

LIPID PROFILE AND CARDIO-PROTECTIVE EFFECTS OF AQUEOUS EXTRACT OF *MORINGA OLEIFERA* (LAM) LEAF ON BROMATE- INDUCED CARDIOTOXICITY ON WISTAR ALBINO RATS

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ABSTRACT

Objective: The present study sought to investigate the lipid profile, cardiotoxicity effect of potassium bromate in dosage of 30mg/kg body weight and the cardio protective effects of 20% and 40% aqueous extract of *Moringa oleifera* leaf on wistar albino rats.

Materials and methods: The rats weighing between 120g and 150g were divided into four groups of five rats each. Group I rats were orally administered with distilled water and rat feed while group II animals were given 30mg/kg/body weight of potassium bromate per day. Similarly group III rats were administered simultaneously with 30mg/kg/body weight of potassium bromate and 20% aqueous extract of *Moringa oleifera* per day, as group IV rats were administered simultaneously with 30mg/kg/body weight of potassium bromate and 40% aqueous extract of *Moringa oleifera* leaf per day. After the expiration of the experiment, the weight of the rats were taken with standard weighing balance, the animals were later sacrificed under chloroform anesthesia and the cardiac tissues were obtained for the determination of the heart's triglyceride (TG), total cholesterol (TC), high density cholesterol (HDL-c), biomarker enzymes (AST, ALT and ALP) and some antioxidant enzymes.

Results: The results showed that potassium bromate significantly and progressively lowered the cardiac tissues SOD, HDL-CHOL and TC, while significantly causing elevation in TRG, ALP, AST, CAT concentrations when compared to the control rats group. The 20% and 40% extracts also significantly lowered the TRG, ALP, AST, CAT on dose-dependent basis.

Conclusion: These results showed the cardioprotective and antioxidants effects of the plant extracts which supports the folkloric use of aqueous extract of the plant in the management of suspected patients with cardiac failure.

Keyword: Cardio-toxicity, cardio-protection, *Moringa oleifera*, antioxidant, enzymes.

INTRODUCTION

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 (De Vriesse *et al.*, 1997). Carcinogenic and mutagenic effects of potassium bromate have been reported in experimental animals (Kurokawa *et al.*, 1987). 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 (Mark, 1988). 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 (Mark, 1988). 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 (Robert and William, 1996). 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 (CSPI, 1999). It is known that potassium bromate induces oxidative stress in tissues (Sai *et al.*, 1991; Watanabe *et al.*, 1992; Parsons and Chipman, 2000). Indeed, oxidative damage appears to be the basis of bromate-induced

carcinogenesis (Chipman et al., 2006). 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 (Paul, 1966). However, Potassium bromate has been banned in several countries including the United Kingdom in 1990, Nigeria in 1993 and Canada in 1994.

The spleen is an organ found in virtually all vertebrate animals. Similar in structure to a large lymph node, it acts primarily as a blood filter. It is possible to remove the spleen without jeopardizing life. The spleen plays important roles in regard to red blood cells (also referred to as erythrocytes) and the immune system. It removes old red blood cells and holds a reserve of blood, which can be valuable in case of hemorrhagic shock, and also recycles iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is removed in the liver (Mebius and Kraal 2005).

The spleen synthesizes antibodies in its white pulp and removes antibody-coated bacteria and antibody-coated blood cells by way of blood and lymph node circulation. A study published in 2009 using mice found that the spleen contains, in its reserve, half of the body's monocytes within the red pulp (Swirski et al., 2009). These monocytes, upon moving to injured tissue (such as the heart), turn into dendritic cells and macrophages while promoting tissue healing (Swirski et al., 2009; Jia and Pamer, 2009 and Natalie, 2009). The spleen is a center of activity of the mononuclear phagocyte system and can be considered analogous to a large lymph node, as its absence causes a predisposition to certain infections (Brender et al., 2005).

The spleen is the organ that is responsible for purifying the blood as well as storing blood cells. It is positioned in the superior abdomen, and is the largest lymphatic organ in the body. The spleen serves a valuable role in immune function because it purifies the blood and helps the immune system with recognize and attack foreign antibodies and disease. The spleen is composed of the red and white pulp. The white pulp produces and grows immune cell as well as blood cells. On the other hand, the red pulp is responsible for purifying the blood and removing dead or old blood cells. The condition known as splenomegaly exists when the spleen becomes enlarged due to disease. When this occurs the spleen can rupture and cause additional damage. The surgical procedure splenectomy, is often needed to remove the enlarged spleen. When the spleen is removed the liver will assume the portion of the spleen's responsibility for fighting germs and coordinating the immune system. Splenomegaly has been associated with the following diseases: thrombocytopenia mononucleosis sickle cell disease cancer.

“*Moringa oleifera*” is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, South Asia, South America and the pacific and Caribbean Islands. Because *Moringa oleifera* has been naturalized in many tropic and subtropics regions worldwide, the plant is referred to number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree, and “Mothers best friend” (Julia, 2008). *Moringa oleifera* is commonly known as “Drumstick”. It is a small or medium sized tree, about 10m height, found in the sub-Himalayan tract (Trapti et al., 2009). *Moringa* leaves contains phytochemical having potent anticancer and hypotensive activity and are considered full of medicinal properties and used in siddha medicine (Monica et al., 2010). The whole *Moringa oleifera* plant is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac, the aqueous extracts of roots and barks were found to be effective in preventing implantation, aqueous extracts of

fruits have shown significant anti-inflammatory activity, methanolic extracts of leaves have shown anti-ulcer activity and ethanolic extracts of seeds exhibited anti-tumour activity (Patel *et al.*, 2010). *Moringa oleifera* is used as drug many ayurvedic practitioners for the treatment of asthma and evaluate the anthelmintic activity of methanolic extract of *Moringa oleifera* in adult Indian earthworms *pheretima posithuma* at different doses (Iswar *et al.*, 2010). The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals. It is very important for its medicinal value. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumour, antipyretic, antiepileptic, anti- inflammatory, antiulcer (Pal *et al.*, 1995). Other important medicinal properties of the plant include antispasmodic (Caceres *et al.*, 1992), diuretic (Morton *et al.*, 1991), antihypertensive (Dahot, 1988), cholesterol lowering (Mehta *et al.*, 2003), antioxidant, antidiabetic, hepatoprotective (Ruckmani *et al.*, 1998), antibacterial and antifungal activities (Nickon *et al.*, 2003).

The present study attempts to assess the toxicity of potassium bromate on the cardiac tissues of Wistar Albino rats and the detoxifying Effects of Aqueous Extract of *Moringa oleifera* in selected bread samples consumed in Ilorin, Central Nigeria with a view to finding out the effect of their consumption on some enzymes of rat liver and kidney. Spices have been used since ancient times not only for increasing the flavor of foods but also for their preservative and medicinal properties. *Moringa oleifera* is a tropical, evergreen tree native to the Moluccas or Spice Island of Indonesia.

MATERIALS AND METHODS

Sample collection and preparation

Plant material: *Moringa oleifera* leaves was collected from Futa area, Akure and were authenticated at Department of Plant Science, Ekiti State, University, Ado-Ekiti.

Extract preparation: The leaves of *Moringa oleifera* were collected and air dried under shade and ground into powder with Marlex Excella laboratory blender and preserved. Different Aqueous extract of *Moringa oleifera* leaves was prepared.

Experimental protocol: The study was performed on twenty (20) wistar albino rats (all males) 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 aqueous *Myristica fragrans* extract. Animals were kept at optimum temperature with a 12 h light/dark cycle and given rat feed and water. The period of drug administration lasted for 21 days.

Group I; Control (Normal Feed)

Group II; Bromate Fed

Group III; Bromate + 20% of *Moringa oleifera* leaf aqueous extract

Group IV; Bromate + 40% of *Moringa oleifera* leaf aqueous extract

Chemicals/Reagent kits

All chemicals and drugs used were obtained commercially and of analytical grade.

All the diagnostic kits are products of Randox Chemical Ltd. England.

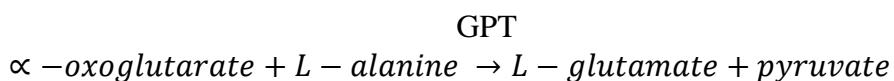
Preparation of Organs homogenate

The animals were quickly dissected; the organs (Heart) were removed. 10% of each organs homogenate were then prepared in 6.7mM potassium phosphate buffer, (pH 7.4) using the Teflon homogenizer. The homogenate was centrifuged at 10,000rpm for 10 minutes at 4°C to obtain a clear supernatant which was stored at 8°C and used for measurement of biochemical contents.

Biochemical Assay

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

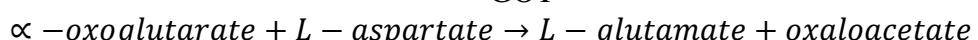
ALT



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

AST

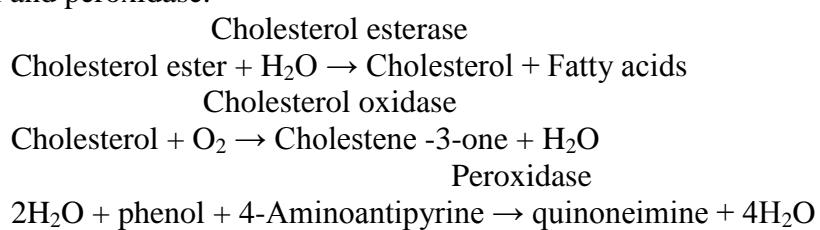
GOT



AST is measured by the monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

CHOLESTEROL

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



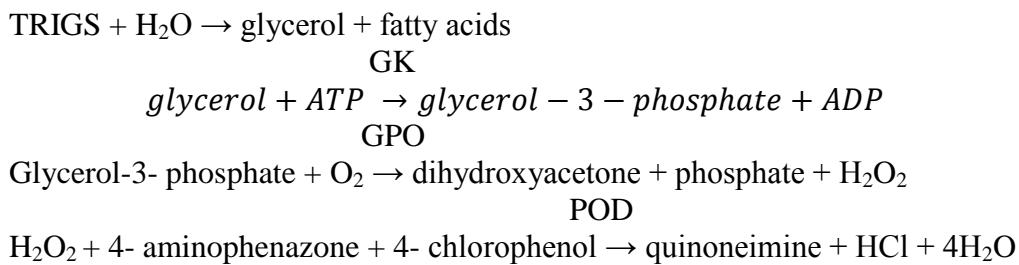
HDL-Cholesterol

Low density lipoprotein (LDL and VLDL) and chylomycron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (High density lipoprotein) fraction, which remains in the supernatant, is determined.

TRIGLYCERIDES

The triglycerides are 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 peroxidase.

lipase



Determination of plasma Malonaldehyde (MDA)

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale (1990). 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 3000g. 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 Adam-Vizi and Seregi (1982). Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{Cm}^{-1}$.

Calculation

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

Determination of Glutathione

The method of Jollow *et al.* (1974) 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 10 minutes 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.

Determination of Catalase activity

This experiment was carried out using the method described by Sinha (1972). 0.2ml of sample was mixed with 0.8ml distilled H₂O to give 1 in 5 dilution of the sample. The assay mixture contained 2mL 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 1mL 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 H₂O₂ by catalase was determined by using the equation for a first-order reaction: $K = 1/t \log S_0/S$, where S₀ is the initial concentration of H₂O₂ 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 Euler and Josephson (1927).

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

Determination of Superoxide Dismutase (SOD)

The level of SOD activity was determined by the method of Misra and Fridovich (1972). 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.

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.

RESULTS AND DISCUSSION

The significant increase ($p < 0.05$) in ALP activity observed in the heart of group II (bromated treated) compared with the control may be attributable to loss of membrane components due to a possible reaction between potassium bromate and the heart tissues. Therefore, enzymes from diseased organs may become manifested in the serum resulting in increased activity since they must have leaked from the diseased organ. The increased activity of the serum enzyme is often accompanied by a corresponding decrease in enzyme activity of the affected organ. The pronounced reduction in ALP activity of the kidney relative to the liver enzyme activity may be attributed to the fact that the latter has a major role of detoxification and hence capable of handling toxic compounds such as potassium bromate.

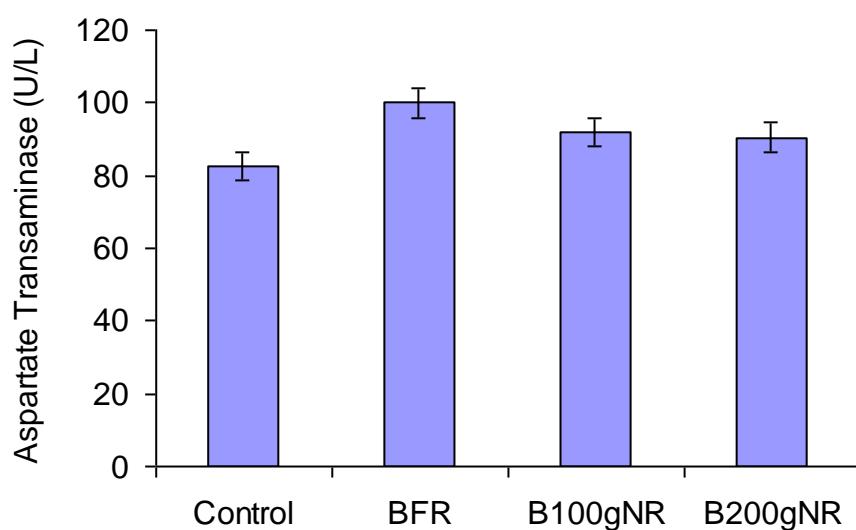


Figure 3.1: Specific activity of aspartate transaminase in Potassium Bromate induced cardiac toxicity in Wistar Albino rats.

The transaminases (AST and ALT) are well-known enzymes used as biomarkers to predict possible toxicity (Rahman *et al.*, 2001). Generally, damage to liver cells will result in elevations of both these transaminases in the serum (Wolf *et al.*, 1972). Furthermore, measurement of enzymic activities of AST and ALT 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 (Singh *et al.*, 2001). In the present study, the observed increase in the activities of heart AST and ALT suggests that there may be a leakage of these enzymes from other organs like liver, thereby causing an increase in the level of the enzymes in the heart. In this study, the degree of restoring concentration of the enzymes to normal in group III (20% Moringa treated animals) is lower to that of group IV (40% Moringa extract treated animals). This suggests that the level of the enzymes is dose dependent. All these are evidences of possible damage to the heart of rat caused by the potassium bromated.

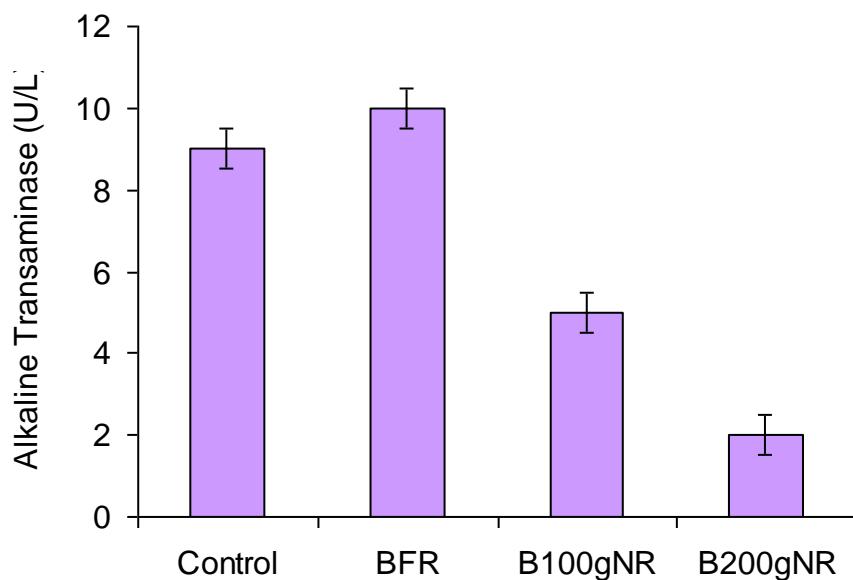


Figure 3.2: Specific activity of alkaline transaminase in Potassium Bromate induced cardiac toxicity in Wistar Albino rats

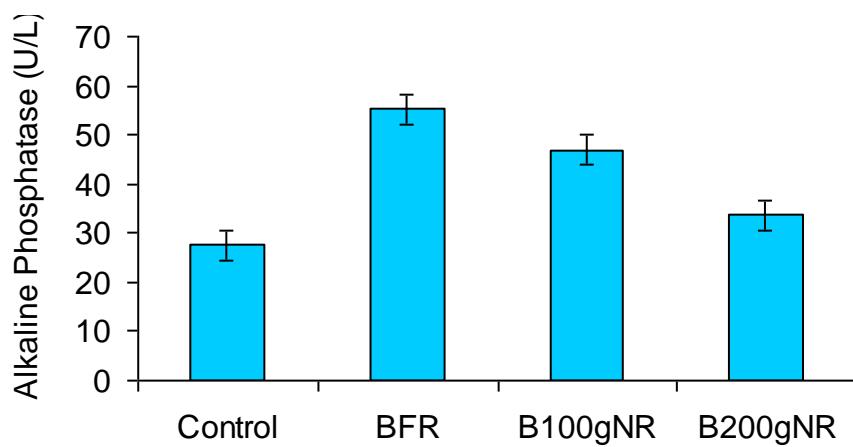


Figure 3.3: Specific activity of alkaline phosphatase in Potassium Bromate induced renal toxicity in Wistar Albino rats

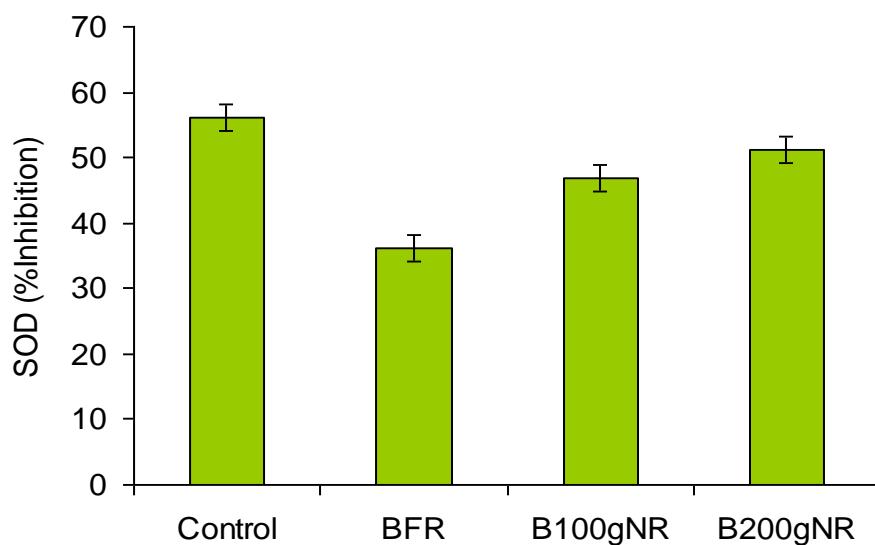


Figure 3.4: % inhibition of superoxide dismutase in Potassium Bromate induced cardiac toxicity in Wistar Albino rats

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 (CuZn-SOD), mitochondria (Mn-SOD) and plasma (EC-SOD) in our body enables SOD to dismutate superoxide radicals immediately and protect the cell from oxidative damage (Fridovich, (1995) and Marklund, (1984)). It is well documental that SOD activity can be decreased by ischemia or hypoxia (Dhaliwal *et al.*, 1991 and Ferrari *et al.*, 1991). This research shows that apart from ischemia and hypoxia, injection of bromated also reduce the activity of SOD. Concomitantly, a significant increase was observed in the activity of SOD after administration of 20% and 40% Moringa extract in group III and IV respectively(Figure 3.4). This also show that the activity of SOD is dose dependent because, a higher increment in the activity of SOD in group IV (40% Moringa extract treated rats) than that of group III (20% Moringa extract treated rats).

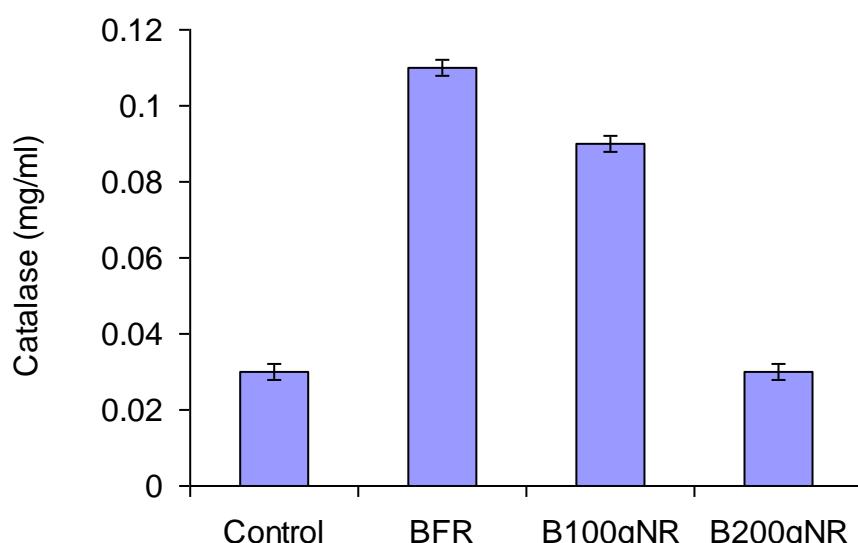


Figure 3.5: Specific activity catalase in Potassium Bromate induced cardiac toxicity in Wistar Albino rats

Figure 3.5 revealed the effects of aqueous extracts of horseradish (*Moringa oleifera*) leaf treatments on the catalase activity of bromate-induced toxicity in rat. The reduction in catalase activity after injection of bromate is another significant finding in this study. Catalase protects cells from the accumulation of H₂O₂ by dismutating it to form H₂O and O₂ or by using it as an oxidant in which it works as a peroxidase (Dhaliwal *et al.*, 1991). The decreased concentration of splenic catalase is attributable in part to the reduced synthesis of this antioxidant enzyme (which functions in the detoxification of hydrogen peroxide) whose concentrations would have fallen with the bromate that was injected into the animals. While treatment with 20% and 40% *Moringa oleifera* leaf aqueous extracts caused slight increase in catalase activity in their respective groups.

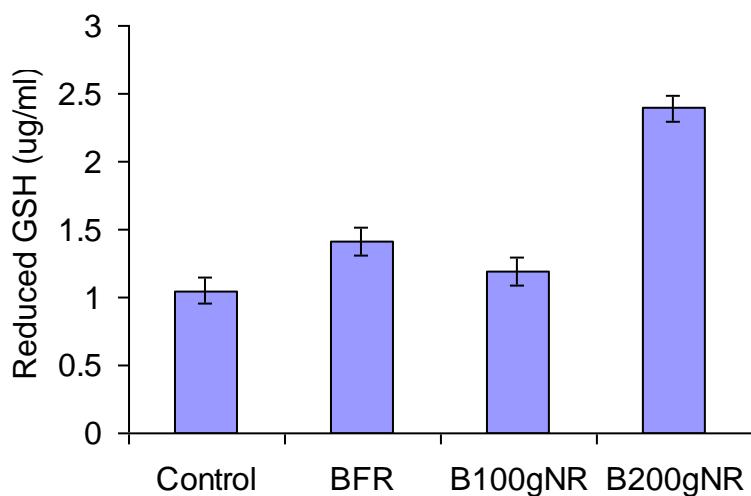


Figure 3.6: Reduced GSH (ug/ml) in Potassium Bromate induced cardiac toxicity in Wistar Albino rats

Another important finding of this study is the increased level of heart GSH in KBrO₃ – treated rats (figure 3.6). Heart GSH level was increased in bromate – treated rats. GSH is a non enzymatic antioxidant against free radicals and has been implicated in immune modulation and inflammatory responses (Kono, 1978). The increased levels of GSH may be attributed to the fact that thiol groups (SH) may not be acting as the main target sites for bromated-induced cellular damage. The increased activities of GSH in the heart at higher doses of bromate injection might be due to stimulation of antioxidant defence system to cope up with the free radicals which was produced in higher amount by bromate. Studies have reported that the responses of tissue cellular protective mechanism during bromate toxicity vary with the nature, dose, duration and route of arsenic exposure.

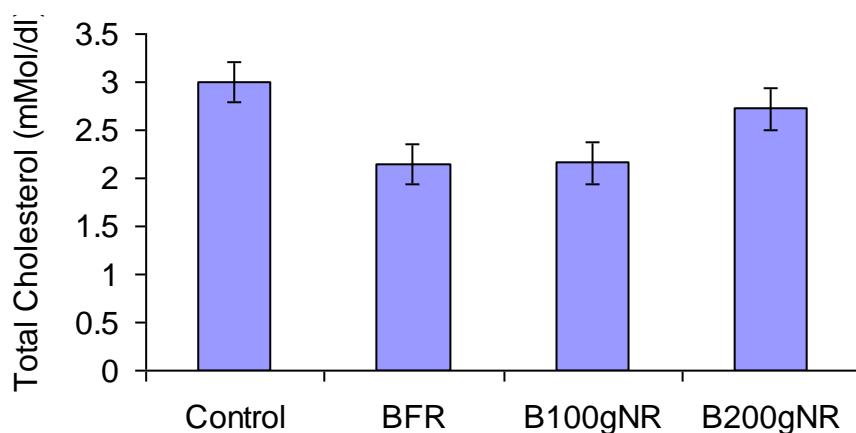


Figure 3.7: Total cholesterol in Potassium Bromate induced cardiac toxicity in Wistar Albino rats

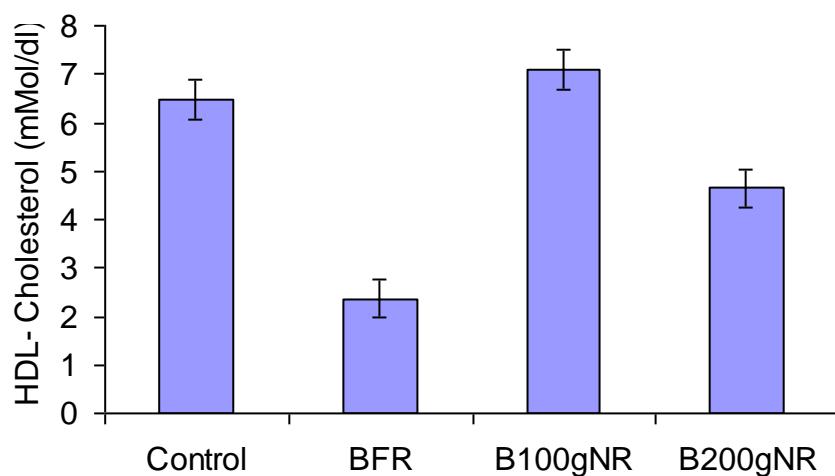


Figure 3.8: HDL-Cholesterol in Potassium Bromate induced cardiac toxicity in Wistar Albino rats

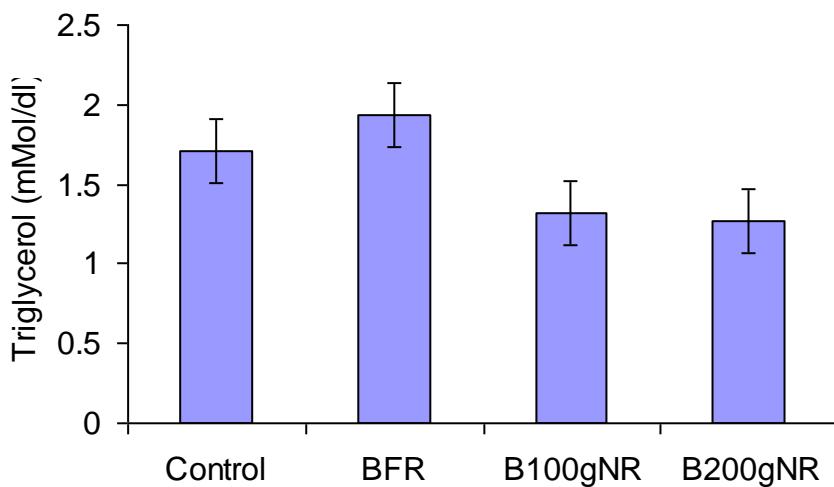


Figure 3.9: Triglyceride in Potassium Bromate induced cardiac toxicity in Wistar Albino rats

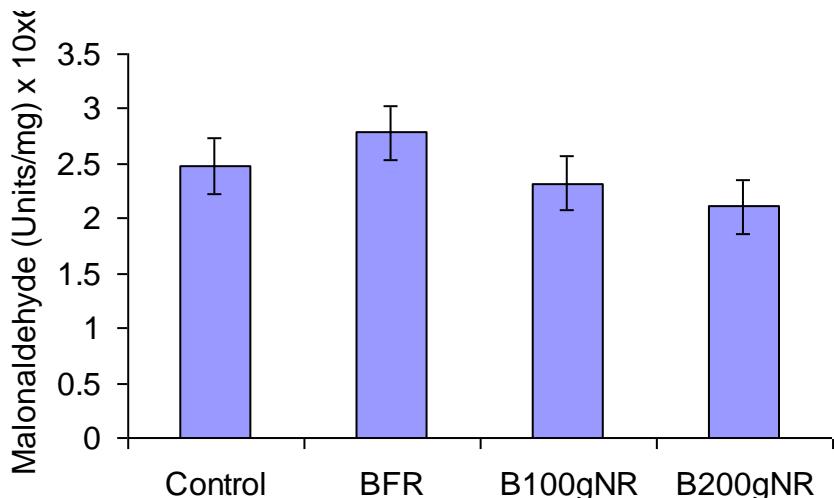


Figure 3.10: Malonaldehyde in Potassium Bromate induced cardiac toxicity in Wistar

Oral administration of potassium bromate ($KBrO_3$) at a dose level of 30mg/kg body weights significantly increased the level of malondialdehyde (representing lipid peroxidation) in heart tissue of bromate treated group (Group I) when compared to the control. $KBrO_3$ has been reported to be highly toxic (Fujie *et al.*, 1988), 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 $KBrO_3$ administration (Malik and Ahluwalia, 1994). Glutamine could also initiate the lipid peroxidation by changing the redox potential of cell and thus favoring the lipogenesis (Malik and Ahluwalia, 1994, Kuldip and Ahluwalia, 2005). Figure 3.10 shows the protective effect of aqueous extract of *Moringa oleifera* leaf. Both concentrations (20% and 40% extract) shows a significant reduction.

Oral administration of Bromated caused significant reductions in the cardiac concentrations of HDL-CHL and TC but caused the reverse effect on the concentration of TRG. The extract also caused significant and progressive elevation in of HDL-CHL and TC values. This shows a dose-related elevation in the cardiac concentrations of HDL-CHL and TC.

The observed significant reduction in the cardiac concentrations of total cholesterol and HDL-cholesterol fractions could also be due to depressed hepatic gluconeogenesis by bromate, although this claim remains a speculation until it is subjected to further scientific validation by the key enzymes regulating this pathway. A positive relationship between gluconeogenesis and lipogenesis has been well documented in literature (Harris and Crabbs, 1982). Any drug that interferes with gluconeogenesis has also been reported to also interfere with lipogenesis. From the foregoing, it is possible for bromate to be inducing its hypoglycemic and hypolipidemic effects via this common pathway. However, this also remains a speculation. In a previous study, *moringa oleifera leaf extract* was reported to reduce total cholesterol and triglycerides in high-fat diet fed rats by interfering with their biosynthesis.

All the above observations, suggested that administration of bromate at dose level of 30mg/kg body weight could induce oxidative stress in heart tissue by altering the activities of RED-GSH, SOD, CAT and other important biomarker enzymes such as TRG, HDL-CHL and TC, thereby being responsible for the initiation of coronary heart disease/ atherosclerosis.

CONCLUSION AND RECOMMENDATION

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

This preliminary study has been able to demonstrate the cardiotoxic effect of bromate and cardioprotective potentials of aqueous extract of *Moringa oleifera* leaf bromated induced-induced cardio toxic rats. The study shows the chemopreventive benefit of extract of *Moringa oleifera* leaf on potassium bromate mediated 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 effect on heart function which might be due to the presence of some bioactive compound in the plant extract. Further investigation should be conducted to identify this bioactive compounds present in *Moringa oleifera* leaf aqueous extract.

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