Moroccan Journal of Biology
Number 16 (2019)

http://www.fst.ac.ma/mjb
e-ISSN: 2351-8456 - p-ISSN: 1114-8756

Effects of simultaneous consumption of food additives on functions and histopathology of Wistar rats Liver

O.P. Femi-Oloye[1], A.M. Olatunji-Ojo[1], A. Owoloye[1], B. Adewumi[1], B.O. Ibitoye[2], F.F. Oloye[3*], F.A Gbore[4]

[1]*Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba-Akoko, Nigeria*
[2]*General Hospital, Owo Ondo State, Nigeria*
[3]*Department of Chemical Sciences, Adekunle Ajasin University, Akungba-Akoko, Nigeria*
[4]*Department of Animal Science, Adekunle Ajasin University, Akungba-Akoko, Nigeria*

Abstract

Histopathological effects of simultaneous consumption of food additives such as sodium benzoate (SB) and ascorbic acid (AA) on liver of Wistar rats were investigated. A total of forty-eight healthy and active male rats (Wistar rats) of weight range80-100 g were allotted into 12 groups (n=4 each). The treatment lasted for 21 non-consecutive days and SB, AA, and there mixtures were orally administered. At the end of the of experiments, Tissue of interest (liver) from both control and treatments groups were excised, blotted and fixed in sample bottles containing 10% buffered formaldehyde. Standard histological procedures were adopted in the assessment of the tissue. The histo-micrographsreveal the presence of degenerated hepatocytes, dilation of the portal vein, cytoplasmic vacuolation, vacuolation of hepatocyte, dilation of central vein and sinusoid as a result of sodium benzoate. However, a high concentration of ascorbic acid was found to be injurious while low concentration ameliorated the effect of sodium benzoate. Also, liver functions were assessed by measuring serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities by colorimetry. It was observed that ascorbic acid has no negative effect on AST, ALT, and ALP activities, because of its anti-oxidative property. However, it lost its anti-oxidative property at high concentrations in Fanta (an example of food with preservatives) due to aggregation. SB had mild or no effect at low concentration on biochemical parameters but had severe consequences as the concentration increases. The effect of SB can easily be ameliorated by AA because AA suppresses the destructive nature of SB.

Keywords: Sodium Benzoate, Ascorbic Acid, Fanta, Histopathology, Liver, Liver function and Wistar rats.

Introduction

As the world population increases, there has been a growing demand for safe food that maintain its nutritional values and organoleptic properties (Lu & Kacew, 2009). Additives are added to food to keep its integrity, in order to meet the demand for safe food (an example is an antioxidant such as ascorbic acid) (Oloye, 2019). These compounds are added into the products for preventing and deferring losses due to microbiologic, chemical or enzymatic changes and for lengthening shelf life (Schacer, 2004). Sodium benzoate is usually used as a preservative in some products from the pharmaceutical, food and cosmetic industries (Lennerz *et al.*, 2015). In the pharmaceutical industry, it is used in the management of various diseases such as liver diseases, multiple sclerosis and disorders of the urea cycle (Yavav *et al.*, 2016). In the food industry, sodium benzoate is used in beverages and foods, as it is effective in preventing the growth of bacteria and fungi during storage (Tsay *et al.*, 2007).

CONTACT F.F. Oloye ✉ bunmi.oloye@aaua.edu.ng 🖃 Department of Chemical Sciences, Adekunle Ajasin University, Akungba-Nigeria

Ascorbic acid (Vitamin C) is a significant antioxidant with several cellular functions. It is found in many fruits and vegetables and it is also synthesised from glucose in the liver of various mammalian species, allowing the maintenance of physiological levels. Ascorbic acid (AA) is involved in a number of metabolic processes in the human body, including those that are important for the optimal functioning of the oxygen energy system. In addition, AA is an important free radical scavenger in extracellular fluids, trapping radicals and protecting bio-membranes from peroxide damage (Salah *et al.,* 2010; Adikwu and Deo, 2013; Jaiswal *et al.,* 2015 and 2017). Vitamin C is chemically adept of reacting with most of the physiologically vital radicals and oxidants and acts as an established hydro-soluble antioxidant (Abraham, 2014). Being an antioxidant, it defends the body from the detrimental effects of free radicals and contaminants. Body needs vitamin C for normal physiological purposes. It aids in the metabolism of tryptophan, tyrosine and folic acid. It aids to lower blood cholesterol and adds to the synthesis of the amino acids carnitine and catecholamine that control nervous system. It is also required for wound healing and tissue growth. Vitamin C can also protect and stimulate biosynthesis of endothelial nitric oxide, which is significant for vascular relaxation (Heller *et al.,* 2001).

Fanta is a brand of fruit-flavoured carbonated drinks produced by The Coca-Cola Company and marketed globally. It is the second oldest brand and is enjoyed more than 130 million times every day around the world (Coca-Cola, 2017). Recently, simultaneous consumption of this soft drink with excess ascorbic acid has been implicated to be poisonous (Sahara Reporters, 2017). However little is known about its pathological effects on liver. Since liver is the major organ responsible for the metabolism and detoxification of a number of xenobiotics in animal and human. The liver is a reservoir for storage of food elements, to be released on demand when required by the body. The important roles done by the liver, not only in the storage and release of nutrients, but also in the neutralization, biotransformation of xenobiotic chemicals and elimination of a variety of toxic substances (Baratta *et al.,* 2009). There is a continuous flow of blood from the bowels-to the liver via the portal vein, causing it to dam up when contaminant overpower. For understanding the pathological conditions of the animal, histological studies pave a way, to have a clear understanding as to how toxicants or chemicals cause injury to the tissues. Also, understanding of liver dysfunction could help to unravel the effect of any chemicals, since liver is the major organ for bio-transformation (Soyinka et al., 2007). This study was therefore conducted to investigate the histopathological and Liver function (serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activity) effects of simultaneous consumption of food additives, as well as consumption of excess AA with a known carbonated soft drinkson the Liver of Wistar rats.

Materials and method

Experimental Animals

Forty-eight (48) healthy and active male rats (Wistar rats) of average weight 80-100 g were purchased from the Department of Animal and Environmental Biology of Adekunle Ajasin University Akungba Akoko, Ondo State, Nigeria. They were acclimatized for two weeks prior to the experiment. The rats were housed in cages and maintained in a ventilated room at 25±2°C with their normal rat pellet ration and portable water.

Experimental Procedure

After two week of acclimatization, the rats were allotted into 12 groups (n=4

each); Basal Control, 1.0 mg of SB, 10 mg of SB, 10 mg of AA, 0.2 mg of AA + 0.5 mg of SB, 0.2 mg of AA + 1 mg of SB, 0.2 mg of AA + 10 mg of SB, 0.2 mg of SB + 0.1 mg of AA, 0.2 mg of SB + 0.5 mg of AA, Fanta + 0.1 mg of AA, Fanta + 1.0 mg of AA and Fanta + 10 mg of AA. The treatment lasted for 21 days and the administration were given orally. The procedures were approved by the by Department of Animal and Environmental Biology, Adekunle Ajasin University Akungba-Akoko, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1985).

Tissue Collection

At the end of the 21th day of treatments, Tissue of interest (liver) from both control and experimental groups were excised, blotted and fixed in sample bottles containing 10% buffered formaldehyde.

Histological Analysis

The livers preserved were subjected to micro techniques using the method of Bucke (1989). The tissue was dehydrated in an ethyl alcohol series of ascending concentrations; they were cleared in xylene and embedded in paraffin. Sagittal sections (5μ) were cut using a rotary microtome and mounted on glass slides. Sections were deparaffinized in xylene and hydratated in ethanol, stained with haematoxylin and alcoholic eosin (H&E 400) for general histological evaluation. Photomicrographs of stained sections were made using photoelectron-microscope (XSZ- 107BN, China). Photomicrographs of control groups were compared with those of exposed groups.

Liver Function Test

Tissues were put in EDTA anticoagulant tubes (ethylene diamine tetra-acetic acid 8.5%), which were placed in the ice bath. Tissues were then homogenized by a hand homogenizer. Liver function was estimated by measuring serum aspartate aminotransferase (AST) alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activity by colorimetric method (Ibekwe et al., 2007; Ramaiah, 2007; Amin *et al.* 2010).

Results

Normal architecture of hepatocyte (HP), sinusoid (S) and central vein (CV) were observed in control and 1 mg AA groups as shown in Figure 1 a, b and c. However, histological examination of the liver sections exposed to 10 mg of AA showed degeneration of hepatocyte (DHP), and cell infiltration. In addition to DHP, 1 mg of SB caused dilation of portal vein (DPV). Likewise, cytoplasmic vacuolation (CPV), was evident in addition to DPV in 10 mg SB group. Vacuolation of hepatocyte (VHP) and dilation of central vein (DCV) where seen in group treated with 0.2 mg AA and 0.5 mg SB. Furthermore, DCV was evident in the groups administered 0.2 mg AA +1 mg SB and 0.2 AA + 10 mg SB. Vacuolization of hepatocytes (VHP) were seen in the liver treated with 0.1 mg AA +0.2 mg SB (traces of DHP was evident) and 0.5 mg AA + 0.2 mg SB (dilation of sinusoid (DS) was also noted). Dilation of central vein (DCV), cell infiltration and dilation of sinusoid (DS) were observed in the livers of Rat administered fanta with AA. Furthermore, figure 2 shows alteration in the AST level with the increment in AST as the SB dosage increases. A slight reduction was noted in the group treated 10mg AA compared with control. Combination of a constant concentration of AA (0.2 mg) with the varied concentration of SB resulted in a slight decrease in alteration of AST compare with the effects induced in the absence of AA. Furthermore, at a fixed concentration of SB, increased concentration of AA resulted in ALT

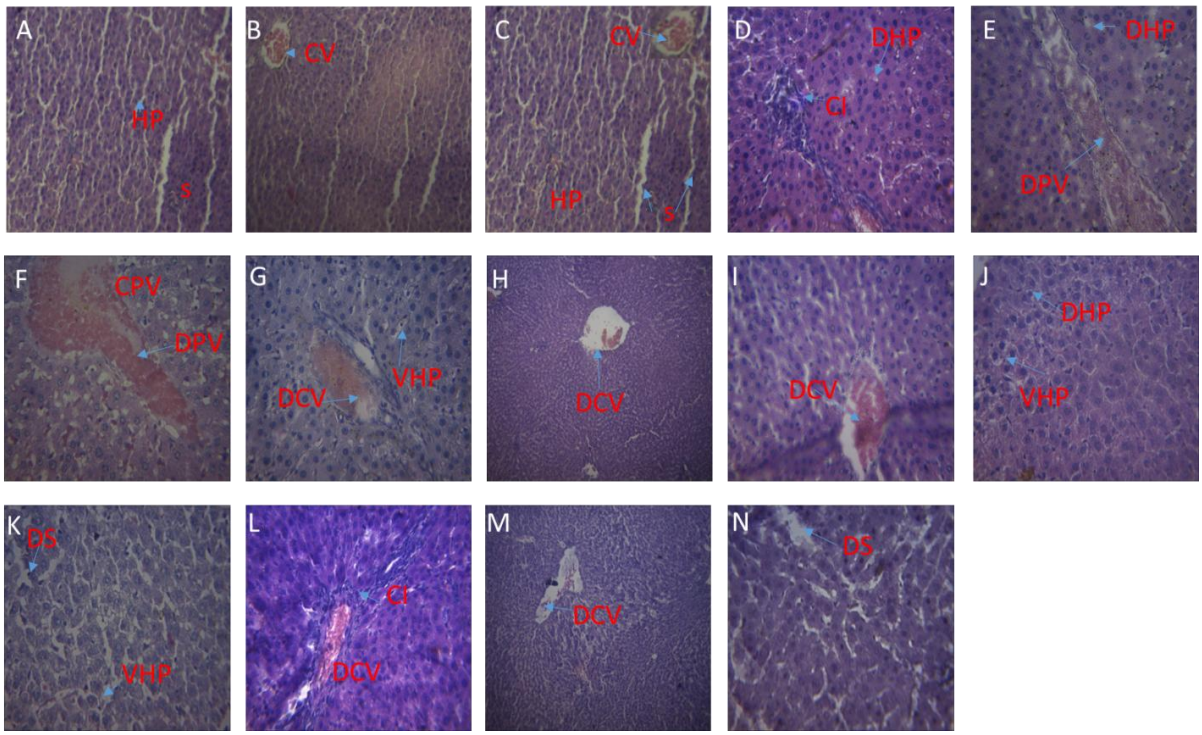

Figure 1. Photomicrograph of Liver sections of Rat (a and b) control showing normal architecture of the hepatocyte (HP), sinusoid (S) and central vein (CV), (c) group containing 1 mg of AA, showing normal architecture like control, (d) 10 mg AA, showing degeneration of hepatocyte (DPH) and cell infiltration (CI), (e) 1 mg SB, showing degeneration of hepatocyte and dilation of portal vein (DPV), (f) 10 mg SB, showing dilation of portal vein and cytoplasmic vacuolation. Mixture of the treatments are shown in g to k. 0.2 mg of AA with varied concentration of SB, (g) 0.5 mg SB, showing dilation of central vein (DCV) and vacuolization of hepatocyte (VHP), (h) 1 mg SB, showing dilation of central vein, (i) 10 mg SB, dilation of central vein. 0.2 mg of SB with varied concentration of AA, (j) 0.1 mg AA, showing degeneration of hepatocyte (DHP) and vacuolization of hepatocyte (VHP), (k) 0.5 mg, showing dilation of sinusoid (DS) and vacuolization of hepatocyte (VHP). Fanta with varied concentration of AA are shown in L-N, (l) 0.1 mg AA, showing cell infiltration, (m) 1 mg AA, showing dilation of central vein, and (n) 10 mg AA, showing dilation of sinusoid. (H & E X 200).

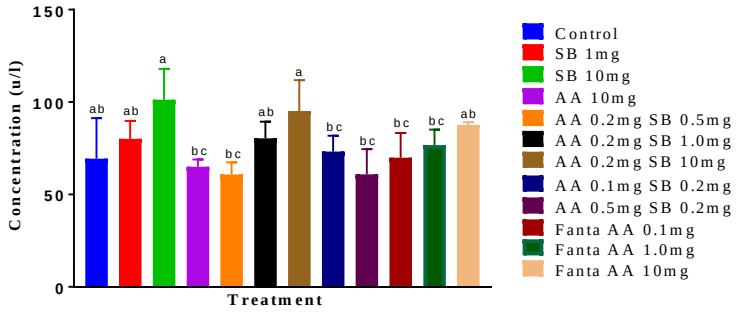

Figure 2. The effect of administered treatment on the serum aspartate amino transferase (AST) level of Wistar albino rats. {Means followed with similar letters within the same column are not statistically different ($p < 0.05$) was considered significant). *significantly different from normal control}.

reduction. High concentration of AA in Fanta caused significant increase in AST level. Figure 3 shows the effect of administered treatments on the serum alkaline phosphatase (ALP**)** of Wistar albino rats. The result shows a gradual increase in the ALP level with the rise in SB concentration and a significant increase in the ALP of the group administered 10mg/kg body weight SB compared with the control. AA (10 mg/kg bw) has no effect on ALP concentration, however, it could hinder SB from increasing ALP level. When the concentration of SB was kept constant, increase in AA concentration did not affects ALP

concentration level. Addition of varied concentration of AA to fanta causes insignificant alteration in ALP concentration level. . The result shows a slight rise in ALT with an increase in SB dosage (Figure 4). Addition of treatments generate an insignificantly alteration in the ALP concentration level, hence no significant change was evident in ALT level.

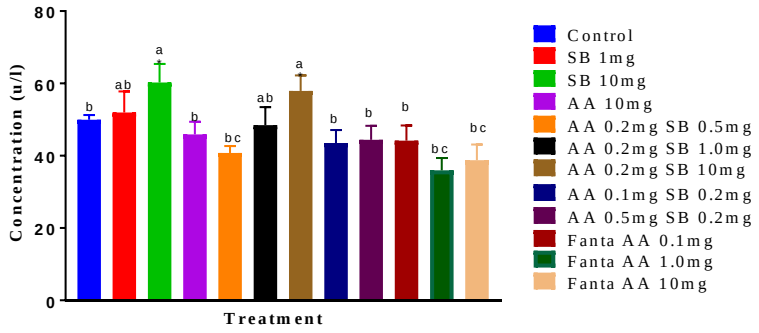

Figure 3. The effect of administered treatment on the serum alkaline phosphatase (ALP) of Wistar albino rats. {Means followed with similar letters within the same column are not statistically different ($p<0.05$) was considered significant). *significantly different from normal control}.

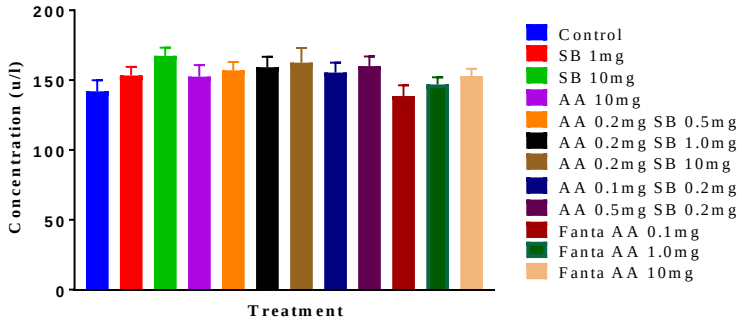

Figure 4. The effect of administered treatment on the serum alanine amino transferase (ALT) level of Wistar albino rats. {Means followed with similar letters within the same column are not statistically different ($p<0.05$) was considered significant). *significantly different from normal control}.

Discussion

Food preservatives used for prolonging shelf life of prepared foods is the prominent chemical exposed by the humans in everyday life. Previous studies have shown that some food additives could be lethal for the living (Kaboglu and Aktac, 2002; Sasaki *et al.*, 2002; Aktac *et al*., 2008). However, studies investigating the effect of effects of simultaneous consumption of common food additives (such as sodium benzoate and ascorbic acid) on liver were not available in literature.

Hepatocytes play an important role in the proper functioning of the liver as the hepatocytes are the key functional cells of the liver. It accounts for 60% to 80% of the liver cell mass and contribute to a wide range of metabolic activity, including protein, lipid, carbohydrate, porphyrin, nucleic acid, metal, vitamin, hormone, glutathione and xenobiotic metabolism; coagulation factor synthesis; biliary secretion; and immune surveillance. The lack of disruption of hepatic cords, even sizes of nucleus in hepatocytes and normal central vein for the control and 1mg AA groups attest that no changes occur in the Rat's liver. Nevertheless cell infiltration and DHP were evident in 10 mg AA group. These observation could be related to the possibility of AA agglomerating at high concentration. Degeneration of hepatocyte is possible when xenobiotic compounds acts like free radical. The hepatocytes showed micro-vesticular fatty degeneration with swollen mitochondria (Saibara *et al*., 1994). Hemorrhage in areas of degenerated hepatocytes after 90 days of sodium benzoate administration at a dose of 1 mg/kg body weight has been reported (Khidr *et al*. 2012). Hence DHP observed

may be because Liver is treating SB as xenobiotic at both low and high concentration, while AA is only consider xenobiotic at very high concentration (10 mg AA). A compromise in the reliability of the hepatocytes could lead to inadequate working of the liver. Any variations in shape and size of hepatocyte´s nucleus could be considered as a sign of increased metabolic activity (Sinha and D'Soza, 2010). According to Michalopoulos, (2013), histopathological changes in liver parenchyma might be due to the accumulations of xenobiotics in hepatocytes following impaired lipid metabolism since liver is the target for detoxification of noxious substances. Sodium benzoate was determined to cause degeneration in organelles in hepatic and cellular metabolism, which could be impaired as the result of these degenerations.

Integrity of cytoplasm is important for regular functioning of intracellular transport mechanisms. These transport mechanisms are considered to be impaired due to cytoplasmic vacuolization. Vacuolation is a morphological phenomenon observed in mammalian cells after exposure to bacteria or viral pathogens as well as to various natural and artificial low molecular weight compounds (Shubin *et al*., 2016). The vacuoles are usually responsible for collecting injurious element and preventing them from interfering with the biological activities of these cells (cytoplasm) (Cheville, 2009). Dilated central vein and portal vein reported could be due to noxious effect of the preservative causing rupture of the vessel wall and may be dose dependent (Khidr *et al.* (2012), since it increased with SB dosage Activities of SB could be slow down with minimal amount of AA, since AA lost its antioxidative properties at higher concentration. Addition of taking Fanta with little AA caused DCV while high amount resulted in hepatic sinusoidal dilation. This is because the newly added AA is in addition to the moderate amount added by the manufacturer, which does coagulate and did not suppress the effect of SB present as preservative in Fanta, but acted as a foreign material.

Sinusoidal dilation or widening of hepatic capillaries is a characteristic of hepatic sinusoidal dilation (Saadoun *et al*., 2004). Hepatic sinusoidal dilation is usually found in the vicinity of hepatic tumours, granulomatous disorders, heart failure, hepatic venous outflow block, and infiltration of sinusoids by several of benign or malignant cells (Saadoun *et al*., 2004). Dilation of sinusoid observed in this study could be as a result of dissociation of sodium benzoate to benzoic acid and sodium ion. This benzoic acid can under stomach condition convert to benzene and carbon (IV) oxide. Benzene, even at little concentration, is capable of causing hepatic tumours since benzene is carcinogenic. It has been reported that there is tendency for the production of benzene in food and beverages when the concentration of benzoic acid is the same with that of ascorbic acid, but when the concentration of the latter is higher, there will be no production of benzene (Vania *et al.*, 2015).

The alteration in AST is obvious as the SB dosage increases when compared with the control. The observed increment in the AST level can be attributed to distinctive alteration in architecture of liver. Sodium benzoate has propensity to cause a distinctive alteration in architecture of liver (Nagy *et al.*, 2015), because it generate reactive oxygen species, which play a role in pathological changes in Liver. Furthermore, such changes has been attributed to hepatocellular damage caused by the toxic effect of xenobiotic agents, which was indicated by vaculation, swelling, necrosis and pyknosis of the liver cells (Nabila *et al.*, 2013; Valchev *et al.* 2016). Increase in both serum AST and ALT of rats was attributed to the changes in liver function and hepatocellular impairment which subsequently caused the release of greater than normal levels of

intracellular enzymes into the blood (Abdel- Rahim *et al.*, 1989). AA did not have negative effect on AST, because it act as oxidant, it rather ameliorate the negative effect of SB when consume simultaneously. However, high concentration of AA also caused slight changes in AST level when consumed with Fanta, because at such high concentration there is possibility of formation of aggregates. This aggregate might be preventing normal biochemical process. AA metabolism mechanism involves decrease in the hepatotoxicity induced by cisplatin, which is embodied in the fact that AA might ameliorate the oxidative damage by decreasing lipid peroxidation and altering antioxidant defense system (El-Gendy *et al*., 2010) or by denoting electrons to free radicals and quenching their reactivity (Bendich, 1990a and 1990b).

Alkaline phosphatse occurs in the canalicular and sinusoidal membranes of the liver, thus damage to the liver will result in elevated serum ALP activity (Ramaiah, 2007). Cholestatic liver disease is characterized by increased level of ALP coupled with high level of bilirubin (Nagy *et al*., 2015). Just like observation in AST, the trend of ALP significantly increase due the SB dosage increase, hence this is an indicator that the hepatic capacity of the liver is grossly affected by sodium benzoate (Inuwa *et al.*, 2011).

Also, the significant elevation of serum aminotransferases under pathological conditions show that the parenchymal cells of hepatic lobules fail to carry out vital functions, which usually results in disturbed or imbalanced intermediatory metabolism. As a result of cellular damage, several enzymes like ALT, AST and ALP beach out into the serum and hence their level indicate the type and extent of damage inflicted (Amin *et al.* 2010). Serum bilirubin concentration may be elevated from acute hepatocellular injury, cholestatic injury, or biliary obstruction (Nabila *et al.*, 2013).

Sodium benzoate caused derangement of liver function as revealed by significant elevation of serum ALT, AST and ALP (Nagy *et al*., 2015). In blood plasma, sodium benzoate has a binding affinity for plasma proteins where it is carried out to different tissues. In the liver, it is metabolized by conjugation with glycine, resulting in the formation of hippuric acid (Kubota & Ishizaki 1991). The observed elevation in the activities of serum enzymes as AST and ALP in response to sodium benzoate are similar to results from rats treated with Nnitrosodiethylamine (Bansal *et al.* 2005) or N-nitrosoamines (Pevicharova *et al.* 1997). Alkaline phosphatase is present on cell surfaces in most human tissues, especially those of the intestine, liver, bones, spleen and kidneys. The specific location of the enzyme within sinusoidal and bile canalicular membranes could account for its serum elevation in the current study in response to sodium benzoate administration.

The ALT enzyme is a strong positive indicator of insulin resistance, diabetes mellitus and obesity which are risk factors for coronary heart disease (Tawfik & Al-Badr, 2012).). The ALT enzyme is also a sensitive marker of liver damage (Al-Mamary *et al.*, 2002). Therefore, the non- significant effect of the preservatives (AA, SB and the combination of the two) on the serum ALT activity might be an indication of its safety and that it may exhibits less toxic effect on liver function and tissues. Cameron and Campbell, (1991) and Khaw *et al.*, *(*2001) found that patients with cancer who received vitamin C supplementation lived longer when compared to control group who did not receive any supplementation. Hence, the non- significant of the group treated AA reflected the benefit of AA. Djurašević *et al*., (2008) observed that AA supplementation dose-dependently increases ascorbate concentration in the blood. In the liver, an increase is present only in the case of a low vitamin C dose,

which implies down-regulation of its hepatic synthesis under the influence of a high dose (Tsao and Young 1989; 1990). Furthermore, long-term feeding with vitamin C increases its concentration in circulation and reduces its synthesis in the liver (Bánhegyi *et al.*, 1997), thus continuous application of AA is beneficial. In this study it is obvious that ascorbic acid reduced the toxicity level of SB against the liver functions. Therefore, administration of AA protects rat against SB- induced liver injury. This is in accordance of Ahmadizadeh *et al*., (2011 and 2015) who stated that the administration of AA protected rat against ST-induces lungs injury.

Conclusion

Ascorbic acid has no negative effect on AST, ALT and ALP, because of its anti-oxidant property. Similarly it has no effect on histopathological architecture of liver at moderate concentration level. However, it lost its anti-oxidative properties at high concentration in Fanta due to aggregation. SB has mild effect at low concentration on biochemical parameters, but has severe consequences as the concentration increases. It also causes several damages to the nature of the histopathological structure of liver, most especially at high concentration level. The effect of SB can easily be ameliorated by AA, because AA suppresses destructive nature of SB. Indiscriminate use of food preservatives for prolonging shelf life of prepared foods should be discouraged; this could lead to the tissues destruction with subsequent abnormality in function.

References

Abdel- Rahim EA, Ashoush YA, Afify AS, Hewedi F (1989) Effect of some synthetic food additives on blood haemoglobin and liver function of rats. Menofia J Agric Res **14(1):** 557-568.

Abraham SE (2014) Biochemistry of free radicals and antioxidants. Sch Acad J Biosci **2(2):** 110-118.

Adikwu E, Deo O (2013) Hepatoprotective effect of vitamin C (ascorbic acid). Pharmacol Pharm **4:** 84-92.

Ahmadizadeh M, Abdolkany E, Afravy M (2015) The preventive effect of vitamin C on styrene-induced toxicity in rat liver and kidney. Jundishapur J Health Sci **7(2):** e26849.

Ahmadizadeh M, Pol T, Boazar M (2011) Effect of vitamin C on styrene induced respiratory toxicity. Int J Occupat Hyg **3(2):** 76-80.

Aktac T, Kaboglu A, Kizilay G, Bakar E (2008) The short-term effects of single toxic citric acid doses on mouse tissues - histopathological study. Fresenius Env Bull **17:** 311-315.

Al-Mamary M, Al-Habori, AM, Al-Aghbari AM, Baker MM (2002) Investigation into the Toxicological Effects of Catha Edulis Leaves: A Short-Term Study in Animals. Phy Res **16:** 127-132.

Amin KA, Abdel Hameid H, AbdElsttar AH (2010) Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food Chem Toxicol **48:** 2994-2999.

Bánhegyi G, Braun L, Csala M, Puskás F, Mandl J (1997) Ascorbate metabolism and its regulation in animals. Free Rad Bio Med **23(5):**793-803.

Bansal AK (2005) Modulation of N-nitrosodiethylamine induced oxidative stress by vitamin E in rat erythrocytes. Human Exp Toxicol **24:** 297-302.

Baratta JL, Ngo A, Lopez B, Kasabwalla N, Longmuir KJ, Robertson RT (2009) Cellular organization of normal mouse liver: a histological, quantitative immunocytochemical, and fine structural

analysis. Histochem Cell Bio **131(6):**713-726.

Bendich A (1990a) Antioxidant vitamins and their functions in immune responses. Adv Exp Med Biol **262:** 35-55.

Bendich A (1990b) Antioxidant micronutrients and immune responses, micronutrients and immune functions. New York Academy of Sciences, New York.

Bucke D (1989) Histopathology. In: Austin B & Austin DA (Eds.), Methods for microbiological examination of fish and shellfish. Ellis Horwood, West Sussex, England, 66-97.

Cameron E, Campbell A (1991) Innovation vs. quality control: an 'unpublishable' clinical trial of supplemental ascorbate in incurable cancer. Med. Hypotheses **36(3):** 185-189.

Cheville NF (2009) Ultrastructural pathology: the comparative cellular basis of disease. 2nd ed. Willey-Blackwell, A John Wiley of Sons, Inc. USA.

Coca cola Company (2017) http:// www.coca-colacompany.com/stories/2017.

Djurašević SF, Djordjević J, Drenca T, Jasnić N, Cvijić G (2008) Influence of vitamin C supplementation on the oxidative status of rat liver. Archives Bio Sci **60(2):**169-173.

EL-Gendy KS, Aly NM, Mahmoud FH, Kenawy A , El-Sebae AH (2010) The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid. Food Chem Tox **48:** 215-221.

Heller R, Unbehaun A, Schellenberg B, Mayer B, Werner-Felmayer G, Werner ER (2001) L-ascorbic acid potentiates endothelial nitric oxide synthesis via a chemical stabilization of tetrahydrobiopterin. J Bio Chem **276:** 40-47.

Ibekwe SE, Uwakwe AA, Monanu MO (2007) In vivo effects of sodium benzoate on plasma aspartate aminotransferase and alkaline phosphatase of Wister albino rats. Sc Res Essays **2(1):** 10-12.

Institute of Laboratory Animal Resources (US) (1985) Committee on Care, Use of Laboratory Animals, & National Institutes of Health (US), Division of Research Resources, Guide for the care and use of laboratory animals, National Academies.

Inuwa HM, Aina VO, Gabi B, Aim Ola I, Ja'afaru L (2011) Determination of nephrotoxicity and hepatoxicity of monosodium glutamate (MSG) consumption. British J Pharm Tox **2(3):** 148-153.

Jaiswal SK, Gupta VK, Ansari MD, Siddiqi NJ, Sharma B (2017) Vitamin C acts as a hepatoprotectant in carbofuran treated rat liver slices in vitro. Tox Reports **4:** 265-273.

Jaiswal SK, Gupta VK, Siddiqi NJ, Pandey RS, Sharma B (2015) Hepatoprotective effect of citrus limon fruit extract against carbofuran induced toxicity in Wistar rats. Chinese J Bio **2015:** 1-10.

Kaboglu A, Aktac T (2002) A study of the effects of sodium benzoate on the mouse liver. Biologia **57:** 375-382.

Khaw KT, Bingham S, Welch A, Luben R, Wareham N, Oakes S, Day N (2001) Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. THE LANCET Conference Alerts **357(9257):** 657-663.

Khidr BM, Makhlouf MMM, Ahmed SMM (2012) Histological and ultrastructural study on the effect of Sodium benzoate on the liver of adult male albino rats. Assiut Univ J Zoology **41(1):** 11-39.

Kubota K, Ishizaki T (1991) Dose-dependent pharmacokinetics of benzoic acid following oral administration of SB to humans. European J Clinical Pharmacol **41(4):** 363-368.

Lennerz BS, Vafai SB, Delaney NF, Clish AB, Deik AA, Pierce KA, Ludwig DS, Mootha VK (2015) Effect of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic

profiles in humans. Mol Genet Metab **114(1):** 73-79.

Lu Frank C, Kacew S (2009) Fundamentals, target organs and risk assessment. Lu's Basic Toxicology. 5th ed., Informa Healthcare, New York.

Michalopoulos GK (2013) Principles of liver regeneration and growth homeostasis. Com physiol **3(1):** 485-513.

Nabila M, Mokrane N, Alami O, Tabet SA, Zaoui C, Kheroua O, Saidi D (2013) A thirteen week ad libitum administration toxicity study of tartrazine in Swiss mice. African J Biotech **12(28):** 4519-4529.

Nagy ST, Hanan MA, Abdalla AA, Shimaa HMF (2015) Adverse effects of some food additives in adult male albino rats. Curr Sci Int **4(4):** 525-537.

Oloye FF (2019) Spectroscopic investigation of the mixture of ascorbic acid and sodium benzoate. Sci J Chem **7(3):** 62-66.

Pevicharova GT, Dimova PI, Atanasova-Goranova VK (1997) Effect of food products on endogenous generation of N-nitrosoamines in rats. British J Nut **78(2):** 325-45.

Ramaiah SK (2007) A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem Tox **45(9):** 1551-1557.

Saadoun D, Cazals-Hatem D, Denninger M-H, Boudaoud L, Pham B-N, Mallet V, Condat B, Brie`re J, Valla D (2004) Association of idiopathic hepatic sinusoidal dilatation with the immunological features of the antiphospholipid syndrome. Case Report Gut **53:** 1516-1519.

Sahara Reporters (2017) http://saharareporters.com/2017/03/13/court-orders-nafdac-compel-nigerian-bottling-company-warn-consumers-dangers-drinking.

Saibara TI, Himeno H, Ueda H, Onishi S, Yamamoto Y, Enzan H, Hara H, Takehara Y, Utsumi K (1994) Acute hepatic failure with swollen mitochondria and microvesicular fatty degeneration of hepatocytes triggered by free radical initiator- laboratory investigation. J Tech Met Pathol **70(4):** 517-524.

Salah SH, Abdou HS, Abdel-Rahim EA (2010) Modulatory effect of vitamins A, C and E mixtures against tefluthrin pesticide genotoxicity in rats. Pest Biochem Physiol. **98:** 191-197.

Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S (2002) The comet assay with 8 mouse organs: results with 39 currently used food additives. Mut Research-Genetic Toxicolog Env Mut **519:** 103-119.

Schacer R (2004) Sialic acids: fascinating sugars in higher animals and man. Zoology (Jena) **107:** 49-64.

Shubin AV, Demidyuk IV, Komissarov AA, Rafieva LM, Kostrov SV (2016) Cytoplasmic vacuolation in cell death and survival. Oncotarget **7(34):** 55863-55889.

Sinha R, D'Souza, D (2010). Liver cell damage caused due to sodium benzoate toxicity in mice. Int J Biotech Biochem **6(4):** 549-554.

Soyinka OO, Adeniyi FA Ajose OA (2007) Biochemical parameters of liver function in artisans occupationally exposed to "vat dyes". Indian J Occupat Env Med **11(2):** 76-79.

Tawfik MS, Al-Badr N (2012) Adverse effects of monosodium glutamate on liver and kidney functions in adult rats and potential protective effect of vitamins C and E. Food Nut Sci **3(5):** 651-659.

Tsao CS, Young M (1989) Effect of dietary ascorbic acid on levels of serum mineral nutrients in guinea pigs. Int J Vitam Nutr Res **59(1):** 72-76.

Tsao CS, Young M (1990) Enzymatic formation of ascorbic acid in liver homogenate of mice fed dietary ascorbic acid. In Vivo **4(3):** 167-9.

Tsay HJ, Wang YH, Chen WL, Huang MY, Chen, YH (2007) Treatment with sodium benzoate leads to malformation of

zebrafish larvae. Neurotoxicol Teratol **29(5):** 562-569.

Valchev I, Grozeva N, Marutsova V, Nikolov Y (2016) Effects of aflatoxin B1 only or co-administerd with mycotox NG on liver function in turkey broilers. Int J Adv Research **4(9):** 1425-1443.

Vania PSS, Andrea MS, Alexandre GT, Karen SP (2015) Benzene as a chemical hazard in processed foods. Int J Food Sci **15:** 1-7.

Yavav A, Kumar A, Das M, Tripathi A (2016) Sodium benzoate, a food preservative, affects the functional and activation status of splenocytes at non cytotoxic dose. Food Chem Toxico **88:** 40-47.