Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 10, Issue 2, 2022

Oxidative Stress, Inflammation and Hepatotoxicity in Male Wistar Rats, exposed to Potassium Bromate and Sodium Nitrite

*Omowumi Oyeronke Adewale, Oluwaseun Abraham Adebisi and Oluwatosin Adefunke Adetuyi

Department of Biochemistry, Faculty of Basic and Applied Sciences,
Osun State University, Osogbo, Nigeria.

*Corresponding Author: adewaleomowumi13@gmail.com

ABSTRACT

The study investigated the hepatotoxic effect of co-exposure liver of male Wistar rats liver to bromate and nitrite. Rats were exposed to Potassium bromate ($KBrO_3$) (20 mg/kg of body weight, twice weekly) and Sodium nitrite ($NaNO_2$) (60 mg/kg of body weight as a single dose) orally and the experiment lasted 28 days. Hepatotoxicity was determined via the activities of the hepatic transaminases, alkaline phosphatase and gamma glutaryl transferase in the serum. Additionally, concentrations of nitric oxide, reduced glutathione, total thiol, malondialdehyde and activities of catalase, superoxide dismutase, and glutathione peroxidase together with the levels of pro-inflammatory markers in the liver were investigated. The results revealed the ability of BrO_3 and $NaNO_2$ to induce hepatotoxicity, oxidative stress and inflammation individually in rats. However, this capacity was augmented when rats were co-exposed to the toxicants as indicated by significant modification in these parameters assessed in the liver compared with the control. This study revealed the danger of hepatotoxicity associated with combined exposure to BrO_3 and $NaNO_2$ present in the environment.

Keywords: Sodium nitrite, potassium bromate, hepatotoxicity, hepatic markers.

INTRODUCTION

The use of chemical additives is fast replacing the conventional use of natural additives in the preservation and enhancement of the organoleptic properties of food substances. Potassium Bromate and Sodium Nitrite are some of the commonly used food additives. Potassium bromate, before its worldwide ban, was occasionally used in most parts of the world as a flour treatment agent which gives bread and bakery products a high rise and uniform finish [1]. Sodium nitrate

on the other hand is widely used in fertilizer and industrially in the manufacture of glass, explosives, charcoal briquettes and in the production of potassium compounds [2]. These additives have found extensive use in food industries as well as other industries. $KBrO_3$ for instance is a component of cold wave hair solutions and is also used in the cosmetics and pharmaceutical industries [3]. Though the acceptable dosages differ, the generally acceptable $KBrO_3$ level according to Food and Drug Administration (FDA) agency should be lower than 75 mg/kg [4].

Owing to their industrial uses, individual exposure to these additives is somewhat inevitable. However, irrespective of their important roles, reports have shown that individual exposure to these additives could be detrimental to human health [5, 6]. $KBrO_3$ for instance is a major tap water pollutant [7]. Exposure to $KBrO_3$ and $NaNO_2$ has been reported to increase the risk of enhanced nephrotoxicity [8]. Oral exposure to potassium bromate has been reported to induce neurobehavioral changes, alter cerebral neurotransmitter levels and impair brain tissue of Swiss mice [9].

The biotransformation of $KBrO_3$is often considered the major mechanism underlying its toxicity [8]. As a result of its biotransformation, $KBrO_3$ generates free radicals and reactive oxygen species (ROS) inducing oxidative stress and causing damage to major macromolecules. This generation of ROS has been attributed to the actions of intracellular reductants, reducing $KBrO_3$ to bromide [8]. Studies supporting the role played by $KBrO_3$ in oxidative stress induction have reported its ability to induce oxidative modification of lipids and proteins in various animal tissues [1]. Oxidative stress induction by $KBrO_3$ is also widely thought to play a role in the carcinogenicity and mutagenicity of $KBrO_3$ [5].

The toxicity of sodium nitrate on the other hand is attributed to its biotransformation to nitrite. During the biotransformation of sodium nitrate to nitrite, several reactive nitrogen species (RNS) are generated. These include $NO^{\bullet -}$ and peroxynitrite (ONOO-) which possess the ability to react with essential macromolecules such as proteins to form nitrotyrosine [10]. Also, nitrite has been reported to react with amines to form nitrosamines such as N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), and N-nitrosomorpholine (NMOR), which are human carcinogens. Specifically, NDMA has been reported to be hepatotoxic, causing fibrosis and tumours [11]. Several studies have reported the alterations of several biochemical parameters by

nitrates. These parameters include glucose, cholesterol, creatinine, LDH, AST, ALT, and alkaline phosphatase (AP) [12, 13]

The liver plays a crucial role in the biotransformation of exogenous substances and can therefore be considered a target organ of exogenous toxicants. Several studies have reported the individual toxicity of $KBrO_3$ and $NaNO_2$ [5,13] as well as the toxicity caused by co-exposure to both additives [7]. It was reported that $KBrO_3$ promoted lesions of several organs, particularly the kidney, as well as posing dangers of mutagenicity and carcinogenicity [5]. It was also reported that individual exposure to $KBrO_3$ and $NaNO_2$ induced renal damage and increased induction of oxidative stress which was further aggravated by co-exposure to these compounds [13]. However, because most of the reported studies examined the effect of $KBrO_3$ and $NaNO_2$ on the kidney, there is a paucity of data on the effect of co-exposure to $KBrO_3$ and $NaNO_2$ on the liver of both human end experimental animals.

This study analyses the effect of co-exposure to $KBrO_3$ and $NaNO_2$ on oxidative stress and inflammatory biomarkers as well as markers of hepatotoxicity in male Wistar rats.

MATERIALS AND METHODS

Chemicals and reagents

Rat C-reactive protein (CRP) Catalog Number.CSBE07922r; and ADA (Adenosine deaminase) rat ELISA kits were supplied by e-Bioscience, Inc. $KBrO_3$(CAS NO: 7758), (99% purity) $NaNO_2$ was supplied by Lab-Tech Chemicals, Osogbo, Nigeria. Glutathione (GSH), 5',5'-dithiobis-2-nitrobenzene (DTNB), 2-thiobarbituric acid (TBA), Biuret and 1 chloro-2, 4-dinitrobenzene (CDNB) and hydrogen peroxide (H_2O_2) were manufactured by Sigma-Aldrich, St Louis, MO, USA. All other reagents and chemicals used in this study were of analytical grade while the water used was glass distilled.

Experimental Animals

Twenty (20) albino Wistar rats were obtained from the animal holding facility of the College of Health Sciences, Osun State University, Osogbo, Nigeria. The animals were allowed to acclimatize for one week before the commencement of the research at 25^{o}C, 45–55% relative humidity and a 12-hour dark-light cycle in plastic cages. The animals were treated following the National Institutes of Health (NIH) guidelines. The ethical committee of the Osun State

University, College of Health Sciences Osogbo, Nigeria, approved the research before its commencement.

Experimental design

Twenty (20) Wistar rats were shared equally into four groups of five animals each and treated as follows:

A – Control

B - Potassium bromate ($KBrO_3$) (20 mg/kg of body weight, twice weekly)

C - Sodium nitrite ($NaNO_2$) (60 mg/kg of body weight as a single dose) orally,

D - Potassium bromate (20 mg/kg) + sodium nitrite (60 mg/kg).

The period of administration lasted 28 days. Twenty-four hours after the last administration, the animals were sacrificed. The serum and tissues were collected for biochemical analysis.

Determination of serum hepatic function biomarkers

Liver function biomarkers, alanine aminotransferases (ALT) and aspartate aminotransferases (AST), activities were determined as described by Reitman and Frankel [14]. Alkaline phosphatase (ALP) activity was determined following the method described by Englehardt [15]. Total protein concentrations of the serum were determined according to the Biuret method as described by Gornall *et al*. [16]. Albumin concentration was determined following the principle reported by Grant [17]. Bilirubin concentration was determined according to the method previously described by Bilirubin [18].

Estimation of antioxidant status

Catalase activity was determined according to the method previously described [19]. Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 and30 °C according to Misra and Fridovich [20]. Reduced glutathione (GSH) was determined according to the method of Jollow *et al*. [21]. The activity of Glutathioneperoxidase (GPx) was estimated following the method reported by Rotruck *et al*., [22], while total thiol (TSH)content in the liver homogenate was determined as described by Ellman [23].

Assay of liver C-reactive Protein and Adenosine Deaminase

Enzyme-linked immunosorbent assay (ELISA), was used to measure the concentration of high sensitive C reactive protein (CRP) by following the instructional manual. Adenosine deaminase (ADA) activity was determined by the method of Giusti and Galanti [22].

Statistical analysis

The data were expressed as mean ± standard deviation (SD) after analysis by one-way analysis of variance (ANOVA) with the aid of GraphPad Prism 6 software. This was followed by Tukey's posthoc multiple comparison test. Differences between mean values of different groups were considered statistically significant at $p< 0.05$.

RESULTS AND DISCUSSION

Table 1 shows a comparison of levels of Liver function markers and protein (TP and Albumin) in rats exposed to Sodium nitrite, Potassium bromate or both.

Table1: Levels of Liver function markers and Protein (TP and Albumin) in rats exposed to sodium nitrite, potassium bromate or both.

Parameters	Control	Bromate	Nitrite	BRO + NIT
T.BIL (mg/dL)	4.50±0.58	8.75±0.50*	7.67±0.58*	8.67±0.58*
C.BILRUBIN (mg/dL)	1.50±0.58	2.75±0.50*	2.67±0.58*	3.33±0.58*
T.PROTEIN (mg/dL)	94±1.15	43.25±1.26*	46.67±4.04*	43.33±3.21*
ALBUMIN (mg/dL)	42±1.41	25.25±2.50*	26±5.57*	21.33±1.53*
AST (U/L)	90±7.07	189±4.55*	179±8.54*	198.33±2.52*
ALT (U/L)	82.5±1.73	125.75±4.35*	116.67±2.52*	130.33±4.73*
ALP (U/L)	26.25±1.89	70.5±6.14*	52.67±3.06*	64.67±4.16*

Results are represented as Mean ± Standard Deviation (* - Significantly different from control $p < 0.05$).

Figure 1 shows the levels of antioxidants (CAT, SOD, GPx, GSH) and Total thiol (TSH) as measured in the liver of rats exposed to sodium nitrite, potassium bromate or both.

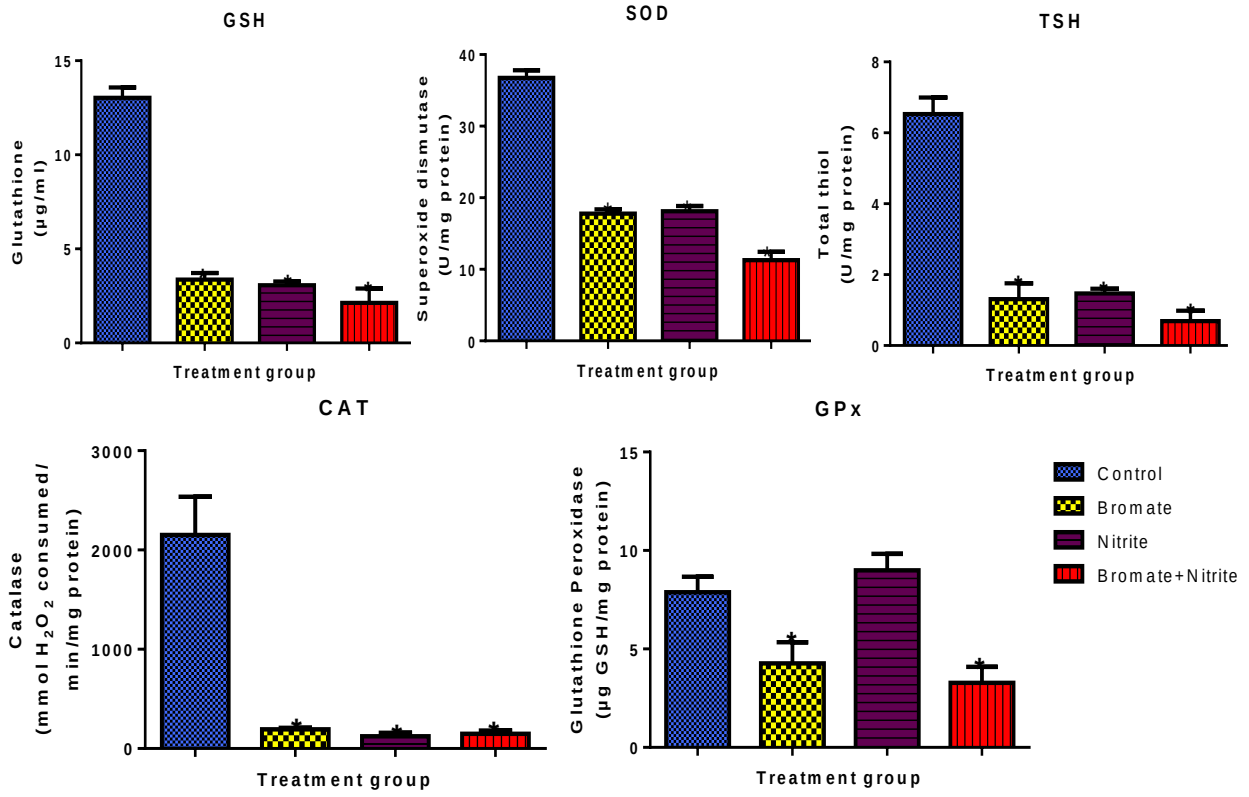

Figure 1: Levels of antioxidants (CAT, SOD, GPx, GSH) and Total thiol (TSH) in the liver of rats exposed to Sodium nitrite, potassium bromate or both. *- Significantly different from control ($p < 0.05$).

$NaNO_2$, $KBrO_3$ and their combination decrease the levels of antioxidants

It was observed that administration of $NaNO_2$, $KBrO_3$ and a combination of both lead to a significant decrease in the levels of glutathione, superoxide dismutase, glutathione peroxidase, and catalase as well as total thiol group when compared to control ($p < 0.05$).

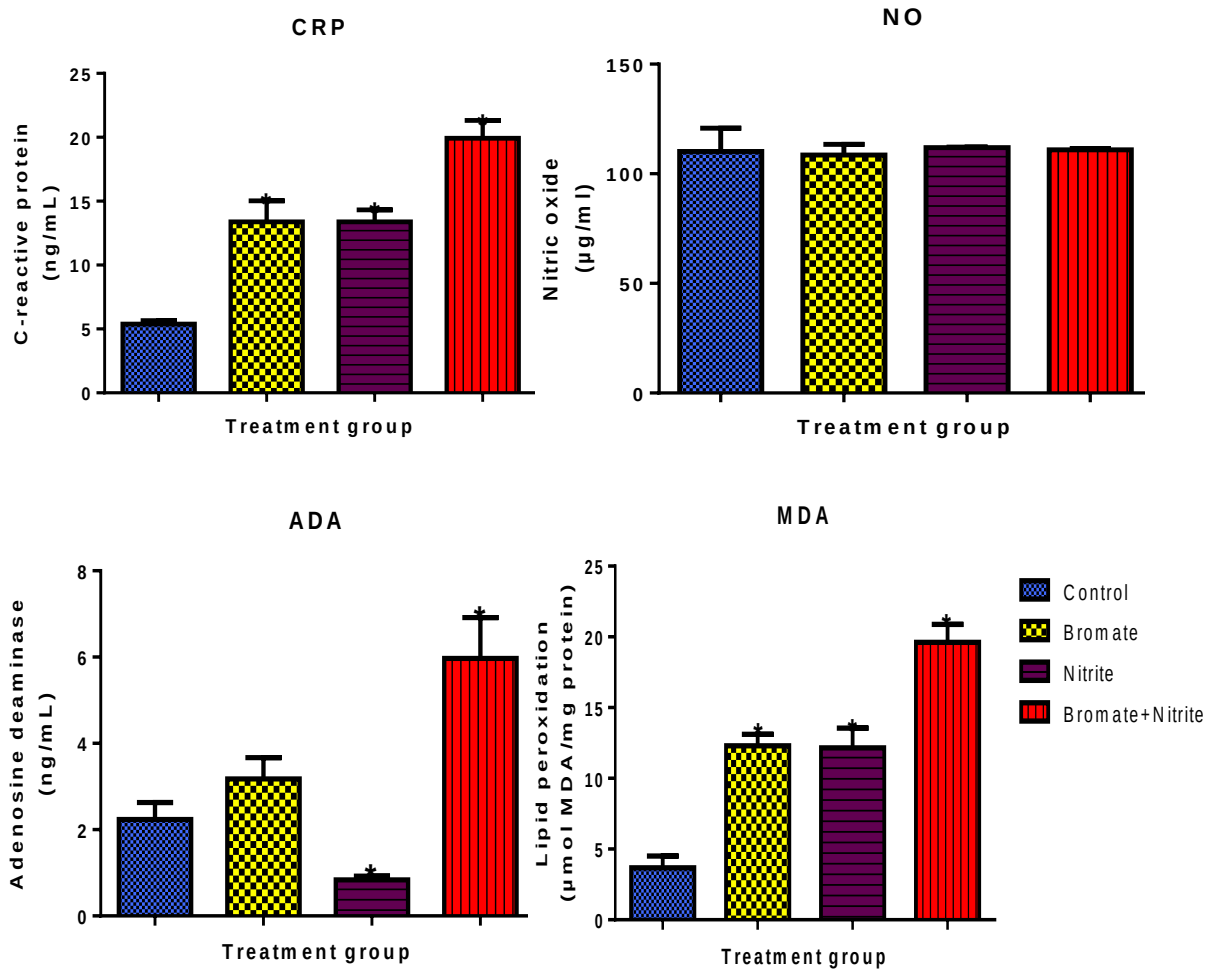

Figure 2: Levels of liver inflammatory markers (CRP, NO and ADA) and Lipid peroxidation of rats exposed to Sodium nitrite, potassium bromate or both. *- Significantly different from control ($p < 0.05$)

$NaNO_2$, $KBrO_3$ and their combination lead to an increase in inflammatory markers and lipid peroxidation in Wistar rats

Administration of $NaNO_2$, $KBrO_3$and their combination lead to an increase in inflammatory markers including C - reactive protein, adenosine deaminase, and lipid peroxidation when compared to control ($p < 0.05$) but there was a significant difference in the levels of nitric oxide across all treatment groups.

The present study highlights the impact of $KBrO_3$, and $NaNO_2$ and their combination on inflammation, oxidative stress and hepatotoxicity in the liver of Wistar rats. Food additives are

important products used in the food industry. The short shelf life of food products, the quest to improve the organoleptic properties of foods and the need for food with great aesthetic value necessitate their continuous use in the industry [25,26]. The function of food additives includes acidity regulators, anticaking agents, antifoaming agents, antioxidants, colourants, and emulsifiers [27]. $KBrO_3$ and $NaNO_2$ use are not limited to the food industry as they are also used in cosmetic and chemical industries. However, the continuous use of food additives is not without health implications and calls for caution in their usage [6].

Oxidative stress represents a lopsidedness in free radicals production including reactive oxygen species and reactive nitrogen species and the body's ability to detoxify them [28]. This state is responsible for a host of diseases including cancer, neurodegenerative diseases, and diabetes [13]. Antioxidants, however, are molecules which when present in certain concentrations can detoxify free radicals thereby conferring protection on the body [29]. They are generally classified as enzymatic and non-enzymatic antioxidants. While enzymatic antioxidants are generally produced endogenously in the cells, non-enzymatic antioxidants are present in some grains, fruits, nuts and cereals [29].

Liver is the major detoxifying organ in the body. It contains phase I and II xenobiotic-metabolizing enzymes including the cytochrome P450 superfamily [30]. Also participating in the synthesis of proteins in the liver are the hepatic transaminases and alkaline phosphatase. Elevated levels of liver function enzymes have been documented to be indicative of hepatic injury or hepatotoxicity [31]. Significant elevation in the levels of liver function enzymes was observed upon $NaNO_2$ and $KBrO_3$ administration and also in their combination as shown in Table 1.

Bilirubin is one of the waste products in the catabolic breakdown of heme in red blood cells [32]. Significant elevation of bilirubin in the liver is indicative of hepatic injury as reflected in Table 1. The elevated level of bilirubin may be a result of the continuous destruction of red blood cells in the liver which is also indicative of hepatic damage.

Figure 1 shows the effect of $KBrO_3$, $NaNO_2$ and their combination on the antioxidant status in the liver. A significant decrease is seen in the levels of catalase, superoxide dismutase, glutathione, glutathione peroxidase and total thiol in the liver of male Wistar rats. Superoxide dismutase and catalase are majorly involved in the detoxification of superoxide radical (O_2^-) via converting it to hydrogen peroxide which is then further split into oxygen and water. Glutathione and glutathione peroxidase also perform a similar function by partaking in the detoxification of

hydrogen peroxide and lipid peroxides converting them into water and lipid alcohols [33]. A significant decline in the activities of these antioxidants is indicative of oxidative stress and hepatotoxicity. The effects of single and combined toxicities of potassium bromate and sodium nitrite in the kidney of Wistar rats have been previously documented [7]. Apart from the studies, other researchers have identified the toxicity effect of potassium bromate and sodium nitrite either singly or in combination [34,35].

Lipid peroxidation as evident by an increase in malondialdehyde is indicative of oxidative stress and damage to lipid-containing substances in the body. This occurrence is in the form of chain reactions and is characterized by a free radical attack on lipids in the body [36]. Sodium nitrite, potassium bromate as well as a combination of both leads to significant elevation in lipid peroxidation in Wistar rats.

Inflammation is generally characterized by an immune response to dangerous stimuli such as toxins, irradiation, and pathogens. This response triggers a host of cellular signaling mechanisms which cascade in an elevation of signaling messengers including nitric oxide, tumour necrosis factor-α, and cyclooxygenase-2 [37]. The levels of nitric oxide in all treatment groups were not significantly elevated in $KBrO_3$, $NaNO_2$ or their combination treatment groups. This indicates that the inflammation induced by $NaNO_2$ and $KBrO_3$ may not be NO-dependent at the doses administered. Furthermore, CRP has been identified to be elevated in several malignancies including those associated with liver cells and is indicative of inflammation [38]. $NaNO_2$, $KBrO_3$ and their combination led to significant elevation in the level of CRP when compared to control.

ADA is an enzyme that controls the deamination of adenosine to inosine. It has two significant is of forms ADA-1 and ADA-2 and is correlated with liver injury [39]. It is significantly elevated in patients with hepatic fibrosis, liver cirrhosis and other diseases and is also indicative of inflammation. $NaNO_2$ and $KBrO_3$ combination significantly elevates the levels of ADA in Wistar rats as shown in Figure 2. It is capable to quell these toxicities and hence should be encouraged in such cases.

SOURCE OF FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

Authors' contributions

Oluwaseun Abraham Adebisi and Omowumi Oyeronke Adewale conceived and designed the study, conducted experiments, provided research materials, collected and organized the data. Oluwaseun Abraham Adebisi and Oluwatosin Adefunke Adetuyi contributed to writing the initial draft of the manuscript and the final review of the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

ETHICAL APPROVAL

All procedures performed in studies involving animals followed the declaration of Helsinki as described by the College of Health Sciences ethical approval committee at Osun State University, Osogbo. The handling and use of all laboratory animals were as per NIH Guide for the care and use of laboratory animals. The authors also have ethics certifications and training with identification number: 68024073.

REFERENCES

1. Ahmad, M.K., Khan, A.A. & Mahmood, R. (2013). Taurine ameliorates potassium bromate-induced kidney damage in rats. *Amino Acids*,45,1109–21.
2. Pokorny, L., Maturana, I.& Bortle, W.H. (2006). Sodium Nitrate and Nitrite. Kirk-Othmer Encycl Chem Technol.
3. IARC (1999). International Agency for Research on Cancer. Potassium Bromate. Monographs, 73.
4. Center for Food Safety and Applied Nutrition. (2006). *Food Additive Status list*. U.S. Food and Drug Administration. https://www.fda.gov/food/food-additives-petitions/food-additive-status-list#ftnP. Retrieved September 4, 2022
5. Starek, A. &Starek-Świechowicz, B. (2016). Toxicological Properties of Potassium Bromate. *J Pharmacol Reports*, 1,1–9.

6. Delgado, M.F.G., Zamora, A.G., Gonsebatt, M.E. & Mata, E.M. (2018). Ecotoxicology and Environmental Safety Subacute intoxication with sodium nitrate induces hematological and biochemical alterations and liver injury in male Wistar rats. *Ecotoxicol Environ Saf. Elsevier Inc*,166, 48–55.
7. Adewale, O.O., Aremu, K.H. & Adeyemo, A.T. (2020). Assessment of combined toxic effects of potassium bromate and sodium nitrite in some key renal markers in male Wistar rats. *Res J Heal Sci*,8, 6–17.
8. Altoom, N.G., Ajarem, J, Allam, A.A., Maodaa, S.N. & Abdel-Maksoud M.A. (2018). Deleterious effects of potassium bromate administration on renal and hepatic tissues of Swiss mice. *Saudi J Biol Sc*i,25, 278–84.
9. Ajarem, J., Altoom, N.G., Allam, A.A., Maodaa, S.N., Abdel- Maksoud, M.A. & Chow B.K.C. (2016). Oral administration of potassium bromate induces neurobehavioral changes, alters cerebral neurotransmitters level and impairs brain tissue of Swiss mice. *Behav Brain Funct. BioMed Central*,12:1–10.
10. Belcastro, E., Gaucher, C., Corti, A., Leroy, P., Lartaud, I. & Pompella, A. (2017). Regulation of protein function by S-nitrosation and S-glutathionylation: Processes and targets in cardiovascular pathophysiology. *Biol Chem*,398,1267–93.
11. Erkekoglu, P. & Baydar, T. (2010). Evaluation of the protective effect of ascorbic acid on nitrite- and nitrosamine-induced cytotoxicity and - genotoxicity in human hepatoma line. 20, 45–52.
12. Seyhan, E., Mert, N. & Mert, H. (2012). The effect of pepper gas (OC) on some biochemical parameters in rats. *Kafkas Univ Vet Fak Derg*, 18, 259–66.
13. Adewale, O.O., Samuel, E.S., Manubolu, M.&Pathakoti, K. (2019). Curcumin protects sodium nitrite-induced hepatotoxicity in Wistar rats. *Toxicol Reports. Elsevier*, 6, 1006–11.
14. Reitman, S. & Frankel, S. A. (1957). colourimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol*, 28, 56–63.
15. Englehardt, L. (1970). Measurement of alkaline phosphatase. *Aerztl Labor*, 16:1.
16. Gornall, A.G., Bardawill, C.J. & David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. *J Biol Chem*, 177, 751–66.
17. Grant, G. (1987). Amino acids and protein; fundamentals of clinical chemistry. Tietz NW WB Saunders Co. Philadelphia USA.

18. Kapitulnik, J. (2004). Bilirubin: An endogenous product of heme degradation with both cytotoxic and cytoprotective properties. *Mol Pharmacol*, 66, 773–9.
19. Manubolu, M., Goodla, L., Ravilla, S., Thanasekaran, J., Dutta, P., Malmlöf, K. et al. (2014). Protective effect of Actiniopteris radiata (Sw.) Link. against CCl 4 induced oxidative stress in albino rats. *J Ethnopharmacol*, 153, 744–52.
20. Misra, H.P. & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*, 247, 3170–5.
21. Jollow, D., Mitchell, J.R., Zampaglione, N. & Gillette, J.R. (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, 11:151–69.
22. Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. & Hoekstra, W.G. (1973). Selenium: Biochemical role as a component of glatathione peroxidase. *Science*,179,588–90.
23. Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, *82*(1), 70–77. https://doi.org/10.1016/0003-9861(59)90090-6
24. Giusti, G. & Galanti, B. (1984). Adenosine deaminase: colorimetric method. *Methods Enzym Anal*, 315–23.
25. Srivastava, R.K. (2018). Enhanced shelf life with improved food quality from fermentation processes. Available from: http://www.alliedacademies.org/food-technology-and-preservation/J Food Technol Preserv, 2,1–7.
26. Malik, A., Erginkaya, Z. & Erten H. (2019). Health and safety aspects of food processing technologies. Heal. Saf. Asp. Food Process. Technol.
27. Blekas, G.A. (2015). Food Additives: Classification, Uses and Regulation. *Encycl Food Hea*l, 731–6.
28. Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V. et al. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxid Med Cell Longev*, 1–14.
29. Kurutas, E.B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. *Nutr J*, 15,1–22.
30. Gu, X.& Manautou, J.E. (2012). Molecular mechanisms underlying chemical liver injury. *Expert Rev Mol Med*,14, 1–15.
31. Ahmed, Z., Ahmed, U., Walayat, S., Ren, J., Martin, D.K., Moole, H. et al. (2018). Liver function tests in identifying patients with liver disease. *Clin Exp Gastroenterol*, 11, 301–7.

32. Kalakonda, A.& John, S. (2018). Physiology, Bilirubin. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29261920StatPearls

33. Tan, B.L., Norhaizan, M.E., Liew, W.P.P. & Rahman, H.S. (2018). Antioxidant and oxidative stress: A mutual interplay in age-related diseases. *Front Pharmacol*, 9, 1–28.

34 Badreldin, H.A., Mohammed Al Za'abi., Turan Karaca., Yousuf Al Suleimani., Khalid A Al Balushi., Priyadarsini Manoj., Mohammed Ashique. & Abderrahim Nemmar. (2018). Potassium bromate-induced kidney damage in rats and the effect of gum acacia thereon. *Am J Transl Res*, 10(1), 126-137

35 Atila Uslu, G., Uslu, H., & Adali, Y. (2019). Hepatoprotective and nephroprotective effects of Trigonella foenum-graecum L. (fenugreek) seed extract against sodium nitrite toxicity in rats. *Biomedical Research and Therapy*, *6*(5), 3142–3150.

36. Ayala, A., Muñoz, M.F. & Argüelles, S. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*, 2014,1–31.

37. Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., et al (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9, 7204–18.

38. Sproston, N.R. & Ashworth, J.J. (2018). Role of C-reactive protein at sites of inflammation and infection. *Front. Immunol*. 2018.

39. Pinheiro, F.V., Pimentel, V.C., De Bona, K.S., Scola, G., Salvador, M., Funchal, C., et al. (2010). Decrease of adenosine deaminase activity and increase of the lipid peroxidation after acute methotrexate treatment in young rats: Protective effects of grape seed extract. *Cell Biochem Funct*, 28, 89–94.