of their products, are dangerous to health. (Vanguard Newspaper, 2018) The Committee also expressed worry that benzoic acid content in Nigeria was much higher than what was obtained in other climes. For instance, while it is 250 milligram per kilogram in Nigeria, it is 150mg per kg in the United Kingdom (vanguard Newspaper, 2018). It is our duty as researchers to inform the society that soft drinks are killing us softly.

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