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The Histological Effects of Ethanolic Leaf Extract of Moringa oleifera on Potassium Bromate Induced Renotoxicity in Adult Wistar Rats

Ezejindu DN[1] Anibeze C.I.P[2] Nduwe GU[2]
1 Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
2 Department of Anatomy, Faculty of Basic Medical Sciences, Abia State University, Uturu, Abia State, Nigeria

Abstract
Much attention has been drawn to the toxic effect of potassium bromate on humans. However, it is clear that the toxicity is related to the vital organs like the liver and kidney, hence the present study is geared towards not only unravelling the toxic effect of potassium bromate on the kidney but more importantly investigating the protective effects of ethanolic leaf extract of *Moringa oleifera* against potassium bromate induced toxicity. Twenty adult wistar rats (185-220g) were divided into four groups of five animals each. Group A was given normal rat feed and water as the control; group B was administered 250mg/kg body weight of ethanolic leaf extract of *Moringa oleifera*, group C was administered 100mg/kg body weight of potassium bromate while group D was administered 100mg/kg body weight of potassium bromate and 250mg/kg body weight of ethanolic leaf extract of *Moringa oleifera*. All the treatments were given daily orally for twenty eight days. Twenty four hours after the last administration, the animals were anasthezised under chloroform vapour and dissected. The kidney tissues were harvested, weighed and fixed in 10% formal saline for histological studies. Histological observation showed that following administration of ethanolic leaf extract of *Moringa oleifera* there was no acute nephritis in the nephrons even in the group treated against potassium bromate. The result of this study shows that ethanolic leaf extract of *Moringa oleifera* did not cause any histopathological lesions in the kidney of adult wistar rats. The extracts may contain antioxidant properties against potassium bromate induced toxicity.
Keywords: Rauwolfia vomitoria, Moringa oleifera, Hepatotoxicity, Renotoxicity, Wistar rats

1. Introduction

Potassium bromate (KBrO3) is a food additive, which exists as a white crystals or powder. It is used primarily as a maturing agent for flour and as a dough conditioner (National Toxicology Program, 1991). It is also generated as a by-product of ozonization of surface water in treated drinking water (Cavanagh *et al*., 1992). Potassium bromate has been evaluated for acceptable level of treatment for flour to be consumed by man. It is used in treating barley in beer making and for improvement of the quality of fish-paste products in Japan. It is generated as a contaminant in drinking water to bromate by ozone which is used as disinfectant (FAO/WHO 1964, Uneno *et al.,* 2000). Several researches on safety evaluation of potassium bromate were carried out. It was found to be a genotoxic and carcinogenic hence was ruled unsafe and banned from the list of food additives (Kurokawa *et al.,* 1990; Sai *et al.,* 1992). Studies have reported that it possess the potential of inducing cancer, kidney failure, deafness, liver failure, redness and pains of the eye and skin (Mack, 1988; De Angelo *et al*., 1998). Recent studies have also reported that the agent is hepatotoxic and renotoxic (Dimkpa *et al*., 2013) thus demonstrating the danger which potassium bromate poses to health if consumed in food or water. In Nigeria, potassium bromate has been declared unsafe and banned from the list of food additives by the National Agency for Food, Drug Administration and Control (NAFDAC). However despite the ban and the awareness created by NAFDAC on the danger of using potassium bromate as flour enhancer, many bakers and water treatment plant owners still use the substance. This poses a great threat to food safety and public health especially to most Nigerians who uses bakery products and commercially prepared water who may be ignorant of adverse effects on body tissues (OEHHA, 2004).

Drugs of plant origin have served through the ages as the mainstay in the treatment of variety of diseases and preservation of human health (Pousset, 1988). The use of herbs to treat diseases is almost universal among non-industrialized societies and is often more affordable than purchasing expensive modern pharmaceuticals. World Health Organization (WHO) estimates that 80% of the population of Asian and African countries presently uses herbal medicine for some aspects of primary healthcare (Lichterman, 2004). The use of and search for drugs and dietary supplement derived from plants have accelerated in recent years. Pharmacologist, Microbiologist, Botanist and natural product chemists are combing the earth for phytochemicals and leads that could be developed for treatment of various diseases. According to the World Health Organization, approximately 25% of modern drugs used in the United States have been from plants (Stepp, 2004).

One of these plants of medicinal value is *Moringa oleifera*. It is the most widely cultivated species of

the genus *Moringa* which is the only genus in the family Moringaceae. The English common names include drumstick tree, horseradish tree and bin oil tree as well as *Okweoyibo* in Igbo, *Zogale* in Hausa and *Igbaele* in Yoruba. The leaves are the most nutritious part of the plant being significant source of B vitamins, vitamin C, provitamin A, vitamin K, manganese and protein among other essential nutrients (Gopalan *et al.,* 1989, Fuglies 1999). *Moringa oleifera* is a highly valued plant distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutrition value. Different parts of this plant contain a profile of important minerals and a good source of protein, vitamins, B carotene and amino acids. In addition to its compelling water purifying powers and high nutritional value, *Moringa oleifera* is very important for its medicinal value. Various part of this plant such as the leaves, roots, seeds, bark, fruit, flowers and immature pods acts as cardiac and circulatory stimulants, possess hepatoprotective, renoprotective, antitumor, antipyretic, antiepileptic, antidiuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, antibacterial and antifungal activities. They are been employed for the treatment of different ailments in the traditional system of medicine (Almad *et al.,* 1998).

The kidneys and liver are the key organs regulating homeostasis in the body. They are involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy production and reproduction. Because of their unique metabolism and relationship to the gastrointestinal tract, they are important target for toxicity produced by drugs, xenobiotics and oxidative stress (Anusha *et al*., 2011; Jaescke *et al*., 2002).

This scenario provides a severe necessity to carry out research on renoprotective effects of ethanolic leaf extract of *Moringa oleifera* and on potassium bromate induced wistar rats.

2. Materials and Methods

2.1. Experimental Animals

Twenty adult wistar rats were procured from the Animals House of Anatomy Department, Nnamdi Azikiwe University, Nnewi Campus. Perspex cages were used to house groups of six (6) animals for routine experiment. Each cage has wire gauze top for cross ventilation. The animals were allowed for a period of two weeks for acclimatization under normal temperature (27^0C -30^0C) at the Animal House of Anatomy Department, Abia State University, Uturu, Abia State before their weights were taken. They were fed ad libitum with water and guinea feed pallets from Agro feed mill Nigeria Ltd. The ethical committee of the College for animal cares and use, Nnamdi Azikiwe University, Nnewi campus approved the study design in compliance with the National regulation for animal research.

2.2. Collection and Preparation of Plant Materials

Moringa oleifera leaves were plucked from Okofia in Nnewi, Anambra State. They were authenticated at Herbarium unit, Botany Department, Abia State University, Uturu, Abia State. They were dried in an oven at a temperature of 50^oC and crushed using laboratory blender. Extraction was done using ethanol. Ethanol was poured into the grinded leafs and was allowed to stay for twenty four hours. It was filtered into a stainless basin with a white cloth and placed in a water bath so as to dry up the ethanol. 250mg/kg body weight of this extract were dissolved in 10mls of distilled water and administered to the animals.

Potassium bromate was procured from Biochemistry Department, Abia State University, Uturu, Abia State. 100mg/kg body weight of potassium bromate was dissolved in distilled water and administered to the animals.

2.3. Experimental Protocols

The twenty animals were weighed and allocated into four groups of five animals each. The groups were designated as groups A, B, C & D. Group A served as the control group and were administered distilled water only. The experimental groups B, C & D were administered different doses of drug as follows: group B received 250mg/kg body weight of leaf extract of *Moringa oleifera,* group C received 100mg/kg body weight of potassium bromate while group D received 100mg/kg body weight of potassium bromate in the first two weeks + 250mg/kg body weight of leaf extract of *Moringa oleifera* in the last two weeks. The extracts were administered once in a day between the hours of 12-3.30pm for a period of twenty eight days. The drugs were administered orally using intubations method. After the twenty eight day, the animals were weighed and their weights recorded. Twenty four hours after the last administration, the animals were anaestathized under chloroform vapour and dissected. Kidney tissues were harvested from the animals, weighed and fixed in 10% formal saline for histological studies.

2.4. Tissues Processing

For easy study of sections under the light microscope, the kidneys tissues passed via several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in 10%

formalin .The tissues were washed overnight in running tap water after four hours in 10% formaldehyde. Dehydration of the fixed tissues was carried out in different percentage of alcohol 50%, and 90% absolute. The tissues were then cleared in xylene and embedded in paraffin wax. Several sections of 5micron thick are obtained using a rotator microtone. The tissue sections were deparaffined hydrated and stained using the routine haematoxylin and eosin method. The stained sections were then examined under the light microscope.

3. Results and Discussion

3.1. Analysis of Body Weight

Table 4.2: Comparison of mean initial and final body weight and weight change in all the groups (A, B, C & D) Mean ± SEM given for each measurement

GROUPS	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	WEIGHT CHANGE
A	180.05±1.05	215.30±2.53	35.25±1.48
B	185.70±6.86	218.08±6.98	32.38±0.12
C	218.28±13.07	174.26±9.07	-44.00±4.00
D	210.10±7.48	231.18±12.10	21.08±4.62
F-RATIO	89.230	48.510	18.211
PROB. OF SIG	<0.005	<0.005	<0.005

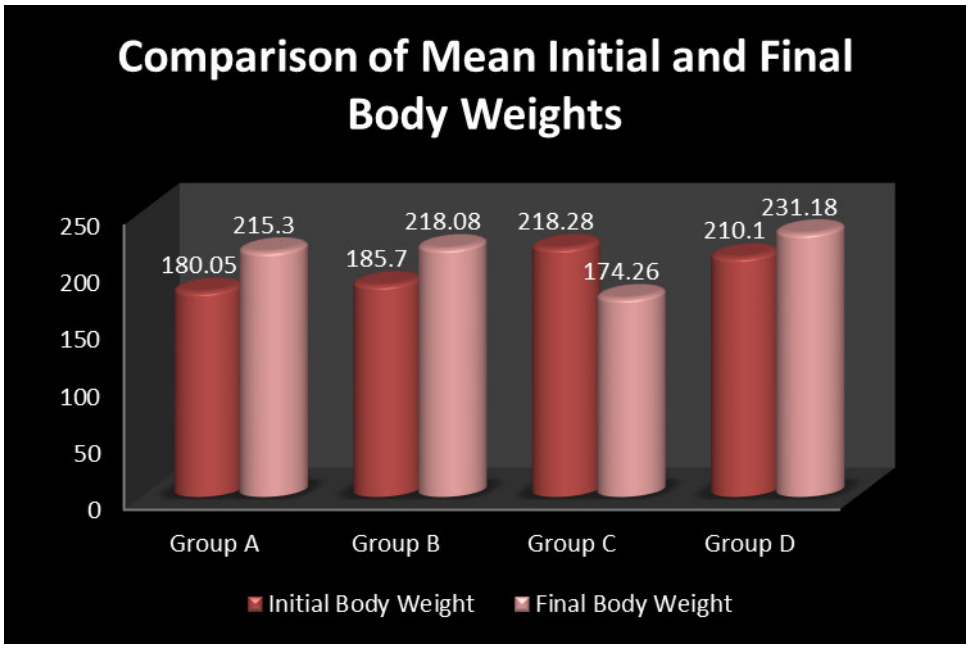

Figure 4.2: Bar Chat Representation of the Mean Initial and Final Body Weights of all the Groups

3.2. Analysis of Kidney Weight

Table 4:3: Comparison of mean relative Kidney Weight for group A (control) and experimental groups (B, C & D)
(Mean ± SEM given for each measurement)

GROUPS	KIDNEY WEIGHTS
A	1.31±0.03
B	1.33±0.04
C	2.20±0.44
D	1.35±0.06
F-RATIO	27.351
PROB. OF SIG.	<0.005

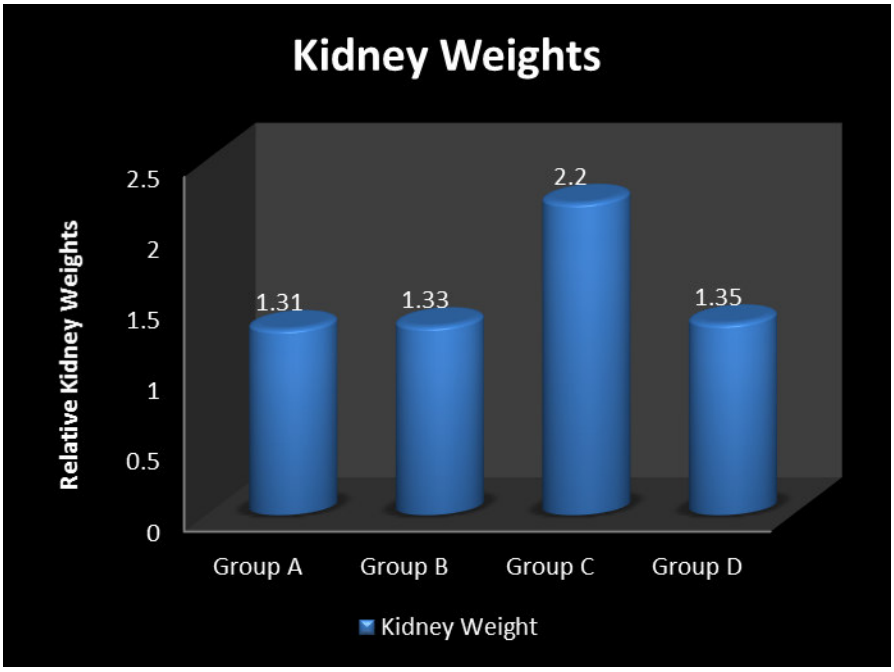

Figure 4.3 Bar Chart Representation of Mean Relative kidney Weight for Groups A (control), B, C & D.

3.3. Histopathological Findings

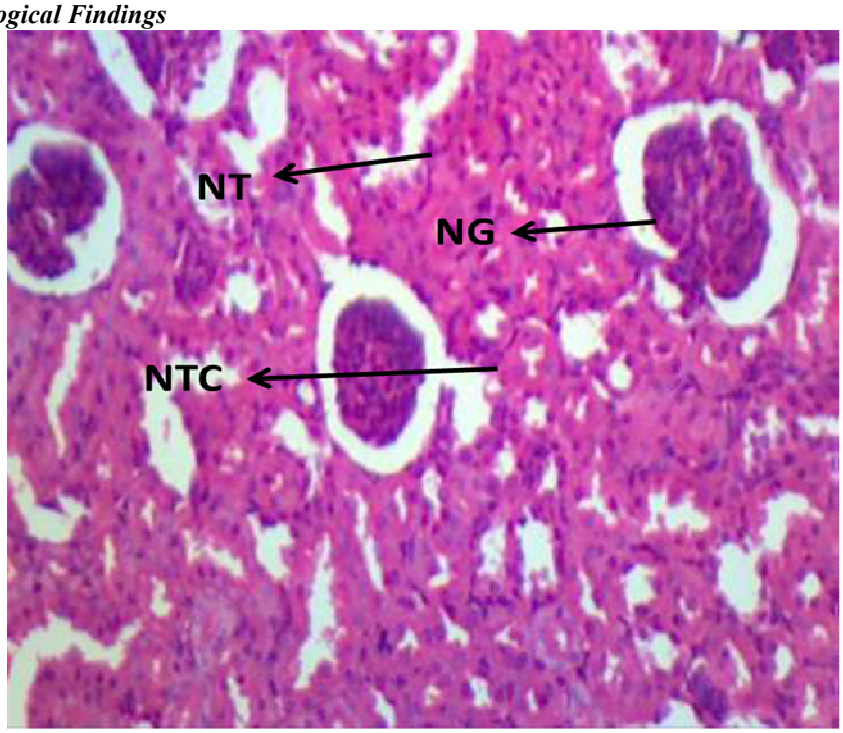

Plate 1: Shows photomicrograph of control Group A kidney section (H/E ×150) composed of normal renal architecture showing normal glomeruli (NG), normal tubules (NT) and normal tubular cells (NTC).

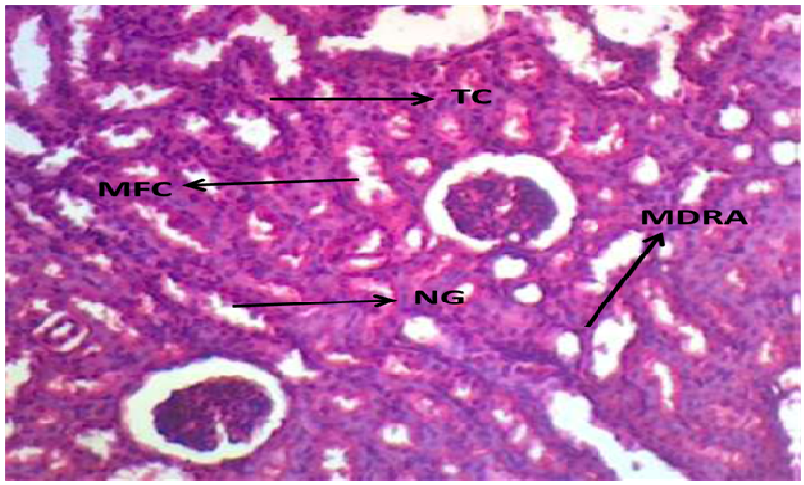

Plate 2: Shows photomicrograph of Group B kidney section administered 250mg/kg of *Moringa oleifera* leaf extract (H/E ×150) shows normal renal architecture with normal glomeruli (NG) and tubular cells (TC). However, there are area of mild fatty change (MFC) and mild distortion of renal architecture (MDRA)

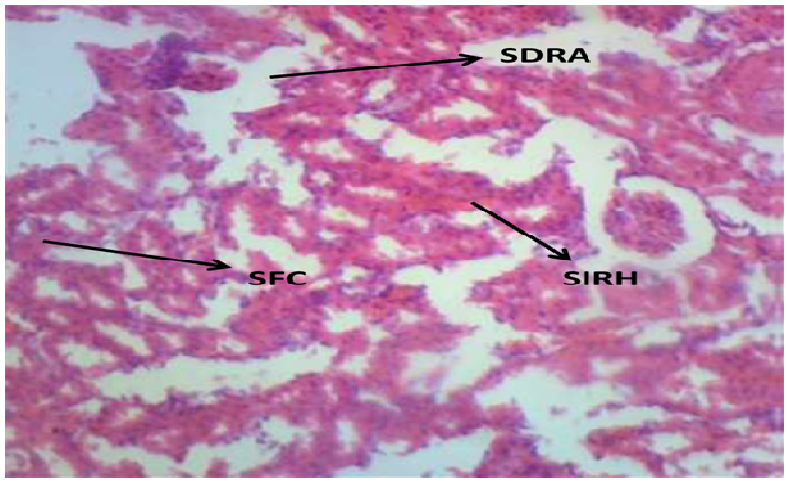

Plate 3: Shows photomicrograph of Group C kidney section administered 100mg/kg of potassium bromate (H/E ×150) shows severe distortion of renal architecture (SDRA) with severe intra renal haemorrhage (SIRH), severe fatty change (SFC) and focal loss of glomeruli (FLG).

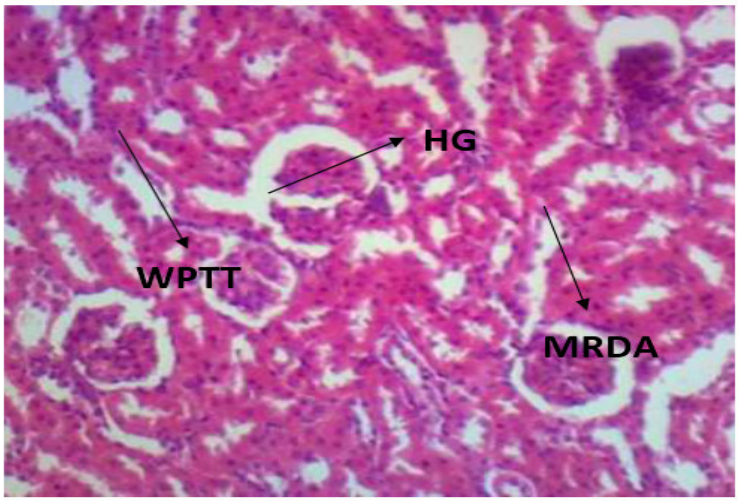

Plate 4: Shows photomicrograph of group D kidney section administered 100mg/kg of potassium bromate and 250mg/kg of *Moringa oleifera* leaf extract (H/E ×150) shows restoration of renal architecture to normal with healthy glomeruli (HG) well perfused tubular tissue (WPTT) and normal tubular cells (NTC). However, there are mild fatty change (MFC) and mild distortion of renal architecture (MRDA) in some areas.

From time immemorial, man depended on plants as medicine. From a historical perspective, it is evident that the fascination for plants is as old as mankind itself. The plant kingdom represent a rich store house of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years. Plants are the richest source of drugs for traditional medicine, modern medicines, nutraceuticals food supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs (Ngaski, 2006).

Observation of the body weight difference in groups reveals increase in body weight of animals in group A. This could be physiological as the only substance they were exposed to was water and feed. The significant body weight increase was also observed in the experimental groups B & D. There was significant reduction in body weight of animals in group C administered with potassium bromate when compared with the control. This is probably as a result of loss of appetite and reduction of food intake by the animals in the group. This result agrees with a previous study Okalie and Ikewuchi (2004) who reported a significant reduction in body weight of rabbits administered with potassium bromate. The groups that were treated with ethanolic leaf extract of *Moringa oleifera* alone, potassium bromate + ethanolic leaf extract of *Moringa oleifera* showed significant increase ($P>0.05$) in body weight similar to the control group. Ethanolic leaf extract of *Moringa oleifera* in this instance functions primarily as a dietary supplement enhancing growth. This result is in line with the report by Bureau of Plant Industry that *Moringa oleifera* and as an outstanding source of nutritional components whose leaves have potassium equivalent of three times that of bananas, calcium equivalent of four times that of milk, the vitamin C content is seven times that of oranges, four times the amount of vitamin A in carrots and two times the protein in milk thus having more than 40 natural antioxidants (Kamal, 2008).

The relative organ weight result also showed significant differences in groups. There was significant ($P>0.05$) relative organ weight increase in group D animals administered potassium bromate when compared with the control group A and groups B & D. The significant relative organ weight was irrespective of the fact that there was total body weight loss. This could have been pathological and one may deduce that the increase in the relative kidney weight of this group was not growth but inflammation. This is in line with the previous work of Kawana *et al*., (1991) who reported increase in kidney, lungs and liver weight above the control organ weights treated with potassium bromate. This is also in line with work done by Watanabe *et al*., (2004) and Abuelgasim *et al*., (2008) which reported relative liver and kidney weight increase in rats administered 100mg/kg body weight of potassium bromate. The result of groups B & D relative organ weight were statistically similar with the control group A. Antioxidant properties of ethanolic leaf extract of *Moringa oleifera* could be responsible for the control or prevention of inflammation in the the groups treated with them. Also it could be as a result of anti-inflammatory properties possessed by ethanolic leaf extract of *Moringa oleifera.*

The histopathological result of group D animals treated with potassium bromate + ethanolic leaf extract of *Moringa oleifera* showed normal kidney tissue architecture. This may be related to the ameliorating importance of vitamin E as an antioxidant in combating the free radical damage mechanism of potassium bromate. This agrees with earlier work of Sai, Hayashi, Takagi *et al.*, (1992) where they studied the suppression of potassium bromate induced micronuclei formation by antioxidants.

The animals in group D gives a particularly interesting observation about the dynamics of reactions to the presence of various substances in our systems. On administration of ethanolic leaf extract of *Moringa oleifera* to the group, no toxicity changes was associated in physical and behavioural observation, there were body weight increase similar with the control, relative organ weight were statistically similar with the control and no histological lesions were observed in the kidney tissues. By these observations, one may deduce that administration of ethanolic leaf extract of *Moringa oleifera* may boost the tolerance capacity for potassium bromate induced toxicity.

4. Conclusion

The ethanolic leaf extract of *Moringa oleifera* showed no histopathological lessons in the kidney tissues of the rats. This study has demonstrated the potential ability of *Moringa oleifera* to protect against potassium bromate induced toxicity in the kidney of adult wistar rats. Rat's tissues are very similar in many aspects to those of human. The findings of this study suggests that leaf extract of *Moringa oleifera* administered to individuals exposed to potassium bromate poisoning could provide some protection against potassium bromate toxicity and perhaps ameliorate the effects of potassium bromate toxicity on the kidney.

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