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PATHOLOGICAL EFFECTS OF FOOD ADDITIVES (SODIUM BENZOATE AND ASCORBIC ACID) ON SELECTED ORGANS OF WISTAR RATS

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ABSTRACT
Food additives are substances added to food to preserve flavor or enhance its taste and appearance. To meet the demand of the rising population, chemical based preservatives and additives are added to the edible items. This study examined the pathological effects of food additives (sodium benzoate and ascorbic acid) on selected organs of Wistar rats. About twenty-eight Wistar rats of both males and females were procured from Osogbo, Osun-state, Nigeria. They were fed on standard rat chow and water having unrestricted access and acclimatized for two weeks, after which they were divided into four groups of 7 rats each. Group A (control) was administered distilled water. Group B was orally administered sodium benzoate at a dose of 100mg/kg body weight. Group C was orally administered ascorbic acid at a dose of 150mg/kg per body weight. Group D was orally administered both sodium benzoate and ascorbic acid at a dose of 100 and 150mg/kg per body weight. At day 28, the rats of each group was allowed to fast overnight, blood samples were taken into EDTA bottles for haematological analysis and the rats were consecutively anaesthetized with ketamine-hydrochloride and then sacrificed. Tissue samples were harvested and fixed immediately with 10% formal saline, thereafter processed using the Automatic Tissue Processor for the histological analysis. There was no significant difference (p > 0.05) in the PCV and monocytes of Wistar rats administered with Sodium-benzoate (SB) additive when compared with the control group but a statistical difference was recorded in all other haematological parameters. There was no significant difference (p > 0.05) observed in the hematological parameters of Wistar rats administered with commonly used food additive (Ascorbic Acid) when compared with the control group. Histological morphology of the kidney, liver, and stomach revealed necrosis, atrophic changes, inflammation, and ulceration. This calls attention to the potential risks associated with consuming foods that contain sodium benzoate. Conclusively, Sodium benzoate (SB) administration is the main trigger that causes effects on gastrointestinal organs and haematological parameters.
Keywords: Ascorbic Acid, Food additives, Hematological parameters, Monocytes, Sodium Benzoate.

INTRODUCTION

Pre-packaged and ready-to-eat foods have become integral to modern society, meeting daily nutritional and lifestyle demands. Food additives are primarily used to extend food's shelf life, stop it from spoiling, and give it the flavor, color, and texture that consumers want (Dey and Nagababu, 2022). Chemical-based additives and preservatives are added to food products to satisfy the growing population's need (Yetuk *et al.,* 2014 and Deepanksha *et al.,* 2018). Food preservatives have been shown in numerous studies to be more detrimental to the body's systems than beneficial (Yetuk *et al.,* 2014). Free radicals, or Reactive Oxygen Species (ROS), are produced by all of these dietary additives and have mutagenic, cytotoxic, and genotoxic effects (Rajesh *et al.,* 2013). Reactive oxygen species (ROS) are highly reactive, oxygen-containing molecules, including radicals like superoxide and hydroxyl radicals, that are produced during normal cellular metabolism and can cause damage to cells if present in excess (Jakubczyk *et al.,* 2020). Sodium benzoate which is a common ingredient of beverage like sodas and fruit juice products, can affect the reaction to vitamin C by reducing its potency (Udo and Alexis, 2023). When vitamin C (ascorbic acid) is combined with sodium benzoate in acidic conditions, it can form benzene, which is a carcinogenic compound

(Piper and Piper, 2017). This reaction can reduce the effectiveness of vitamin C and potentially cause harm to the body (Walczak-Nowicka and Herbet, 2022). Additionally, sodium benzoate has been reported to cause allergic reactions in some individuals, which can further affect their response to vitamin C. Therefore, it is important to avoid consuming vitamin C supplements or foods that contain both vitamin C and sodium benzoate (EFSA, 2016). By interfering with normal metabolic processes, daily consumption of food treated with food additives raises their concentration in the body above the acceptable daily intake (ADI), which in turn promotes aging, kidney stones, cancer, ulcerative colitis, asthma, hypertension, and urinary diseases, among other conditions (Rajesh *et al.,* 2013).

The potential of food additives to enhance the stability and quality of food is one of their useful qualities. When food is processed with other things, such as water, minerals, carbon sources, and other compounds, its physio-chemical characteristics helps to define its nature. The use of food additives is growing daily in contemporary food technology (Deepanksha *et al.,* 2018). Coloring and sweetening are the primary purposes of food additives. Through alterations in their enzymatic and chemical characteristics, or microbiological processes, the preservatives stop the loss of nutrients. They keep food's quality or shelf life intact (Novais *et al.,* 2022). The preservatives work as an anti-microbiological in food such as energy drink jams, cold drink, vine among others. Food additives have become more common. Therefore, several studies had revealed that various processed foods make nearly 75% of the western diet.

Consuming preservatives has been linked to a number of negative consequences of food additives, including dermatitis, nausea, vomiting, eczema, and brain disorders (Warner, 2024). According to research, food additives that act as antimicrobial agents have also been shown to be genotoxic (Deepanksha *et al.,* 2018).

Sodium benzoate, a white, odorless, crystalline salt that can be obtained as powder or grain, is a salt of benzoic acid with the European name E211 and the chemical formula $C_7H_5NaO_2$ (Davidson et al., 2021). This substance barely dissolves in ethanol but dissolves readily in water. Its molecular weight is 144.1 gmol-1, and raising the temperature of the water makes it more soluble. This chemical is composed of 84.7% benzoic acid and 99% dry (Shahmohammadi *et al.,* 2016). Usually, it serves as a preservative in some food, pharmaceutical, and cosmetic items (Lennerz *et al.,* 2015).

Because it is easy to apply and effectively inhibits the growth of bacteria and fungi during storage, sodium benzoate is used in food products and beverages (Tsay *et al.,* 2007). Margarine, sauces, marmalades, gelatin, liqueurs, beers, fruit juices, and soft drinks are among the items that it is recommended for preserving (Zhang and Ma, 2013). According to population research, soda and carton-packaged juice are the primary dietary sources of this preservative, although it is also found in various other foods (Pongsavee, 2015).

Because sodium benzoate is very soluble in water and has good stability, it has been used as a preservative for many years (Ren *et al.,* 2014). According to Food and Drug Administration (FDA), sodium benzoate is generally regarded as safe for human consumption if found in foods at levels not higher than 0.1% concentration (Lennerz *et al.,* 2015).

Benzoate is thought to be capable of decarboxylating into poisonous benzene, particularly when combined with vitamin C, which can subsequently develop into a highly toxic, mutagenic, and tetragenic chemical (Piper and Piper, 2017).

With the chemical formula $C_6H_8O_6$, ascorbic acid, also referred to as vitamin C, has the European nomenclature E300. It is a water-soluble vitamin, antioxidant, and necessary co-factor that involves dioxygenases for collagen biosynthesis, carnitine and catecholamine metabolism, and dietary iron absorption (Khalife *et al.,* 2019). Ascorbic acid is a vital part of the human diet and can be found in many different foods, particularly fresh fruits and vegetables (Berretta *et al.,* 2020). Thus, vitamin C shortage causes scurvy, which manifests as hematological abnormalities, bleeding, and hyperkeratosis (Carvalho *et al.,* 2019). Although research has been conducted on the effects of these combined food additives, their impact on gastrointestinal organs and the brain has not been thoroughly explored. The aim of this study was to examine the effects of food additives (sodium benzoate and ascorbic acid) on selected organs of Wistar rats.

MATERIALS AND METHODS

Study area

This study was carried out at the animal house of the Department of Medical Laboratory Science, Adeleke University, Ede, Osun State. Adeleke University is a private institution located in Ede, a town in Osun State, southwestern Nigeria. The university is situated in a serene and conducive environment for learning, approximately 10 kilometers from Osogbo, the state capital. It lies within the tropical rainforest region of Nigeria, characterized by a humid climate with distinct wet and dry seasons. Ede, where the university is located, is historically significant and home to the Timi of Ede, a traditional ruler. The town is easily

accessible from major cities like Ibadan (about 100 kilometers away) and Lagos (approximately 200 kilometers away) via well-connected road networks. Adeleke University's location provides a peaceful and academic-friendly atmosphere, making it an ideal place for higher education and research.

Study design

In the research, we used experimental study design.

Ethical consideration

The protocol for this study was applied and approved by the Ethics and Research Committees of Adeleke University, Ede, Osun State, with reference number AUERC/2023/04/33MLS-UG/18.

Procurement of sodium benzoate and ascorbic acid

Sodium benzoate and Ascorbic acid were procured from Azchem Resources, Nigeria and authenticated at the Department of Biochemistry, Adeleke University, Ede.

Experimental animals

About twenty-eight wistar rats of which 16 were males and 12 females were procured from the animal house of Adeleke University, Ede, Osogbo, Osun-State, Nigeria. The rats were kept in cages in a ventilated room with a 12-hour light/12-hour dark cycle at 25±2°C. For two weeks, they were acclimated and provided regular rat feed and water with unlimited access. The animals were treated humanely in compliance with the regulations governing the use and care of laboratory animals.

Time frame Adopted from (Kehinde *et al.,* 2018). This study was concluded within a period of three months, between March and June, 2023.

Experimental design

After two weeks of acclimatization, the rats were allotted into four groups (n=7 each);

Group A (4 males and 3 females) were the negative control, received 1ml of distilled water.

Group B (4 males and 3 females) were administered sodium benzoate orally at a dose of 100mg/kg body weight.

Group C (4 males and 3 females) were administered ascorbic acid orally at a dose of 150mg/kg per body weight.

Group D (4 males and 3 females) were administered both sodium benzoate and ascorbic acid orally at a dose of 100 and 150mg/kg per body weight respectively.

Oral administration was used for the 28-days course of therapy. Adhering to the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, the procedures were authorized by Adeleke University's institutional review ethical committee. Using an animal weighing balance, the initial and final body weights were recorded.

Table 1: Distribution of Wistar rats into various groups (the experimental design).

GROUP	TREATMENT
A (4 males and 3 females)	Distilled water
B (4 males and 3 females)	Sodium benzoate
C (4 males and 3 females)	Ascorbic acid
D (4 males and 3 females)	Sodium benzoate + Ascorbic acid

Sample collection

At day 28, the rats of each group was allowed to fast overnight and were consecutively anaesthetized with ketamine-hydrochloride and then sacrificed. Blood samples were collected from the heart region immediately into EDTA bottle. Organs were harvested and preserved immediately in 10% buffered formalin.

Histopathological analysis

Representative tisue samples were placed in tissue cassettes and processed using the Automatic Tissue Processor containing 12 beakers.

Staining Procedure

Haematoxylin and Eosin technique was used for staining the tissue sections.

Sections were dewaxed (an hour) in xylene and hydrated in descending grade of alcohol (100%, 95%, 90%, 80% and 70% alcohol) then water. They were stained in Harris heamatoxylin for 5minutes and rinsed in water. The sections were briefly differentiated in 1% acid alcohol, rinsed in tap water, and blued in running tap water for 10minutes.

The sections were further counterstained with 1% eosin for about 2minutes, rinsed in tap water. They were dehydrated in ascending grades of alcohol (70%, 80%, 90%, 95%, absolute alcohol), cleared in xylene and mounted using DPX and cover slips.

Hematological analysis

Following the blood sample collection into an EDTA bottle, it was mixed to a good ratio of the

anticoagulant to blood. The sample was analyzed using a hematological analyzer (3-Part Mindray) by means of aspiration.

Statistical analysis

The data complied with the equality of variance assumption and were regularly dispersed. SPSS 20.0 was used to do a one-way Analysis of Variance (ANOVA) on the differences between the groups. Standard Deviation (SD) ± Mean was used to express the results. Post Hoc analyses were performed using the Duncan multiple range test, and a significance level of $p \leq 0.05$ was considered.

RESULTS

There was no significant difference ($p > 0.05$) in the PCV and monocytes of Wistar rats administered with Sodium-benzoate (SB) additive when compared with the control group but a statistical difference was recorded in all other haematological parameters hence the sodium benzoate has effect on the haematological assessment (Table 2).

There was no significant difference ($p > 0.05$) observed in the hematological parameters of Wistar rats administered with commonly used food additive (Ascorbic Acid) when compared with the control group (Table 3).

This shows that there was no significant difference ($p > 0.05$) in the hematological parameters of Wistar rats administered with the mixture of commonly used food additive (Ascorbic Acid) and sodium benzoate compared with the control group (Table 4).

No significant difference was recorded ($p > 0.05$) in the neutrophil, lymphocyte and eosinophils of Wistar rats administered with SB additive when compared with the group treated with the mixture of AA and SB but a statistical difference was recorded in all other haematological parameters (Table 5).

Table 2: Comparison of haematological parameters between groups A (control) and B (Sodium Benzoate)

Parameter	Control (Group A)	Sodium Benzoate (Group B)	*p*- value
TLC	8.3 ± 1.53	13.0 ± 1.00	0.01*
NEUT %	44.3 ± 6.66	61.3 ± 2.52	0.01*
LYMP %	51.3 ± 7.37	37.0 ± 3.61	0.04*
MONO %	2.0 ± 1.00	0.33 ± 0.30	0.05*
EOS %	2.3 ± 0.58	0.7 ± 0.58	0.03*
PLT X109/L	507.3 ± 28.80	812.3 ± 70.15	0.01*
PCV %	43.3 ± 1.53	44.0 ± 1.00	0.54
RBC	8.9 ± 1.32	5.7 ± 0.76	0.02*
HB g/L	14.3 ± 1.05	9.5 ± 0.66	0.01*

Significant at $p \leq 0.05$

Table 3: Comparison of the hematological parameters between groups A (control) and C (Ascorbic Acid).

Parameter	Control (Group A)	Ascorbic Acid (Group C)	*P*-Value
TLC	8.3 ± 1.53	5.87 ± 0.50	0.06
NEUT %	44.3 ± 6.66	50.0 ± 4.36	0.28
LYMP %	51.3 ± 7.37	47.0 ± 4.36	0.43
MONO %	2.0 ± 1.00	1.70 ± 0.58	0.68
EOS %	2.3 ± 0.58	2.70 ± 1.15	0.64
PLT $X10^9$/L	507.3 ± 28.80	468.3 ± 26.50	0.46
PCV %	43.3 ± 1.53	47.3 ± 2.89	0.10
RBC	8.9 ± 1.32	10.2 ± 1.18	0.27
HB g/L	14.3 ± 1.05	13.8 ± 0.26	0.47

Significant at $p \leq 0.05$

Table 4: Comparison of the hematological parameters between groups A (control) and D (Sodium benzoate + Ascorbic acid).

Parameter	Control (Group A)	Sodium benzoate + Ascorbic acid (Group D)	*p*-value
TLC	8.3 ± 1.53	5.9 ± 0.50	0.06
NEUT %	44.3 ± 6.66	60.0 ± 7.21	0.05*
LYMP %	51.3 ± 7.37	38.0 ± 7.00	0.09
MONO %	2.0 ± 1.00	1.0 ± 1.00	0.16
EOS %	2.3 ± 0.58	1.0 ± 1.00	0.12
PLT $X10^9$/L	507.3 ± 28.80	463.7 v 40.62	0.44
PCV %	43.3 ± 1.53	42.7 ± 6.33	0.88
RBC	8.9 ± 1.32	8.27 ± 0.78	0.52
HB g/L	14.3 ± 1.05	13.5 ± 0.72	0.34

Significant at $p \leq 0.05$

Table 5: Comparison of the haematological parameters between groups B (Sodium Benzoate) and D (Ascorbic Acid + Sodium Benzoate).

Parameter	Sodium Benzoate (Group B)	Ascorbic Acid + Sodium Benzoate (Group D)	*p*-value
TLC	13.0 ± 1.00	5.9 ± 0.50	0.01*
NEUT %	61.3 ± 2.52	60.0 ± 7.21	0.78
LYMP %	37.0 ± 3.61	38.0 ± 7.00	0.84
MONO %	0.33 ± 0.30	1.0 ± 1.00	0.02*
EOS %	0.7 ± 0.58	1.0 ± 1.00	0.68
PLT $X10^9$/L	812.3 ± 70.15	463.7 v 40.62	0.01*
PCV %	44.0 ± 1.00	42.7 ± 6.33	0.74
RBC	5.7 ± 0.76	8.27 ± 0.78	0.02*
HB g/L	9.5 ± 0.66	13.5 ± 0.72	0.01*

Significant at $p \leq 0.05$

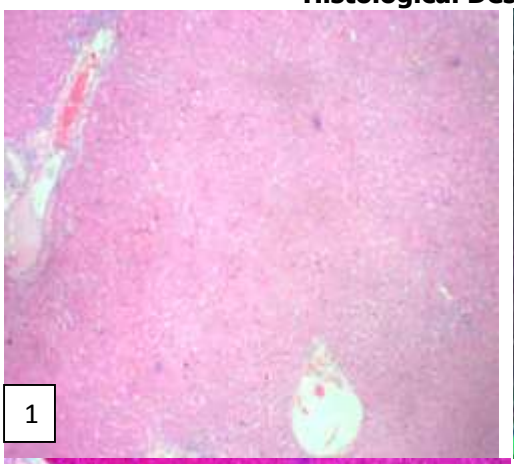
There was no significant difference ($p > 0.05$) in the haematoligical parameters of Wistar rats administered with commonly used food additive (Ascorbic Acid) compared with the group treated with mixture of AA and SB (Table 6).

Table 6: Comparison of the hematological parameters between groups C (Ascorbic Acid) and D (Ascorbic Acid + Sodium Benzoate).

Parameter	**Ascorbic Acid (Group C)**	**Ascorbic Acid + Sodium Benzoate (Group D)**	***p*-value**
TLC	5.9 ± 0.50	5.9 ± 0.50	1.00
NEUT %	50.0 ± 4.36	60.0 ± 7.21	0.11
LYMP %	47.0 ± 4.36	38.0 ± 7.00	0.13
MONO %	1.70 ± 0.58	1.0 ± 1.00	0.11
EOS %	2.70 ± 1.15	1.0 ± 1.00	0.13
PLT $X10^9$/L	468.3 ± 26.50	463.7 v 40.62	0.87
PCV %	47.3 ± 2.89	42.7 ± 6.33	0.32
RBC	10.2 ± 1.18	8.27 ± 0.78	0.08
HB g/L	13.8 ± 0.26	13.5 ± 0.72	0.53

Significant at $p \leq 0.05$

Histological Description of Photomicrographs



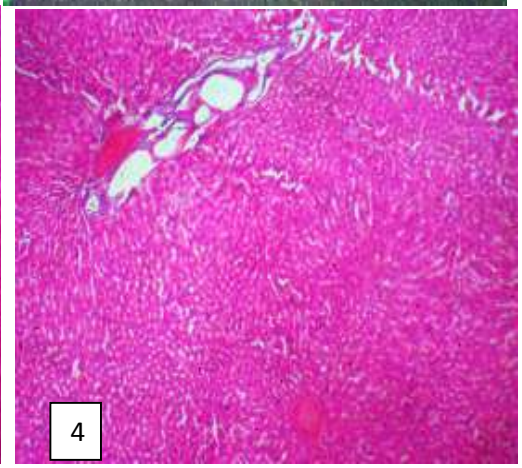
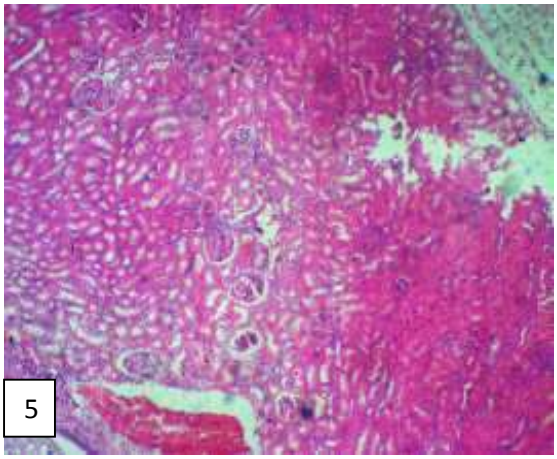
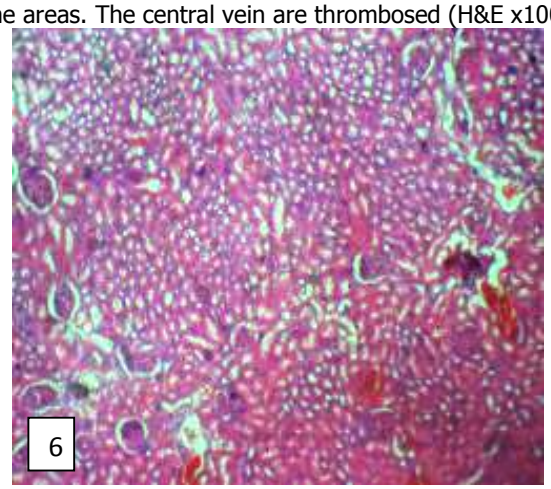
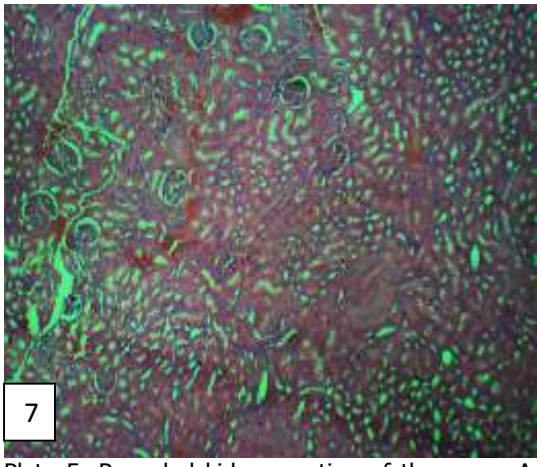


Plate 1: Revealed liver section of the control group fed with rat feed and water shows vascular relationship is preserved (H&E x100).
Plate 2: Revealed liver section of the group B (Sodium benzoate) Photomicrograph shows that vascular relationship is preserved and there is no necrosis (H&E x100).

Plate 3: Revealed liver section of the group C (Ascorbic acid), shows that vascular relationships are preserved; there are inflammatory cells within the hepatocytes lobules. The central vein is surrounded by inflammatory cells with accompanying necrosis at the hepatocytes (H&E x100).
Plate 4: Revealed liver section of the group D (Sodium benzoate + Ascorbic acid), shows that vascular relationship are preserved. There is centrilobular necrosis in some areas. The central vein are thrombosed (H&E x100).







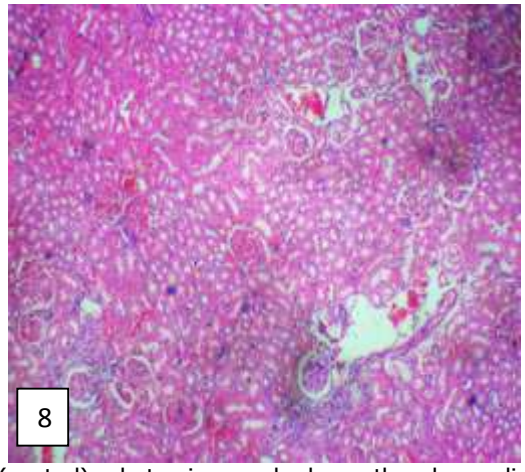
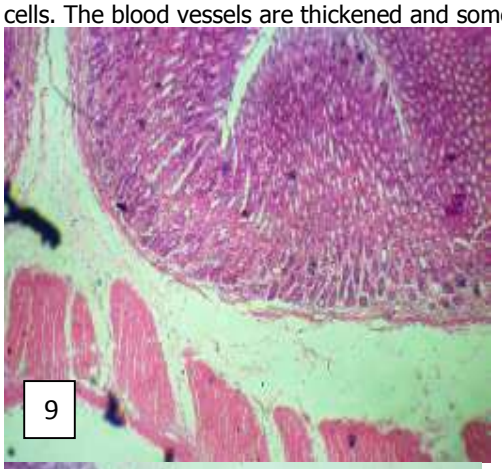
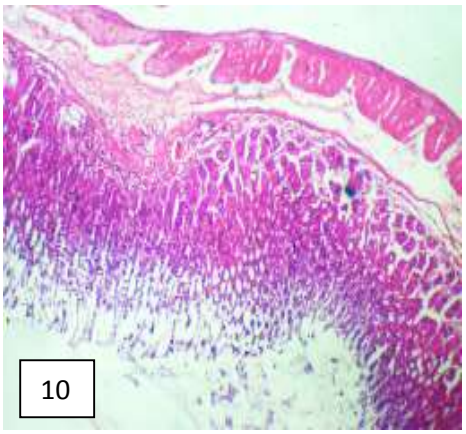
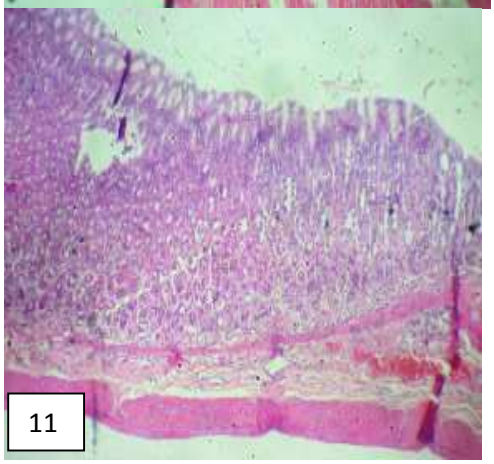


Plate 5: Revealed kidney section of the group A (control), photomicrograph shows the glomeruli are essential normal. (H&E x100).
Plate 6: Revealed kidney section of the group B (Sodium benzoate), photomicrograph shows the glomeruli are essentially normal and the blood vessels are thickened (H&E x100).
Plate 7: Revealed kidney section of the group C (Ascorbic acid), majority of the glomeruli showed atropic changes and appear lobulized, others showed hypercellular mesangial cells. The renal tubule especially at the periphery showed tubular necrosis. The interstation contain inflammatory cells (H&E x100).
Plate 8: Revealed kidney section of the group D (Sodium benzoate + Ascorbic acid), photomicrograph shows; The glomeruli are hypercellular due to increase in capillary proliferation. The interstition contains few inflammatory cells. The blood vessels are thickened and some shows thrombosis (H&E x100).







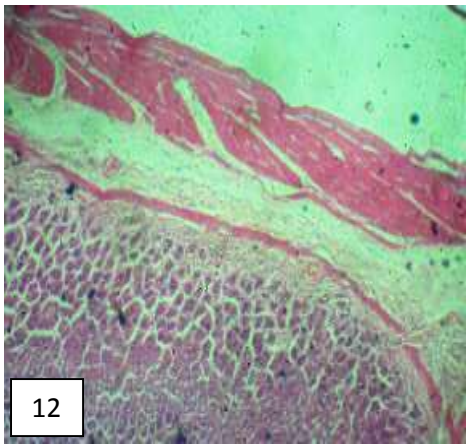


Plate 9: Revealed stomach section of the group A (control), photomicrograph revealed the mucosa epithelia is preserved and normal (H&E x100).
Plate 10: Revealed stomach section of the group B (Sodium benzoate), Photomicrograph shows no mucosal epithelial ulceration (H&E x100).
Plate 11: Revealed stomach section of the group C (Ascorbic acid), Photomicrograph shows there is no ulceration present however, there are no foci of mucosal erosion (H&E x100).
Plate 12: Revealed stomach section of the group D (Sodium benzoate + Ascorbic acid), Photomicrograph shows there is no ulceration of the epithelial mucosa lining down to muscularis mucosa (H&E x100).

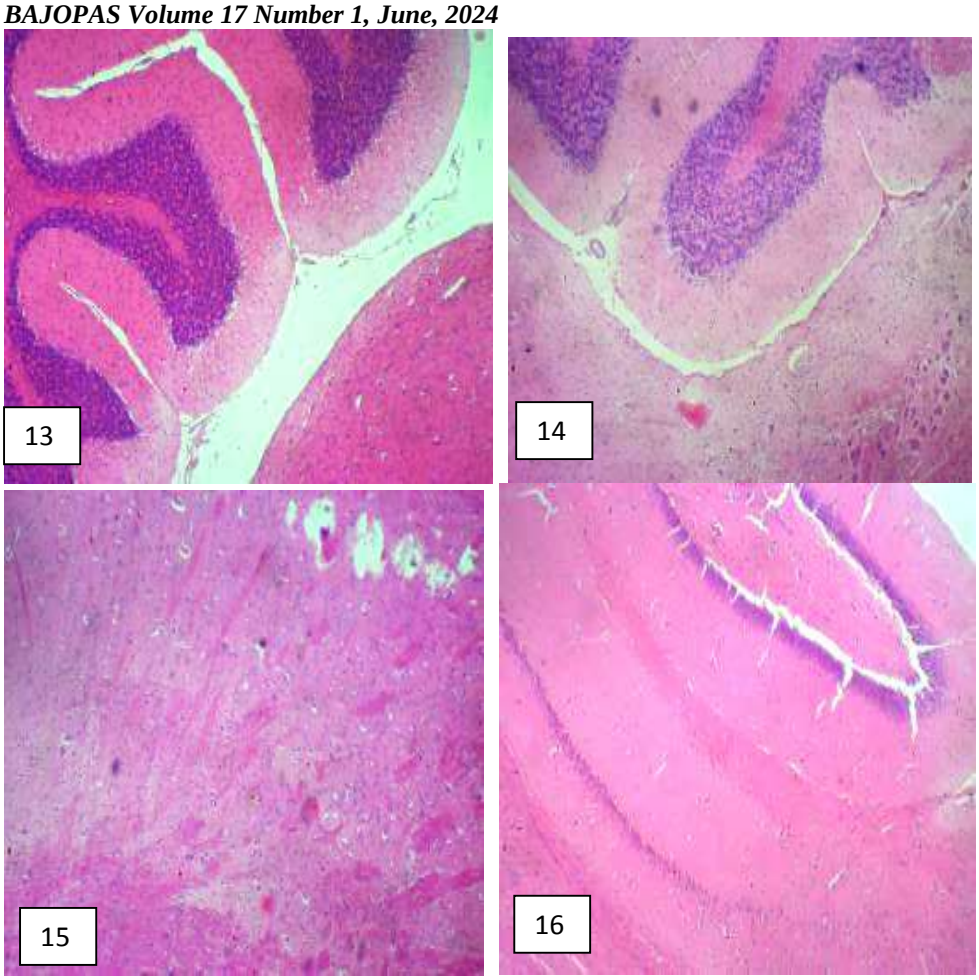


Plate 13: Revealed brain section of the group A (control), Photomicrograph shows a normal study of the cerebellum (H&E x100).
Plate 14: Revealed brain section of the group B (Sodium benzoate), Photomicrograph shows no abnormality in the cerebellum (H&E x100).
Plate 15: Revealed brain section of the group C (Ascorbic acid), Photomicrograph shows no abnormality (H&E x100).
Plate 16: Revealed stomach section of the group D (Sodium benzoate + Ascorbic acid), Photomicrograph shows no abnormality (H&E x 100).

DISCUSSION

Sodium benzoate is an agent that inhibits the growth of bacteria and fungi in acidic environments. When it enters cells, it interacts with anaerobic energy production pathways, thereby suppressing the proliferation and development of microorganisms that cause food spoilage (Pongsavee, 2015). Typically, a concentration of approximately 0.1% of sodium benzoate is sufficient for preserving food (Lennerz *et al.,* 2015). Additives are commonly used in combination with other preservation agents, and the mixture of multiple chemicals can cause shifts in UV spectroscopy, such as red or blue shifts, indicating potential interactions between the substances. These interactions may have adverse effects on the health of organisms consuming the products, and the specific outcomes can vary among different species (Jacob *et al.,* 2018).

In this study, the values of hematological parameters for the control group fell within the normal ranges commonly reported in the literature for rats (Adebayo *et al.,* 2010; Etim *et al.,* 2014; Jacob *et al.,* 2018 and Gbore *et al.,* 2020;). Exposure to toxins can lead to alterations and damage to the hematological profile and hematopoietic system in both humans and animals (Gbore et al., 2020). White blood cells (WBCs) play a crucial role in combating infections and diseases in the body. Low WBC counts may indicate compromised immunity, while excessively high WBC counts could be a sign of underlying diseases or the introduction of foreign substances triggering an increase in WBC counts. Antioxidants are molecules that neutralize harmful free radicals produced through various reactions (Tanko *et al.,* 2013). Free radicals can be detrimental to living cells, cause food spoilage, and damage materials like rubber, gasoline, and lubricating oil. The WBC count of rats in the control group was higher than the counts reported by Barbeieri *et al.* (2001); Adedapo *et al.* (2004) and Adedapo *et al.* (2007). Which can

be attributed to variations in WBC counts among different species of animals used for the study. In this study, the administration of 150mg of ascorbic acid (AA) per kilogram of body weight did not significantly reduce WBC counts compared to the control group. This finding suggests that ascorbic acid, an antioxidant, might have the ability to prevent excessive production of WBCs (George and Adegoke, 2012). On the other hand, the significant increase in WBC count in the group treated with 100mg of sodium benzoate per kilogram of body weight compared to the control group indicates that sodium benzoate was recognized as a foreign substance. Hence, at high concentrations, sodium benzoate has the potential to stimulate WBC synthesis. The elevated WBC counts in Wistar rats treated with sodium benzoate may be due to an inflammatory response triggered as a defense mechanism (Ekanem *et al.,* 2015). Ascorbic acid used in this study did not significantly affect WBC counts. However, the addition of a small quantity of ascorbic acid to the sodium benzoate mixture before consumption significantly reduced WBC counts, likely due to the antioxidative properties of ascorbic acid.

Typically, there is an expected temporary increase in lymphocyte levels following an infection as they play a vital role in fighting diseases, which are recognized as antigen by the body. Low lymphocyte values can be a result of cold or other infections, as well as intense physical exercise, severe stress, or malnutrition. In this study, the observed low lymphocyte levels in the group B treated with 100 mg of sodium benzoate per kilogram of body weight were not statistically significant. However, the group D treated with a combination of ascorbic acid and sodium benzoate revealed a non-significant reduction in lymphocyte levels. This could be attributed to the xenobiotic activity of sodium benzoate, which can trigger an immune response at higher concentrations. Therefore, the lymphocyte levels in the other groups were not significantly different when compared to the control group or the group treated with ascorbic acid.

Red blood cells (RBCs) play a crucial role in the body by transporting oxygen throughout the system and removing carbon dioxide, which is exhaled in the lungs. Deficiency in RBCs lead to anemia, a condition characterized by reduced oxygen-carrying capacity. In this study, the significant decrease in hemoglobin concentration and reduced RBC count observed may be attributed to the intoxicating effects of sodium benzoate, indicating the initiation of anemia in the rats treated with sodium benzoate. The decrease in RBCs in the blood of rats exposed to sodium benzoate could be due to various factors, including impaired supply of cells from hematopoietic tissues, which might have been affected by the destructive impact of the foreign substance, as reported by Tuormaa, (2004). Additionally, the xenobiotic compound of sodium benzoate may have a harmful effect on the membranes of red blood cells, leading to hypoxemia. High doses of sodium benzoate can directly increase erythrocyte destruction and catabolism of hemoglobin

In general, when the hemoglobin concentration falls below the typical values (9-14 g/dL), it results in anemia. Specifically, toxins can decrease hemoglobin synthesis by inhibiting aminolevulinic acid dehydratase and ferrochelatase activities (Ashour *et al.,* 2007). Thus, at high concentrations, sodium benzoate may induce anemia by interfering with hemoglobin synthesis and weakening RBC survival. The increase in sodium benzoate consumption is associated with a decrease in hemoglobin levels, while the addition of ascorbic acid was found to mitigate the effects of sodium benzoate in this study. Similar results have been reported by Al-Hamdany, (2010), who suggested that exposure to xenobiotics can cause significant increase in WBC counts and decrease in hemoglobin, leading to inflammation-induced anemia in rats. The slight increase in hemoglobin and RBCs observed when ascorbic acid was added to sodium benzoate suggests the potential benefits of incorporating ascorbic acid and sodium benzoate food additives for fast foods or drinks.

The packed cell volume (PCV), which represents the average mass of packed red blood cells (RBCs) compared to the whole blood, is an important parameter. In this study, the PCV of the control group (43.3 ± 1.53%) fell within the previously reported ranges (Maaroufi *et al.,* 2016). The PCV values of the groups treated with ascorbic acid, sodium benzoate, and a combination of sodium benzoate and ascorbic acid were not significantly different compared to the standard control group. This similarity suggests that the observed effects of the food additives on both the cells and plasma contribute to the comparable PCV values. When the number of red blood cells and plasma volume decrease simultaneously, the hematocrit (PCV) may show little or no significant difference. The reduction in plasma volume could be attributed to dehydration caused by the effects of sodium benzoate and ascorbic acid (Yadav *et al.,* 2016). However, it is possible that an increase in sodium benzoate dosage can lead to a decrease in hematocrit levels.

The observed increase in platelet (PLT) counts in the group treated with sodium benzoate could be attributed to the stimulation of erythropoietin, which is triggered by increased metabolic activity or damage to respiratory membranes leading to a greater demand for oxygen and carbon dioxide transport (Zaki *et al.,* 2018). On the other hand, the reduction in platelet count observed in the group treated with a combination of ascorbic acid and sodium benzoate may be associated with thrombocytopenia (Ognjanovic *et al.,* 2003 and Achukwu *et al.,* 2009) and this have shown that ascorbic acid plays a significant role in preventing the adverse effects of sodium benzoate on hematological values.

The significant decrease in red blood cell (RBC) and hemoglobin (Hb) values, with no variations in hematocrit (HCT), observed in the treated groups indicates that prolonged oral administration of sodium benzoate could lead to macrocytic hypochromic anemia.

Statistical analysis revealed a significant difference in the means of neutrophils, monocytes, and eosinophils between the sodium benzoate treated animals when compared with the control group. The decrease in monocyte and eosinophil levels could be attributed to the toxic effect of sodium benzoate on these cell types, similar to what was observed in erythrocytes. Consequently, it is plausible to suggest that sodium benzoate may have a suppressive effect on hematopoietic cells, given that most of the major cell lines were affected. Thus, it can be inferred that sodium benzoate possesses some degrees of myelosuppressive effect on the bone marrow.

Specifically, the kidneys of Wistar rats in Group C and D, which were treated with sodium benzoate were subjected to histological analysis. The results of this analysis revealed majority of the glomeruli showed atropic changes and appear lobulized. Others showed hypercellular mesangial cells. The renal tubule especially at the pheriphery showed tubular necrosis. In contrast, the glomeruli of the control group's Wistar rats appeared fundamentally normal.

The administration of sodium benzoate in animals had noticeable effects on liver and kidney parameters. Furthermore, histopathological changes and dose-dependent alterations in biochemical markers of liver damage were observed (Khodaei *et al.,* 2019). The result of this study agrees with a study by Zeghib and Boutlelis, (2021) which stated that both kidney and liver histology were affected with sodium benzoate showing a greater impact on the kidneys than the liver. Similar to previous studies, rats exhibited histological changes such as glomerular and tubular necrosis and atrophy accompanied by a decrease in antioxidant defense. Another in vivo study confirmed the detrimental effects of sodium benzoate on the liver, as demonstrated in this investigation (Ibekwe *et al.,* 2007). It is evident from this study that the administration of sodium benzoate resulted in distinctive subcellular alterations in hepatocytes as reported by Khidr *et al.* (2012). Furthermore, the liver of the rats exhibited toxic effects caused by benzoate, in accordance with previous research. Histological changes were consistent with previous studies, confirming the adverse effects on the liver and kidneys (Agarwal *et al.,* 2016 and Radwan *et al.,* 2020).

Considering the stomach samples, the control group underwent histological investigations, which revealed an essentially normal stomach lining with no indications of mucosal epithelial necrosis or ulcers. In contrast, the stomach samples from Group C and D, exhibited no mucosal epithelial ulceration, however the attached skin shows extensive ulceration of the epidermis with accompanying intense inflammation. However, in Group B, there was no mucosal epithelial ulceration, and the attached skin was essentially normal although attenuated.

This study revealed that benzoate caused an increase in the release of allergic mediators, such as histamine and prostaglandins, from the mucosa, leading to damage to gastrointestinal cells compared to the control group. This is in agreement with the study by Walczak-Nowicka and Herbet, (2022) who also suggested that allergic reactions associated with benzoate might be mediated by prostacyclins and histamine.

This study found no abnormalities in any of the brain samples from both the control and treatment groups. This contradicts the findings of Asejeje *et al.* (2022), who reported that sodium benzoate exposure resulted in significant anxiogenic-like behavior, impaired locomotor and exploratory activities, and memory deficits. The pathological changes observed in their study may be attributed to the higher concentrations of sodium benzoate used to induce effects in the animals.

CONCLUSION

The findings of this study demonstrated that the administration of sodium benzoate (SB) to wistar rats has a notable impact on various haematological parameters, including white blood cell count, lymphocyte value, neutrophil count, monocyte count, eosinophil count, red blood cell count, haemoglobin concentration, and platelet values. However, when sodium benzoate is administered at high dosage, it can lead to aneamia. No significant effect of sodium benzoate was observed on the packed cell volume (PCV) of

the animals. On the other hand, the use of ascorbic acid as a food additive did not show any significant impact on the studied parameters.

Ethical Clearance

The protocol for this study was applied and approved by the Ethics and Research Committees of the Adeleke University, Ede, Osun State, with reference number AUERC/2023/04/33MLS-UG/18.

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Availability of data and materials: All data and materials that were collected during this study are available with the corresponding author upon reasonable request.

Authors' contributions: AAB and OVO conceived the idea. AAB and OVO designed the study methodology. AAB and OVO conducted the study. AAB and OVO analyzed the data. AAB and OVO interpreted the results. AAB and OVO wrote the draft manuscript. AAB and OVO revised and edited the final manuscript. AAB and OVO approved the manuscript.

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