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Potassium Bromate Induced Renal Toxicity in Wistar Albino Rats: Effects of Aqueous Extract of Nutmeg (*Myristica fragrans* Houtt)

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Authors' contributions

This work was carried out in collaboration between all authors. Author OAO designed the study, performed statistical analysis, correct and update the manuscript. Author SAO was involved in experimental and samples analyses. Author ASKI managed sample analyses, literature searches and first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Much attention has been drawn to the toxic effect of bromate on the organs like liver, spleen etc. However, it is not clear whether or not the toxicity of bromate is related to other vital organs like the kidney. Hence, the present work is geared towards not only unraveling the toxic effect of bromate on the kidney but more importantly investigate the protective effect of African nutmeg *Myristica fragrans* against bromate induced toxicity in this organ. Twenty wistar albino rats (180 to 200 g) were divided into 4 groups. Group I was given normal rat feed with water as control; group II was administered with 1.0mL Potassium bromate ($KBrO_3$) solution (30 mg/kg body weight); groups III and IV were simultaneously administered with 1.0mL of $KBrO_3$ each and 1.0mL of 20% and 40% aqueous extract of African nutmeg respectively. All the treatments were given daily for two weeks. Enzyme biomarkers such as Aspartate Transaminase (AST), Alkaline Transaminase (ALT), Alkaline Phosphatase (ALP), Superoxide Dismutase (SOD), Catalase (CAT), reduced glutathione (GSH), Cholesterol (CHOL), High Density Lipoprotein-Cholesterol (HDL-CHOL), Triglycerides (TRIG), and

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Malondialdehyde (MDA) were measured in the kidney homogenate. Results obtained showed that although bromate exerted significant ($P < 0.05$) toxic effects on the kidney homogenate, administration of the aqueous seed extract of African nutmeg caused a marked reversal in the toxicity of bromate in a dose dependent fashion. Since, the introduction of bromate caused an alteration in enzyme biomarkers in the kidney homogenate, this indicates that the seed is a potential antioxidant against bromate toxicity of the kidney tissues.

Keywords: Myristica fragrans; Potassium bromated; toxicity; antioxidant; enzyme biomarkers.

1. INTRODUCTION

*Myristica fragrans (*Houtt.) of the family Myristiceae is a spice seed from the fruit of an evergreen tree called *Myristica fragrans* Houtt. tree [1]. Both *M. fragrans* (nutmeg) and mace (its sister spice) are native to tropical Asia and Australia. Nutmeg is the actual seed of the tree, while mace is the dried “lacy” reddish covering on the seed. It is the species used for culinary and medicinal purposes and grew naturally only on a small group of island called the Bandas. Nutmeg and mace taste similar though nutmeg is sweeter in flavour and mace more delicate.

Many countries use nutmeg as a seasoning. In India, it is used in sweet dishes. In the Middle East, nutmeg spices savoury dishes. Europeans use it in most dishes to season potatoes, eggs, meats and even spinach, sauces and baked goods. Nutmeg had been reported to have aphrodisiac [2], stomachic, carminative [3,4], tonic [5], nervous stimulant [6], aromatic, narcotic, astringent, hypolipidemic, antithrombotic, antifugal, antidysentric and anti inflammatory [2] properties.

Nutmeg is used by Arabs of Israel and people of its Jewish communities, especially Yemenites' as a drug of their folk medicine, as well as a spice and as an important ingredient in love-portions. It is used against vomiting and to regulate the movements of the bowels; it is good for liver and for the spleen. It is used in the treatment of tuberculosis, against colds, fever, and in general for respiratory ailments. It is said to be antihelminthic and also used against skin diseases like eczema and scabies [7].

The nutmeg tree is any of several species of trees in genus *Myristica*. The most important commercial species is *Myristica fragrans*, an evergreen tree indigenous to the Banda Islands in the Moluccas (or Spice Islands) of Indonesia. The nutmeg tree is important for two spices derived from the fruit: nutmeg and mace. Nutmeg is the seed of the tree, roughly egg-shaped and about 20 to 30 mm (0.8 to 1.2 in) long and 15 to 18 mm (0.6 to 0.7 in) wide, and weighing between 5 and 10 g (0.2 and 0.4 oz) dried, while mace is the dried "lacy" reddish covering or aril of the seed.

The first harvest of nutmeg trees takes place 7–9 years after planting, and the trees reach full production after 20 years. Nutmeg is usually used in powdered form. This is the only tropical fruit that is the source of two different spices. Several other commercial products are also produced from the trees, including essential oils, extracted oleoresins, and nutmeg butter. The common or fragrant nutmeg, *Myristica fragrans*, native to the Banda Islands of Indonesia, is also grown in Penang Island in Malaysia and the Caribbean, especially in Grenada. It also grows in Kerala, a state in southern India. Other species of nutmeg include Papuan nutmeg *M. argentea* from New Guinea, and *M. malabarica* from India.

Bromate is an oxidizing agent, used in the neutralizing solution of permanent-waving kits. Accidental or deliberate ingestion of bromate has rarely been reported, but is potentially severe [8]. Carcinogenic and mutagenic effects of potassium bromate have been reported in experimental animals [9]. Lethal oral doses of bromate in humans have been estimated to be between 154 and 385 mg/kg body weight while serious poisoning results at doses of 46–92 mg/kg body weight [10].

Oral doses of 185–385 mg/kg body weight results in irreversible toxic effects like renal failure and deafness in humans while lower doses are associated with vomiting, diarrhea, nausea and abdominal pain [10]. Potassium bromate is extremely irritating and injurious to tissues especially those of the central nervous system and kidneys. The pathologic findings include kidney damage and haemolysis [11]. Bromate was first found to cause tumour in rats in 1982.

Subsequent studies on rats and mice confirmed that it causes tumour of the kidney, thyroid and other organs [12]. It is known that potassium bromate induces oxidative stress in tissues [13,14,15].

Indeed, oxidative damage appears to be the basis of bromate-induced carcinogenesis [16]. Several cases of accidental poisoning in children resulting from ingestion of bromate solution and sugar contaminated with bromate were reported as the source of an outbreak of mild poisoning in New Zealand [17]. However, Potassium bromate has been banned in several countries including the United Kingdom in 1990, Nigeria in 1993 and Canada in 1994.

The present study attempts to assess the toxicity of potassium bromate on the renal tissues of Wistar Albino rats and the detoxifying effects of aqueous extract of *Myristica fragrans* with a view to finding out the effect of their consumption on kidney and antioxidant enzymes, lipid peroxidation and some basic compounds status of rat kidney.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Myristica fragrans seeds were collected from the local market in Ado-Ekiti, Nigeria and were authenticated at Department of Plant Science, Ekiti State University, Ado-Ekiti. The seeds of *Myristica fragrans* were collected and air dried under shade and was pulverized with Marlex Excella laboratory blender. The two doses of aqueous extract of *Myristica fragrans* seed were made by weighing 100g and 200g of the powder into 500mL of distilled water in a cocked bottles respectively, shaken and allowed to stand overnight with constant stirring to give 20% and 40% aqueous solutions of the seed.

2.2 Experimental Design and Protocol

The study was performed on twenty (20) male wistar albino rats housed in ventilated cages in the Animal House of Biochemistry Department, Ekiti State University, Ado-Ekiti, Nigeria. They were acclimatized for two weeks before administration of the drugs. Animals were divided into four groups of five rats each. Group I served as the control and received distilled water and rat feed. Groups II, III and IV were administered 30mg/kg body weight potassium bromate daily. However, the rats in group III and IV were treated daily with oral administration of 1.0ml aqueous *Myristica fragrans* extract. Animals were kept at optimum temperature with a 12 h light/dark cycle with rat feed and water. The period of drug administration lasted for 14 days.

Group A; Normal Feeding (Control)
Group B; Bromate fed (BFR)
Group C; Bromate + 20% aqueous extract of *Myristica fragrans* (B100gNR)
Group D; Bromate + 40% aqueous extract of *Myristica fragrans* (B200gNR)

2.3 Biochemical Assay

Standard Randox kits were used to determine Triglycerides, Cholesterol, HDL-Cholesterol, Total protein, Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Aspartate Transaminase (AST) and Alkaline Transaminase.

ALT

$$\propto -\text{oxoglutarate} + \text{L} - \text{alanine} \xrightarrow{\text{GPT}} \text{L} - \text{glutamate} + \text{pyruvate}$$

Alanine Aminotransferase was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

AST

$$\propto -\text{oxoglutarate} + \text{L} - \text{aspartate} \xrightarrow{\text{GOT}} \text{L} - \text{glutamate} + \text{oxaloacetate}$$

Aspartate Transaminase was measured by the monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

2.4 CHOLESTEROL

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestene -3-one} + H_2O$$

$$2H_2O + \text{phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{quinoneimine} + 4H_2O$$

2.5 HDL-Cholesterol

Low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomychron fractions were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the High density lipoprotein (HDL) fraction, which remained in the supernatant was determined.

2.6 Triglyceride

The triglyceride was determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of perioxidase.

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol} - 3 - \text{phosphate} + \text{ADP}$$

$$\text{Glycerol-3- phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone} + \text{phosphate} + H_2O_2$$

$$H_2O_2 + \text{4- aminophenazone} + \text{4- chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + HCl + 4H_2O$$

2.7 Determination of Plasma Malondialdehyde (MDA)

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of [18]. An aliquot of 0.4mL of the plasma or other organ homogenates was mixed with 1.6mL of Tris-KCl buffer to which 0.5mL of 30% trichloroacetic acid (TCA) was added. Then 0.5mL of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C. This was then cooled on ice and centrifuged at 3000*g*. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532nm. The MDA level was calculated according to the method of [19]. Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}Cm^{-1}$.

2.7.1 Calculation

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance} \times \text{volume of mixture}}{E_{532nm} \times \text{volume of sample} \times \text{mg protein}}$$

2.8 Determination of Reduced Glutathione

The method of [20] was followed in estimating the level of reduced glutathione (GSH). 0.2mL of sample was added to 1.8mL of distilled water and 3mL of the precipitating solution was mixed with sample. The mixture was then allowed to stand for approximately 10minutes and then centrifuged at 3000g for 5 minutes. 0.5mL of the supernatant was added to 4mL of 0.1M phosphate buffer. Finally 0.5mL of the Ellman's reagent was added. The absorbance of the reaction mixture was read within 30 minutes of colour development at 412nm against a reagent blank.

2.9 Determination of Catalase Activity

This experiment was carried out using the method described by [21]. 0.2mL of sample was mixed with 0.8ml distilled H_2O to give 1 in 5 dilution of the sample. The assay mixture contained 2.0mL of solution (800µmol) and 2.5mL of phosphate buffer in a 10mL flat bottom flask. 0.5mL of properly diluted enzyme preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1.0mL portion of the reaction mixture was withdrawn and blown into 1mL dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide content of the withdrawn sample was determined by the method described above. The mononuclear velocity constant, K, for the decomposition of H_2O_2 by catalase was determined by using the equation for a first-order reaction: $K = 1/t \log S_0/S$, where S_0 is the initial concentration of H_2O_2 and S is the concentration of the peroxide at t min. The values of the K are plotted against time in minutes and the velocity constant of catalase $K_{(0)}$ at 0 min determined by extrapolation. The catalase contents of the

enzyme preparation were expressed in terms of Katalase feiahigkeit or 'Katf' according to von [22].

$$\text{Kat. f} = \frac{K_{(0)}}{\text{mg protein/ml}}$$

2.10 Determination of Superoxide Dismutase (SOD)

The level of SOD activity was determined by the method of [23]. 1mL of sample was diluted in 9mL of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

2.10.1 Calculation

$$\text{Increase in absorbance per minute} = \frac{A_3 - A_0}{2.5}$$

where

A_0 = absorbance at 0 second
A_3 = absorbance 150 seconds

$$\% \text{ inhibition} = \frac{\text{increase in absorbance for substrate}}{\text{increase in absorbance of blank}} \times 100$$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute.

2.10.2 Statistical Analysis

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

3. RESULTS

Fig. 1 reveals the specific activity of aspartate transaminase in Potassium Bromate induced renal toxicity in Wistar Albino rats, it is evidenced that aqueous extract of the nutmeg caused a reversal of the increased concentration of aspartate transaminase in the bromate fed rat on dose dependent approach.

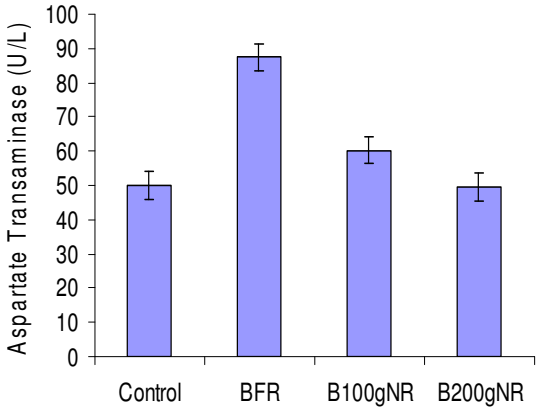

Fig. 1. Specific activity of aspartate transaminase in Potassium Bromate induced renal toxicity in Wistar Albino rats

Fig. 2 also shows specific activity of alkaline transaminase in Potassium Bromate induced renal toxicity in Wistar Albino rats, it is evidenced that aqueous extract of the nutmeg caused a reversal of the decreased concentration of alkaline transaminase in the bromate fed rat on dose dependent approach.

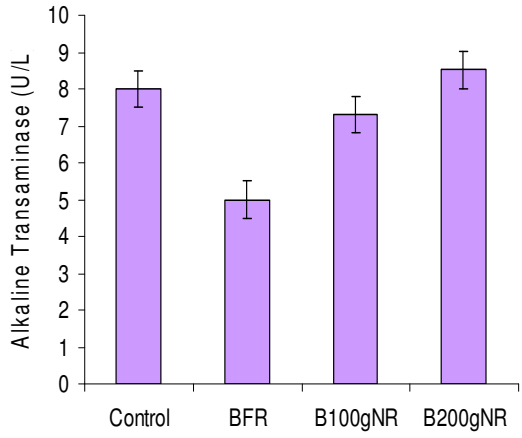

Fig. 2. Specific activity of alkaline transaminase in Potassium Bromate induced renal toxicity in Wistar Albino rats

Fig. 3 shows the specific activity of alkaline phosphatase in Potassium Bromate induced renal toxicity in Wistar Albino rats, it can be seen that the aqueous extract of the nutmeg caused a

reversal of the increased concentration of alkaline phosphatase in the bromate fed rat on dose dependent manner.

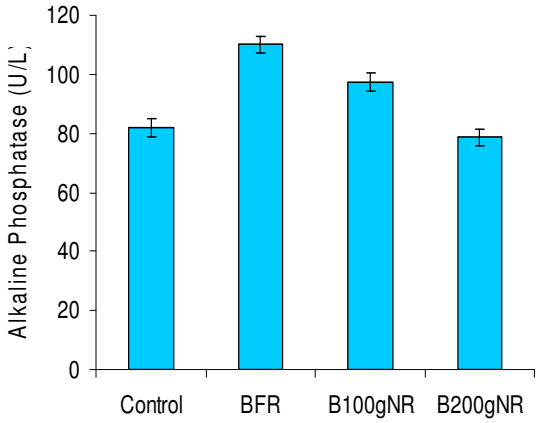

Fig. 3. Specific activity of alkaline phosphatase in Potassium Bromate induced renal toxicity in Wistar Albino rats

Fig. 4 shows the percentage inhibition of superoxide dismutase in Potassium Bromate induced renal toxicity in Wistar Albino rats. There is also reversal of the effect of the bromate fed on the organ which also manifest in dose dependent mode.

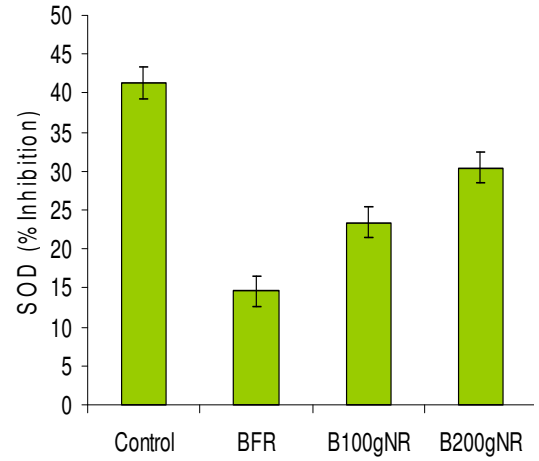

Fig. 4. % inhibition of superoxide dismutase in Potassium Bromate induced renal toxicity in Wistar Albino rats

Fig. 5 reveals the specific activity of catalase in Potassium Bromate induced renal toxicity in Wistar Albino rats. As observed, there is also a reversal of the effect of the fed bromate on the organ which is evident on dose dependent mode.

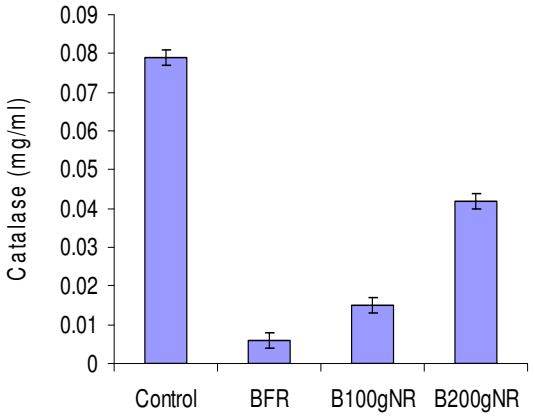

Fig. 5. Specific activity of catalase in Potassium Bromate induced renal toxicity in Wistar Albino rats

Fig. 6 shows the concentration of reduced GSH (ug/ml) in Potassium Bromate induced renal toxicity in Wistar Albino rats, there is also a reversal of the effect of the fed bromate on the organ which also manifest in dose related manner.

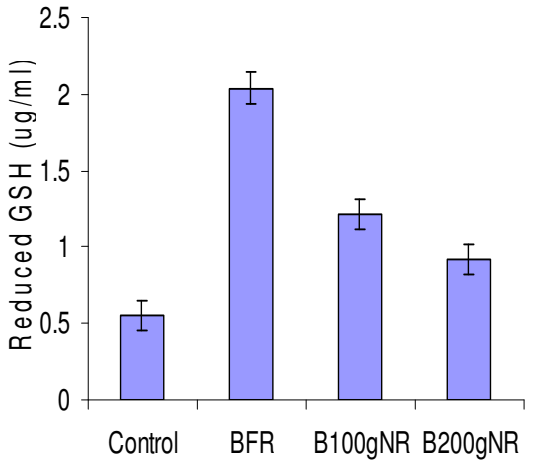

Fig. 6. Reduced GSH (ug/ml) concentration in Potassium Bromate induced renal toxicity in Wistar Albino rats

Fig. 7 shows the concentration of total cholesterol in Potassium Bromate induced renal toxicity in Wistar Albino rats. The reversal of the consequence of the fed bromate was evidenced in the nutmeg aqueous extract fed group which can also be seen in dose dependent mode.

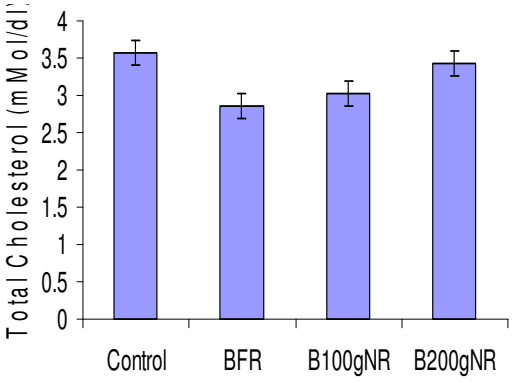

Fig. 7. Total cholesterol concentration in Potassium Bromate induced renal toxicity in Wistar Albino rats

Fig. 8 shows the concentration of HDL-Cholesterol in Potassium Bromate induced renal toxicity in Wistar Albino rats, the 20% aqueous extract of nutmeg reversal of the bromate fed effect was almost nullified by the 40% aqueous extract.

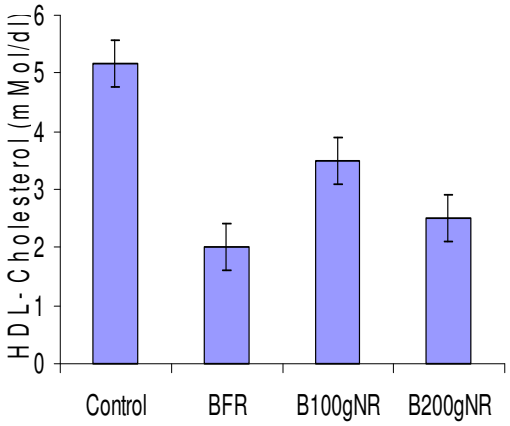

Fig. 8. HDL-Cholesterol concentration in Potassium Bromate induced renal toxicity in Wistar Albino rats

Fig. 9 Triglyceride in Potassium Bromate induced renal toxicity in Wistar Albino rats. It is evidenced that both concentrations of the aqueous extracts caused a reversal of the effect of the fed bromate in the organ homogenate.

Fig. 10 shows the concentration of malondialdehyde in Potassium Bromate induced renal toxicity in Wistar Albino rats. Both concentrations of aqueous extracts caused a great reversal in lipid peroxidation observed in bromate fed organ.

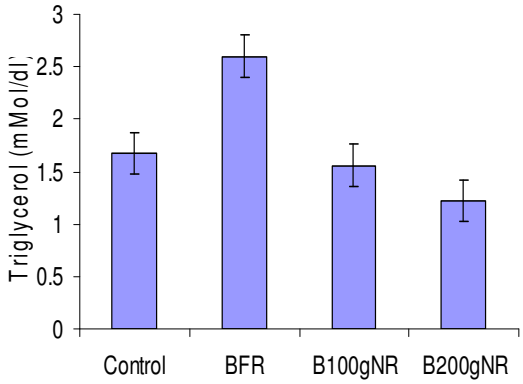

Fig. 9. Triglyceride concentration in Potassium Bromate induced renal toxicity in Wistar Albino rats

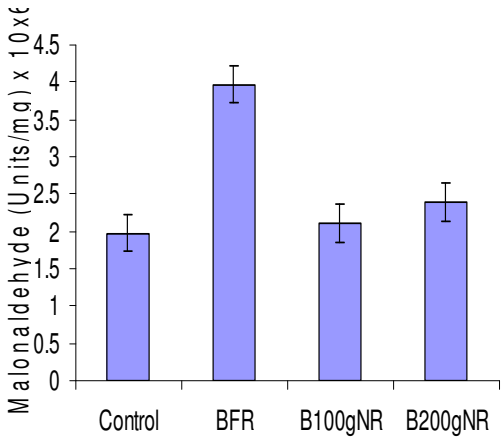

Fig. 10. Malondialdehyde concentration in Potassium Bromate induced renal toxicity in Wistar Albino rats

4. DISCUSSION

CAT, SOD, GST, GSH and GSH-PX represents an armoury of antioxidants produce by the body to neutralize or 'mop up' free radicals that can harm the cells and hence defend it against oxidative stress. The ability of the body to produce these antioxidants is controlled by genetic makeup and influenced by exposure to environmental factors such as diet and chemicals [24]. Potassium bromate ($KBrO_3$) causes renal cell and thyroid carcinomas in rats, hamsters and mice when exposed chronically [25]. It has been investigated that $KBrO_3$ produces free oxygen radicals which causes oxidative stress and DNA damages [26]. $KBrO_3$ causes nephrotoxicity and hepatotoxicity; decreases the tissue soluble proteins, antioxidant enzymes. The decrease of

antioxidant enzymes (SOD and CAT) are due to reactive oxygen species (ROS) produced by metabolism of $KBrO_3$. $KBrO_3$ depleted glutathione (GSH) content in various tissues which causes decrease in phase II metabolizing enzymes like glutathione peroxidase (GSH-Px) and glutathione reductase (GSR). It also increases thiobarbituric acid reactive substances (TBARS) contents, causes lipid peroxidation and disrupts liver profile including gammaglutamyltransferase (γ-GT), alkaline phosphatase (ALP) and protein concentration [27].

Results of the present study revealed that *Myristical fragrans* showed chemo-protection ability against $KBrO_3$- induced renal toxicity in rat. The increased concentrations of AST, ALP and MDA by potassium bromate also indicates its adverse effects on kidney function in rats. The observed nephrotoxicity brought about by potassium bromate in this study is similar to earlier observations [28,29]. The significant recovery of renal antioxidant content and reversal in the enhancement of ALT, SOD, CAT, GSH, HDL-Cholesterol by both concentrations used in this study suggest that *Myristical fragrans* is a potent chemo-preventive agent against oxidative stress and may suppress potassium bromate-mediated renal oxidative damage in rat [30]. Results of the present investigation revealed that $KBrO_3$ significantly increased renal AST, ALP, TRG and MDA, [31] observed the similar increase in urine protein, RBC and WBC showing renal injuries that half kidney is damage. The present study revealed that oral administration of *Myristica fragrans* significantly improved the concentrations of ALT, SOD, CAT, Cholesterol and HDL-cholesterol and decreased the elevated levels of AST, ALP, GSH and TRG, this study also revealed that administration of $KBrO_3$ caused marked impairment in renal function along with significant oxidative stress in the kidneys. The significant increase in ALP activity observed in the serum of rats fed with bromate-containing diet compared with the control in some other studies may be attributable to loss of membrane components due to a possible reaction between potassium bromate and the membranes of kidney cells, causing leakage of the enzyme into the blood stream. This observation was supported by [32] who reported that any damage done to the cell membrane may lead to leakage of ALP, which is a marker enzyme in the plasma membrane, into extracellular fluid. The transaminases (AST and ALP) are well-known enzymes used as biomarkers to predict possible toxicity [33]. Generally, damage to kidney cells will result in elevations of both these transaminases in the serum [34], the same was observed in this study too. Furthermore, measurement of enzymic activities of AST, ALP and ALT is of clinical and toxicological importance as changes in their activities are indicative of kidney damage by toxicants or in diseased conditions [35]. In the present study, the observed decrease in the activities of kidney ALT, SOD and CAT activities could suggests that there may be a leakage of these enzymes from the kidney to the blood stream [36]. The reduction in the activities of ALT in the kidney may be due to the interference with protein metabolism in the cells or inhibition of the enzyme [37]. Increased activities of serum enzymes have been reported in conditions of tissue damage [36]. Normally, enzymes will not always be found in the serum except there is damage to one or more organs of the body. Therefore, enzymes from diseased organs may become manifested in the serum resulting in increased activity since they must have leaked from the diseased organ. Therefore, the reduction in concentration of some of these parameters maybe due to leakage of these enzymes into the blood stream. For the concentrations of *Myristic fragrans* used in this study, there are appreciable differences observed at the level of restoring the concentrations of the AST, ALT, CAT, Total Cholesterol, TRG and MDA to normal level. These however show the chemoprotective effects of these concentrations of the aqueous extracts. However, there are differences in the level of the concentrations of parameters like ALP, SOD, RED. GSH and HDL- Cholesterol between the group III animals treated with 20% extract (B100gNR) and Group IV animals treated with 40% extract (B200gNR).

5. CONCLUSION

Potassium bromate is a potent nephrotoxic agent as it enhances lipid peroxidation with significant reduction in the activities of renal antioxidant capacity. It also caused renal dysfunction as revealed in marked increase in renal AST and ALP. The present study has also show that potassium bromate portend serious damaging effects on kidney cells as evidenced by reduced activities of ALT, SOD and CAT in the studied tissue. Direct consumption of potassium bromate or any foods that contain potassium bromate may result in kidney damage and as such should be avoided.

The study also showed that *Myristic fragrans* has phyto-preventive benefit on potassium bromate mediated renal oxidative damage in rat as they significantly reduced the extent of antioxidant loss and restoration of renal dysfunction caused by potassium bromate in rat.

6. RECOMMENDATIONS

Though the results obtained in this study portend protective effect of aqueous extract of *Myristic fragrans* on kidney function which might be due to the presence of some bioactive compounds in the plant extract. Further investigations however should be conducted to identify the bioactive compounds present in aqueous extract of *Myristic fragrans*.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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