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Research Article

THE PROTECTIVE EFFECTS OF AQUEOUS EXTRACT OF AFRICAN NUTMEG (*Myristica fragrans*) IN BROMATE-INDUCED SPLEEN AND CARDIAC TISSUE TOXICITIES USING MALE WISTAR ALBINO RATS

^{1*}Oseni Oatunde Abass, ²Olagboye Sulaiman Adeoye, ³Adams Olusegun Timothy, ¹Maikasuwa Bamaiyi Sunday

¹Department of Medical Biochemistry, College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria

²Department of Chemistry, Faculty of Science, Ekiti State University, Ado-Ekiti, Nigeria

³Department of Anatomy, College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria

ABSTRACT

This study was aimed at investigating the toxic effects of potassium bromate on the spleen and heart of Wistar albino rat and to evaluate the protective effects of aqueous extract of African nutmeg (*Myristica fragrans*) against potassium bromate induced toxicity in the two organs. Twenty-four (24) male wistar albino rats weighing between 180g and 200g were divided into four groups of six animals each. Group I animals were served with rat feed and water as the control group, group II were administered daily with 30mg/kg bw potassium bromate while groups III and IV animals were administered with 0.5mL of 20% and 40% aqueous extracts of nutmeg seed in addition with 30mg/kg body weight potassium bromate respectively for two weeks. The animals were kept at optimum temperature within a 12 hours light/dark cycle while the experiment lasted. Enzyme biomarkers such as Aspartate Transaminase, Alanine Transaminase, Alkaline Phosphatase; antioxidant enzymes such as, Superoxide dismutase, Catalase; reduced Glutathione; some lipid profiles like Cholesterol, Triglyceride and malondialdehyde were measured in the spleen and heart homogenates of the animals in all the groups. Results of this study showed that potassium bromate exerted significant ($P < 0.05$) toxic effects on the spleen and heart homogenates while the administration of aqueous extracts of African nutmeg seed caused a marked reversal in the toxicity in a dose dependent manner. However, the results of this study showed that aqueous extract of the seed of African nutmeg is a potential antioxidant against potassium bromate toxicity in the two organs.

Keywords: Toxicity, Potassium bromate, *Myristica fragrans*, Cardiac tissue, Antioxidant

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*Address for Correspondence:

Oseni Oatunde Abass, Department of Medical Biochemistry, College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria

INTRODUCTION

Apart from the use of potassium bromate as a laboratory reagent and oxidizing agent in permanent-wave compounds, as a food additive and in explosives;^{1,2} it has also been used severally in many countries of the world as flour improver to strengthen the dough and allowing higher rising.³ Carcinogenic and mutagenic effects of potassium bromate have been reported in experimental animals⁴ and extremely toxic to tissues especially those of the central nervous systems and

kidney.⁵ Similarly, it has also been shown that it possess the potential of inducing kidney failure, deafness, redness and pains of the eye and skin.^{6,7}

The mechanism of action of potassium bromate has been investigated by Fujii.⁸

Potassium bromate was found to be a nephrotoxic, neurotoxic, genotoxic, and a carcinogenic agent as it causes lipid peroxidation (LPO) and oxidative DNA damage.^{9,10,4} The LD₅₀ (median lethal dose, 50%) of

potassium bromate was reported to be 150-385 mg/kg body weight. In humans, oral doses of 185-385 mg/kg body weight leads to irreversible renal damage and deafness while lower doses leads to gastrointestinal disturbances and abdominal pain.⁶ Though nutmeg was reported to inhibit prostaglandin production and contains hallucinogens that may affect fetus if consumed in large quantities. Nutmeg was once considered an abortifacient, but may be safe for culinary use during pregnancy; its uses have expanded with time.

In low doses, nutmeg produces no noticeable physiological or neurological response, but in large doses, raw nutmeg has psychoactive effects. In its freshly-ground (from whole nutmegs) form, nutmeg contains myristicin, a monoamine oxidase inhibitor and psychoactive substance. Myristicin poisoning can induce convulsions, palpitations, nausea, eventual dehydration, and generalized body pain.¹¹ It is also reputed to be a strong deliriant.¹² Fatal myristicin poisonings in humans are very rare, but two have been reported: one in an 8-year-old child¹³ and another in a 55-year-old adult, the latter case attributed to a combination with flunitrazepam.¹⁴

In a case reports, raw nutmeg produced anticholinergic-like symptoms, attributed to myristicin and elemicin.^{15,13,16}

The potential toxicity of bromate has given rise to various forms of speculations which led to its ban in Nigeria by National Agency for Food and Drug Administration Control¹⁷ and even in some other countries by their regulatory bodies. World Health Organization also banned the use of potassium bromate in 1993.¹⁸

The present study however attempts to assess the toxic effects of potassium bromated on the

spleen and cardiac tissue of albino rats and to determine the protective effects of aqueous extract of *Myristica fragrans* on these effects by assaying for the activities of some carefully selected 'marker' enzymes (alkaline phosphatases, alkaline transaminase and aspartate transaminase); catalase, superoxide dismutase, glutathione, cholesterol, triglyceride and malondialdehyde.

MATERIALS AND METHODS

Sample collection: Nutmeg was bought from the Erekesan market in Ado-Ekiti, Ekiti State, Nigeria and made to powder using hand grater. While wistar-albino rats were obtained from animal house of Pharmacology Department, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The rats were brought and housed in ventilated cages in the Animal House of Biochemistry Department, Ekiti State University, Ado-Ekiti, Nigeria where they were acclimatized and fed with standard rat pellets and water for two weeks before administration of the inducer and treatments.

Chemical reagents: The alloxan and potassium bromate (KBrO₃) used were of analytical grade, distilled water was used in the analyses; injection water was obtained from a Pharmaceutical Store in Ado-Ekiti. All the diagnostic kits used were products of Randox Chemical Ltd. England.

Animal Grouping: The study was performed on twenty-four (24) wistar-male albino rats housed in ventilated cages and grouped into four of six rats each.

Group A served as the control. Groups B, C and D were administered 30mg/kg body weight potassium bromate. In addition, Group C and Group D animals were treated with oral administration of 20% and 40% aqueous extract of nutmeg daily respectively. All the animals were kept at optimum temperature with a 12 hour light/dark cycle and given water and rat feed for the three weeks period of the experiment. Rats were sacrificed at the end of the 21days experimental period by anaesthetized chloroform and quickly dissected to harvest the required organs.

Preparation of Organs homogenate: Spleen and heart were removed, placed on ice-bath and weighed. 10% of each organs homogenate were prepared in 6.7mM potassium phosphate buffer, (pH 7.4) using top driven electrical homogenizer. The homogenate was centrifuged at 3,000 rpm for 10 minutes to obtain a clear supernatant which was stored at 8⁰C for the estimation of Aspartate Transaminase (AST), Alkaline Transaminase (ALT), Alkaline Phosphatase (ALP), Cholesterol, High Density Lipoprotein-Cholesterol (HDL-Chol), Triglyceride (TGD) were done using Fortress analytical kits from England while Superoxide dismutase (SOD) was determined using the method of Misra and Fridovich,¹⁹ Catalase (CAT) using the method described by Sinha,²⁰ reduced Glutathione (GSH) was done with Jollow *et al.*, method²¹ and Malondialdehyde (MDA) was done by Varshney and Kale method²²

Statistical Analysis

The experimental results of the analyses were obtained in triplicates with means and standard deviations. Various formulae were used to calculate the individual parameter and enzyme activities. The means and standard deviations of the triplicates results were determined using Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Results

The results of Aspartate Transaminase (AST), Alkaline Transaminase (ALT), Alkaline Phosphatase (ALP) in potassium bromate induced-spleen and cardiac tissue toxicities in male Wistar albino rats were assessed as shown in Table 1. The concentration of these studied enzymes biomarkers were observed to reduce in the spleen while it was shown to increase in the heart of the bromate fed group. The treatment with aqueous extracts of the nutmeg however produced a reversal to the bromate effect induced on the spleen and heart.

Table 1: Effects of some enzyme biomarkers on potassium bromate-induced toxicity in the spleen and cardiac tissue of Wistar albino rats

ANIMAL GROUPS	AST (IU/L)		ALT (IU/L)		ALP (IU/L)	
	SPLEEN	HEART	SPLEEN	HEART	SPLEEN	HEART
A	30.00±0.50	16.50±0.72	8.00±0.22	9.00±0.20	18.20±0.62	9.11±0.51
B	20.00±0.30	20.00±0.50	7.00±0.14	10.00±0.20	9.60±0.30	18.20±0.65
C	30.00±0.40	18.50±0.44	6.00±0.21	8.00±0.30	18.11±0.21	9.60±0.33
D	40.00±0.40	19.20±0.52	2.00±0.10	7.00±0.40	21.80±0.28	9.90±0.26

Mean ±SD of triplicate results

- A Control (Normal Feed)
 B Bromate Fed
 C Bromate + 20% Nutmeg aqueous extract
 D Bromate + 40% Nutmeg aqueous extract

Table 2: Effects of some antioxidant enzymes on potassium bromate-induced toxicity in the Spleen and Cardiac Tissue of Wistar albino rats.

ANIMAL GROUPS	SOD (% Inhibition)		CAT (mg/dl)		RED.GSH (µg/ml)	
	SPLEEN	HEART	SPLEEN	HEART	SPLEEN	HEART
A	77.30±0.96	56.00±0.80	0.20±0.01	0.03±0.00	2.19±0.04	1.41±0.04
B	34.06±0.51	36.06±0.62	0.03±0.00	0.01±0.03	1.15±0.05	1.09±0.03
C	41.67±0.42	39.30±0.44	0.13±0.00	0.03±0.00	3.10±0.01	1.40±0.03
D	52.30±0.65	43.00±0.51	0.38±0.01	0.05±0.00	3.38±0.10	2.42±0.06

Mean ±SD of triplicate results

- A Control (Normal Feed)
 B Bromate Fed
 C Bromate + 20% Nutmeg aqueous extract
 D Bromate + 40% Nutmeg aqueous extract

The results of Superoxide dismutase (SOD), Catalase (CAT), reduced Glutathione (GSH) in potassium bromate induced spleen and cardiac tissue toxicities in male Wistar albino rats were assessed as shown in Table 2. The concentration of these studied antioxidant

enzymes were observed to reduce in both the spleen and the heart in bromate fed group. The treatment with aqueous extracts of the nutmeg however produced a reversal to the bromate effects on the spleen and heart.

Table 3: Some Lipid Profile parameters of Potassium Bromate-induced Toxicity in the Spleen and Cardiac Tissue of Wistar albino rats.

ANIMAL GROUPS	CHOL (mMol/dl)		HDL-CHOL (mMol/dl)		TGD (mMol/dl)		MDA (Unit/ mg) x 10 ⁻⁶	
	SPLEEN	HEART	SPLEEN	HEART	SPLEEN	HEART	SPLEEN	HEART
A	3.74±0.13	2.95±0.21	1.92±0.05	6.47±0.18	2.14±0.02	1.70±0.07	3.13±0.10	2.48±0.12
B	2.73±0.12	2.13±0.17	1.48±0.07	2.36±0.05	2.44±0.06	1.93±0.04	7.35±0.50	4.08±0.11
C	3.02±0.06	2.87±0.09	1.92±0.06	1.66±0.03	1.89±0.06	2.09±0.03	1.38±0.11	2.78±0.22
D	3.05±0.03	2.77±0.08	4.20±0.11	3.06±0.13	2.49±0.10	1.55±0.02	4.16±0.15	3.39±0.24

Mean ±SD of triplicate results

- A Control (Normal Feed)
 B Bromate Fed
 C Bromate + 20% Nutmeg aqueous extract
 D Bromate + 40% Nutmeg aqueous extract

The results of cholesterol, High Density Lipoprotein-Cholesterol (HDL-Chol), Triglyceride (TGD) and Malondialdehyde (MDA) in Potassium Bromate induced-Spleen and Cardiac tissue toxicities in male Wistar Albino rats were assessed as shown in Table 3. The concentration of these studied parameters were observed to increase in the spleen (except in CHOL)

whereas the concentration of CHOL and HDL-Chol decreased while that of TGD and MDA increased in the heart of the bromate fed group. The treatment with aqueous extracts of the nutmeg however produced a reversal to the bromate effect induced on the spleen and heart.

Discussion

In Table 1, the increase in the concentration of AST, ALT and ALP activity observed in the heart of animals in group B compared with the control in group A may be attributed to some impairment to the integrity of the organ as a result of a possible reaction between potassium bromate and the heart tissues whereas the decrease in AST, ALT and ALP activity observed in the spleen of group B as compared with the control in Group A may be attributed to loss of some components of the spleen tissue due to possible reaction with potassium bromate. The observed changes in the concentrations of the enzymes however might not pose any serious danger to the organs as the values rarely exceed the normal concentrations.

The enzymes from diseased organs which appeared to have been leaked into the serum resulting in increased activity and reduced activity in diseased organ might become evidenced. The pronounced reduction in ALP activity of the spleen relative to the heart enzyme activity may be attributed to the fact that the latter has a major role of oxygenation and distribution of blood throughout the various organs in the system and hence capable of handling toxic compounds such as potassium bromate.

The transaminases such as AST, ALT and ALP are well-known enzymes used as biomarkers to predict possible toxicity in liver and other organs.²³ Furthermore, measurement of enzymatic activities of AST, ALT and ALP is of clinical and toxicological importance as changes in their activities are indicative of heart damage just as observed in the liver damage by toxicants or in diseased conditions.²⁴ In the present study however, the observed slight increase in the activities of heart AST, ALT and ALP suggests that there may be some side reactions resulting in the increase in the level of these enzymes in the heart. The degree of restoring concentration of AST to normal in group C (20% nutmeg extract treated animals) is however similar to that of group D (40% nutmeg extract treated animals), whereas the degree of restoring concentration of the enzyme (ALT) to normal in group C (20% Nutmeg extract treated animals) is the same to that of group D (40% nutmeg extract treated animals) while the degree of restoring concentration of ALP to normal in group C of 20% Nutmeg extract treated animals is also similar to that of group D (40% nutmeg extract treated animals). This suggests that the level of the enzymes concentrations is slightly dose dependent and the evidences of possible damage to the spleen and heart of the rat caused by the potassium bromate were addressed and reversed by the extracts dosages. It has been reported severally by earlier works by many researchers that many plant extracts reversed the effects of tissue damaged by restoring the activities of AST, ALT and ALP in such organs accordingly. The observation in this study however corroborates what was reported earlier by²⁵ in the study on effects of aqueous extract of nutmeg on potassium bromate induced renal toxicity in Wistar albino rats. Similar trends have been reported by^{26,27,28} in their various works.

In Table 2 Superoxide dismutase (SOD), one of the important intracellular antioxidant enzymes, present in all aerobic cells has an antitoxic effect against superoxide anion. The presence of SOD in various fractions such as cytosol, mitochondria and plasma in our body enables SOD to dismutate superoxide radicals immediately and protect the cell from oxidative damage.^{29,30} This research shows that bromate significantly reduce the activity of SOD for both the spleen and heart. Concomitantly, a significant increase was observed in the activity of SOD after administration of 20% and 40% nutmeg extract in group C and D respectively. This also show that the activity of SOD is dose dependent because, a higher increment in the activity of SOD in group D (40% nutmeg extract treated rats) was more than that of group C (20% nutmeg extract treated rats).

The effect of aqueous extracts of Nutmeg treatments on the catalase activity was also observed in bromate-induced rats. There was observable significant reduction in catalase activity in the spleen and heart of the bromate-fed compared to group A. Catalase protects cells from the accumulation of H₂O₂ by converting it to H₂O and O₂ or by using it as an oxidant in which it works as a peroxidase.³¹ The significant decrease in the concentration of catalase in the spleen and that of the heart in the bromate fed group may attributable in part to the reduced synthesis of this antioxidant enzyme (which functions in the detoxification of hydrogen peroxide) which was caused by the bromate given to the animals. Whereas, the 20% and 40% nutmeg aqueous extracts given to the rats caused an increase in catalase activity in the respective groups.

It could also be deduced from this study that there was a decrease in the concentration of reduced GSH level in the spleen and heart of bromate-fed rats in group B compared to group A. reduced GSH is a non-enzymatic antioxidant against free radicals and has been implicated in immune modulation and inflammatory responses.³² The increased levels of reduced GSH is likely attributed to the fact that thiol groups (SH) may not be acting as the main target sites for bromate-induced cellular damage. The reduced concentration of reduced GSH in the spleen and heart observed in this study when the rats were induced with bromate might suggest that bromate produced some oxidant effects on the tissues. Whereas, treatments with the extract of nutmeg reversed this effects by reversing the effects of the bromate effects on the tissues which might be due to stimulation of antioxidant defense system to cope up with the free radicals which were produced in higher amount by bromate in the bromated-fed rats. Studies have reported that the responses of tissue cellular protective mechanism during bromate toxicity vary with the nature, dose, duration and route of exposure.

The nutmeg aqueous extract actually improves the antioxidant status of these organs after the bromate induction. This is in accordance with the work of Palanivel²⁸ in their study on hepatoprotective and antioxidant effect of *Pisonia aculeata* against CCl₄-Induced Hepatic Damage in Rats, similar observations were reported by Singh and Sharma³³ in their work on

hepatoprotective effect of curcumin on Lindane-induced oxidative stress in male Wistar rats.

It was observed in Table 3, that the concentration of CHOL and HDL-CHOL were reduced in the spleen and heart of bromate-fed rats (Group B) when compared with Group A. But when the rats were fed with 20% and 40% concentrations Nutmeg aqueous extracts respectively there was significant and progressive elevation in the value CHOL and HDL-CHOL thereby reversing the effects of the bromate posed to the spleen and the heart which shows a dose-related ratio.

It was also observed that the concentration of TGD in both the spleen and heart of the bromate-fed rats (group B) was slightly increased when compared with normal control group A. Treatment with 20% concentration of nutmeg aqueous extracts respectively however produced a slight decrease in the spleen but slight increase in the heart. The 40% treatment of the nutmeg aqueous extracts however produced an observable increase in the concentration of TGD in the spleen while the same treatment caused a reduction in the heart of the animals. There was not much observable potassium bromate-induced toxicity effects on the lipid profiles of the organs as observed in Table 3.

Also in Table 3, there was an increase in the level of MDA (representing lipid peroxidation) in spleen and heart tissue of bromate fed group B when compared to the control in group A. Bromate has been reported to be highly toxic,³⁴ which can result in the peroxidation of membrane lipids by increasing the events responsible for glucose oxidation, which in turn promotes NADPH dependent thiobarbituric acid reactive substances (TBARS) in the presence of cytochrome P450. The increase in lipid peroxidation level could be due to increased level of glutamine following bromate administration.³⁵ The administration of the extracts especially 20% aqueous extract however brings a significant reduction in the amount of MDA in the two organs of the rats while 40% nutmeg aqueous extract produced effects similar to the bromate.

From the biochemical analyses in this study, it could be deduced from the above observations that potassium bromate could induce toxic effects to the spleen and the

heart tissues by altering the activities of AST, ALT, ALP, reduced GSH, SOD, CAT and other important lipid biomarker compounds such as CHOL, TGD, HDL-CHOL and lipid peroxidation index (MDA), thereby being responsible for affecting the heart performance and probably leads to the initiation of heart diseases and spleen complications. Consequently, the 20% and 40% nutmeg extracts also produced effects capable of reversing the effects of the bromates on the organs though 40% dose in some cases may also cause negative effects in the organs.

CONCLUSION AND RECOMMENDATION

Potassium bromate is a powerful spleen and cardio toxic agent as it enhances lipid peroxidation with significant reduction in the activities of heart antioxidant ability. It also caused cardiac dysfunction as revealed in marked increase in heart AST, ALT and ALP. This study has also shown that potassium bromate induce serious damaging effects on the spleen and heart cells as evidenced by reduced activities of SOD and CAT in the studied tissue. Consumption of potassium bromate or any foods that contain potassium bromate may result in heart and spleen problems and as such should be avoided.

It has been observed in this study that spleen and cardio toxic effects of bromate as well as spleen and cardio protective effects of aqueous extract of nutmeg on bromate induced toxic rats. The study shows the chemopreventive benefit of extract of nutmeg on potassium bromate mediated spleen and cardiac oxidative damage in rat as they significantly reduced the extent of antioxidant loss and restoration of cardiac dysfunction caused by potassium bromate in rat. The results of this study show protective effects on heart and spleen functions which might be due to the presence of some bioactive (phytochemicals) compound in the plant extract. Further investigation should be conducted to identify, isolate, purify and characterize these bioactive compounds present in the nutmeg aqueous extract.

Conflicts of Interest: The Authors declare that no conflict of interest in relation to the publication of this manuscript

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