

Annona muricata Mitigates Kidney Damage in Rats following Chronic Exposure to Bromate

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Mobile Number: +234 8077413177 DOI: 10.5281/zenodo.17428609

Abstract

Kidney disease ranks among the leading causes of death globally, steering research towards finding safe, natural, and affordable regimens for its cure. *Annona muricata* leaves have been used in traditional medicine and contain a myriad of antioxidants, which contribute to their pharmacological properties. This study investigated the effect of *Annona muricata* methanol leaf extract on potassium bromate (KBrO₃)-induced renal toxicity in male Wistar albino rats.

Forty-two male albino rats, divided into seven groups of six rats each, were used. Group 1 (Control) received normal saline, group 2 received only 125 mg/kg body wt. KBrO₃, while groups 3 and 4 received 150 and 300 mg/kg body wt. extract only respectively. Groups 5, 6 and 7 received 150, 300, and 450 mg/kg wt. extract along with KBrO₃, respectively. All treatments were administered orally for ninety days. Using standard methods, serum urea and creatinine, tissue levels of malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities were assayed. Kidney histopathology and haematology analysis. The extract significantly ($P < 0.05$) lowered urea, creatinine and MDA levels while GSH level, and activities of GPx, CAT, and SOD. Although there were no significant changes in white blood cells (WBCs), red blood cells (RBCs) and platelets (PLTs), histopathological analysis revealed fairly normal glomeruli and tubules, with mild interstitial congestion. The results from this study suggest that *Annona muricata* methanol leaf extract effectively restored kidney function by improving antioxidant enzyme activity and can serve as a source of natural products for drug design.

Keywords: Bromate, Antioxidants, *Annona muricata*, Kidney damage, Natural product

Introduction

Kidney disease is a non-communicable disease (NCD) recently identified by the World Health Organization (WHO) as a global health priority. Over the past two decades, the incidence of deaths resulting from kidney disease has increased steadily [1]. Chronic kidney disease (CKD) is a progressive condition that eventually leads to kidney failure, at which stage kidney replacement therapy (KRT) like dialysis or transplantation is essential for survival. Individuals with CKD also face an increased risk of developing other NCDs, particularly cardiovascular disease.

Potassium Bromate (KBrO₃) is a common kidney toxicant, an oxidizing agent used as a neutralizer in home permanent cold wave hair kits [2], serves as a food additive for dough enhancement in bread production [3], a conditioner in fish paste and in beer or cheese [4,

5]. Its application is also found in pharmaceutical industries as well as in the cosmetic industry [5, 6]. Despite its usefulness, bromate intoxication results in a severe and irreversible sensorineural hearing loss as well as renal failure, methemoglobinemia and kidney cancer [2, 4, 7]. Its mechanism of toxicity is largely via the production of radicals, which result in oxidative stress, the underlying cause of many known diseases. Indeed, bromate has been categorized as a genotoxic carcinogen in line with positive results of the Ames test [8], as well as chromosome aberration and micronucleus tests [9]. The compound also induces 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation both *in vitro* and *in vivo* [10]. 8-OHdG is the most abundant oxidative DNA adduct, and it induces mutations like GC to TA transversions during DNA polymerase-catalysed replication [11]. Although Nigeria, in 1993, banned potassium



bromate just like in several countries, including the United Kingdom in 1990, and Canada in 1994, it is still being used in some bakeries in Nigeria to maximize profit [12, 13, 14, 15, 16]. However, in the United States, the use of bromate is under prescribed conditions for bread making and malting of barley [17].

Annona muricata Linn, popularly identified as *graviola*, soursop, *guanabana*, or corossol, is a tropical tree with heart-shaped fruits. The plant belongs to the family *Annonaceae*, and it is native to Central America, Brazil, Mexico, Peru, Cuba, Venezuela, and Colombia. It also grows in sub-Saharan African countries within the tropics. Presently, soursop is grown in some areas of Southeast Asia and the Pacific Islands [18, 19]. Traditionally, the leaves are used to manage insomnia, headaches, diabetes, cystitis, liver problems, and as anti-inflammatory, anti-dysenteric and anti-spasmodic. The fruit is also used for the treatment of diarrhoea, cough, hypertension, rheumatism, tumours, cancer, asthma, tranquillizer, skin rashes, parasites, worms, liver problems, and arthritis. A growing body of evidence has shown that *Annona muricata* leaves are rich in antioxidants, flavonoids, saponins, and glycosides [20, 21]. They contain a group of phytochemical compounds called annonaceous acetogenins comprising murihexocin and annocuricin [22, 23, 24]; annopentocin A, B and C, (2,4-cis)-annomuricin-D-one, murihexocin A and B (2,4-tranmurihexocin, annocuricin, and annopentocin A and B [25, 26, 27], as well as essential oils which exert parasitocidal, anti-diarrhoeal, rheumatological and anti-neuralgic effects [28]. It has been reported that water infusion of the leaves of *Annona muricata* exerted anti-plasmodic, astringent and gastroprotective properties [29, 30]. Other studies reported that the leaf infusion was effective in the management of diabetes, stomach upset [31], as well as jaundice [32]. It has also been revealed that the leaves produced hepatoprotective effect against carbon tetrachloride- and acetaminophen-induced liver damage [33] and in streptozotocin-treated diabetic rats [25], parasitocidal, neuralgic, and anti-rheumatic effects when used internally, while the topical application of boiled leaves, was effective against rheumatism and abscess [34, 35]. *In vitro* studies have indicated that annonaceous acetogenins from *Annona muricata* leaves may be potent and promising new anticancer agents [35]. Acetogenins were found to be selectively toxic

against various types of cancer cell lines without interfering with healthy cells [36, 37, 38, 39, 40, 41]. Studies by [42] proved that the methanolic extract of *Annona muricata* leaves suppresses tumour initiation and promotion [43]. Due to the high mortality of kidney disease in low- and middle-income countries [20], there is a need to continually search for safer, natural and affordable medicines in herbs. The present study was designed to investigate the effect of the methanolic extract of *Annona muricata* leaves on bromate-induced kidney toxicity in Wistar rats.

Materials and Methods

Collection of Annona muricata Leaves and Preparation of Methanol Extract

Fresh leaves of *Annona muricata* were collected within Oredo Local Government area, Benin City, Edo State. They were separated from the stalk, air-dried at room temperature, crushed, and macerated in methanol for seventy-two hours. Using a cheese cloth, the mixture was filtered to obtain the filtrate, which was concentrated in a rotary evaporator at 40°C. The concentrate was further evaporated to dryness in a water bath at the same temperature. The dried extract was stored in an air-tight container and placed in a refrigerator at 4°C until needed for the experiment. An appropriate amount of extract for each animal was obtained by calculation and reconstituted in normal saline before administering it to the animals at their respective doses.

Experimental Animals

Forty-two male albino rats weighing 120-140g were acquired from the Animal House of the Pharmacology and Biochemistry Department, University of Benin. They were kept in wooden cages in the animal house of the Biochemistry Department to acclimatize for fourteen days. Thereafter, they were placed in seven groups of six rats each and fed rat pellets throughout the experiment. The animals had access to feed and clean water *ad libitum* under a 12-h light/12-h dark photoperiod. Group 1 (Control) received normal saline, group 2 received of 125 mg/kg body wt. KBrO₃ alone, groups 3 and 4 were administered extract only, at doses of 150 and 300 mg/kg body wt. Groups 5, 6 and 7 each received the extract at doses of 150, 300, and 450 mg/kg wt., respectively, along with KBrO₃ at a dose of 125 mg/kg body. All treatments were administered orally for ninety days (thirteen weeks), thereafter



sacrificed under light anaesthesia. Blood samples were collected via cardiac puncture, while the kidneys were excised. Portions of the kidney were fixed in 10% formaldehyde for histopathological analysis, while the remaining portions were placed in sample bottles containing 0.9% NaCl for biochemical analysis.

Biochemical and Antioxidant Assays

Protein levels in serum were measured using the Biuret method as described by [44] with some modifications. Urea in serum is hydrolysed to ammonia in the presence of urease. Urea was determined by [45]. Cayman's Creatinine (serum) Assay was used to measure creatinine levels in serum in line with [46]. Urea and creatinine were measured in line with the procedure outlined in the assay kit manual.

Superoxide dismutase (SOD) activity was assayed by the method [47]. Catalase activity was assessed as described by [48]. Reduced glutathione (GSH) levels were measured according to the method of [49]. Kidney GST was determined according to the procedure of [50]. Lipid

peroxidation was assayed according to the method of [51].

Haematological Analysis

Haematological analysis of the rat blood was carried out using a hemocytometer. A sample of blood was diluted with an isotonic solution (2% acetic acid), and the number of red blood cells in a fraction of this diluted blood was counted, and then multiplied by a correction factor.

Tissue Histology

Kidney tissues fixed in 10% formalin were processed for light microscopy by a Pathologist who read, interpreted the slides and prepared relevant tissue photomicrographs. Analysis was done in accordance with the method of [52].

Statistical Analysis

Data are presented as mean $\pm$ SD (n=6). Differences among and within groups were analysed for statistical significance using analysis of variance (ANOVA) and the student t-test, respectively. Values of $p < 0.05$ were taken as indicative of significant differences.

RESULTS

Table 1: Effect of *Annona muricata* Methanol Leaf Extract on Serum Urea and Creatinine Concentrations

Groups	Description	Urea (mg/dl)	Creatinine (mg/dl)
1	Control (Normal saline)	44.63 $\pm$ 0.36	1.04 $\pm$ 0.12
2	KBrO ₃ (125mg/kg)	92.12 $\pm$ 5.65 ^a	7.32 $\pm$ 1.79 ^a
3	Extract (150mg/kg)	43.11 $\pm$ 4.30 ^b	0.66 $\pm$ 0.59
4	Extract (300mg/kg)	44.38 $\pm$ 2.15 ^b	0.36 $\pm$ 0.05
5	Extract (150mg/kg) + KBrO ₃ (125mg/kg)	49.77 $\pm$ 3.5 ^b	0.90 $\pm$ 0.65 ^b
6	Extract (300mg/kg) +125mg/kg KBrO ₃	30.26 $\pm$ 2.35 ^{a,b}	0.60 $\pm$ 0.19 ^b
7	Extract 450mg/kg + KBrO ₃ (125mg/kg)	29.23 $\pm$ 4.57 ^{a,b}	0.30 $\pm$ 0.08 ^b

Values are Mean $\pm$ SD (n = 6). a=significant compared with Group 1(control); b= significant compared with Group 2. (p <0.05)



Table 2: Effect of *Annona muricata* Methanol Leaf Extract on Some Biochemical Indices and Antioxidant Status

Group	Description	Serum protein (g/dl)	MDA $\times 10^{-5}$ ($\mu\text{g}/\text{mg}$ protein)	Catalase ($\mu\text{moles}/\text{mg}$ protein)	Reduced glutathione ($\mu\text{g}/\text{ml}$)	Glutathione peroxidase ($\mu\text{g}/\text{mg}$ protein)	Superoxide dismutase (mg/ml)
1	Control (Normal saline)	16.042 $\pm$ 0.57	2.33 $\pm$ 0.10	20.88 $\pm$ 2.98	3.67 $\pm$ 0.14	13.11 $\pm$ 0.39	9.07 $\pm$ 0.35
2	KBrO ₃ (125mg/kg)	18.506 $\pm$ 0.49 ^a	5.72 $\pm$ 1.01 ^a	19.34 $\pm$ 1.74 ^a	2.63 $\pm$ 0.53 ^a	12.16 $\pm$ 0.45 ^a	7.22 $\pm$ 0.79 ^a
3	Extract (150mg/kg)	18.081 $\pm$ 1.15	1.82 $\pm$ 0.33 ^b	38.88 $\pm$ 5.14 ^a	3.00 $\pm$ 0.71	12.63 $\pm$ 0.32 ^a	7.98 $\pm$ 0.29 ^a
4	Extract (300mg/kg)	17.77 $\pm$ 0.11 ^{a,b}	1.96 $\pm$ 0.17 ^b	68.34 $\pm$ 5.52 ^{a,b}	2.50 $\pm$ 0.71 ^a	12.95 $\pm$ 0.24 ^b	8.35 $\pm$ 0.15 ^{a,b}
5	Extract (150mg/kg) + KBrO ₃ (125mg/kg)	17.516 $\pm$ 1.43	1.79 $\pm$ 0.26 ^{a,b}	61.34 $\pm$ 1.77 ^{a,b}	2.38 $\pm$ 0.53 ^{a,b}	12.37 $\pm$ 0.01	8.53 $\pm$ 0.32 ^{a,b}
6	Extract (300mg/kg) + 125mg/kg KBrO ₃	18.759 $\pm$ 0.69 ^a	2.48 $\pm$ 0.56 ^{a,b}	74.10 $\pm$ 1.77 ^{a,b}	2.83 $\pm$ 0.14 ^{a,b}	12.17 $\pm$ 0.17 ^{a,d}	8.52 $\pm$ 0.50 ^{a,b}
7	Extract (450mg/kg) + KBrO ₃ (125mg/kg)	18.144 $\pm$ 0.19 ^a	3.39 $\pm$ 1.09 ^a	119.00 $\pm$ 5.71 ^{a,b}	3.63 $\pm$ 0.18 ^b	12.39 $\pm$ 0.22 ^a	8.55 $\pm$ 0.77

Values are Mean $\pm$ SD (n = 6). a=significant compared with Group 1(control); b= significant compared with Group 2. (p <0.05)

Table 3: Effect of *Annona muricata* Methanol Leaf Extract on Haematology Parameters

Groups	Description	WBC ($\times 10^3/\text{ul}$)	RBC ($\times 10^6/\text{ul}$)	Hgb (g/dl)	MCV (fL)	PCT (%)	MPV (fL)	Leukocytes ($\times 10^3/\text{ul}$)	Granulocytes ($\times 10^3/\text{ul}$)
1	Control (Normal saline)	4.65 $\pm$ 0.21	6.36 $\pm$ 0.50	11.45 $\pm$ 0.78	50.17 $\pm$ 2.32	0.33 $\pm$ 0.11	5.83 $\pm$ 0.55	1.87 $\pm$ 0.83	1.533 $\pm$ 0.83
2	KBrO ₃ (125mg/kg)	9.00 $\pm$ 0.28	7.76 $\pm$ 0.73	7.76 $\pm$ 0.73	55.6 $\pm$ 1.56	0.28 $\pm$ 0.05	5.95 $\pm$ 0.21	4.70 $\pm$ 0.71	2.80 $\pm$ 0.71
3	Extract (150mg/kg)	8.07 $\pm$ 2.80	7.51 $\pm$ 0.39	13.9 $\pm$ 0.65	54.5 $\pm$ 3.8	0.31 $\pm$ 0.019	6.20 $\pm$ 0.17	4.90 $\pm$ 1.74	2.2 $\pm$ 0.70
4	Extract (300mg/kg)	5.40 $\pm$ 1.41	7.76 $\pm$ 0.09	14.6 $\pm$ 0.46	53.47 $\pm$ 1.90	0.255 $\pm$ 0.03	6.07 $\pm$ 0.32	3.15 $\pm$ 0.64	2.60 $\pm$ 1.70
5	Extract (150mg/kg) + KBrO ₃ (125mg/kg)	7.80 $\pm$ 0.99	7.39 $\pm$ 0.95	13.97 $\pm$ 1.92	56.37 $\pm$ 2.34	0.296 $\pm$ 0.01	6.10 $\pm$ 0.36	4.85 $\pm$ 1.06	2.50 $\pm$ 1.21
6	Extract (300mg/kg) + 125mg/kg KBrO ₃	7.57 $\pm$ 0.81	8.41 $\pm$ 0.32	16.1 $\pm$ 0.89	53.9 $\pm$ 2.10	0.296 $\pm$ 0.01	6.00 $\pm$ 0.10	4.97 $\pm$ 0.31	1.87 $\pm$ 0.55
7	Extract 450mg/kg + KBrO ₃ (125mg/kg)	6.4 $\pm$ 0.54	7.38 $\pm$ 0.13	13.5 $\pm$ 0.25	60.10 $\pm$ 0.15	0.34 $\pm$ 0.04	6.30 $\pm$ 0.51	3.60 $\pm$ 1.38	1.80 $\pm$ 1.20

Effect of *Annona muricata* Methanol Leaf Extract on Kidney Histology

Plate 1 shows the effect of *Annona muricata* methanol leaf extract on kidney histology. Treatment with bromate alone, resulted in cloudy

tubular epithelial cell swelling and hyperplasia. While control group showed normal glomeruli, tubules and interstitial space. Bromate and extract co-treatment, revealed mild interstitial congestion and fairly patent tubules.



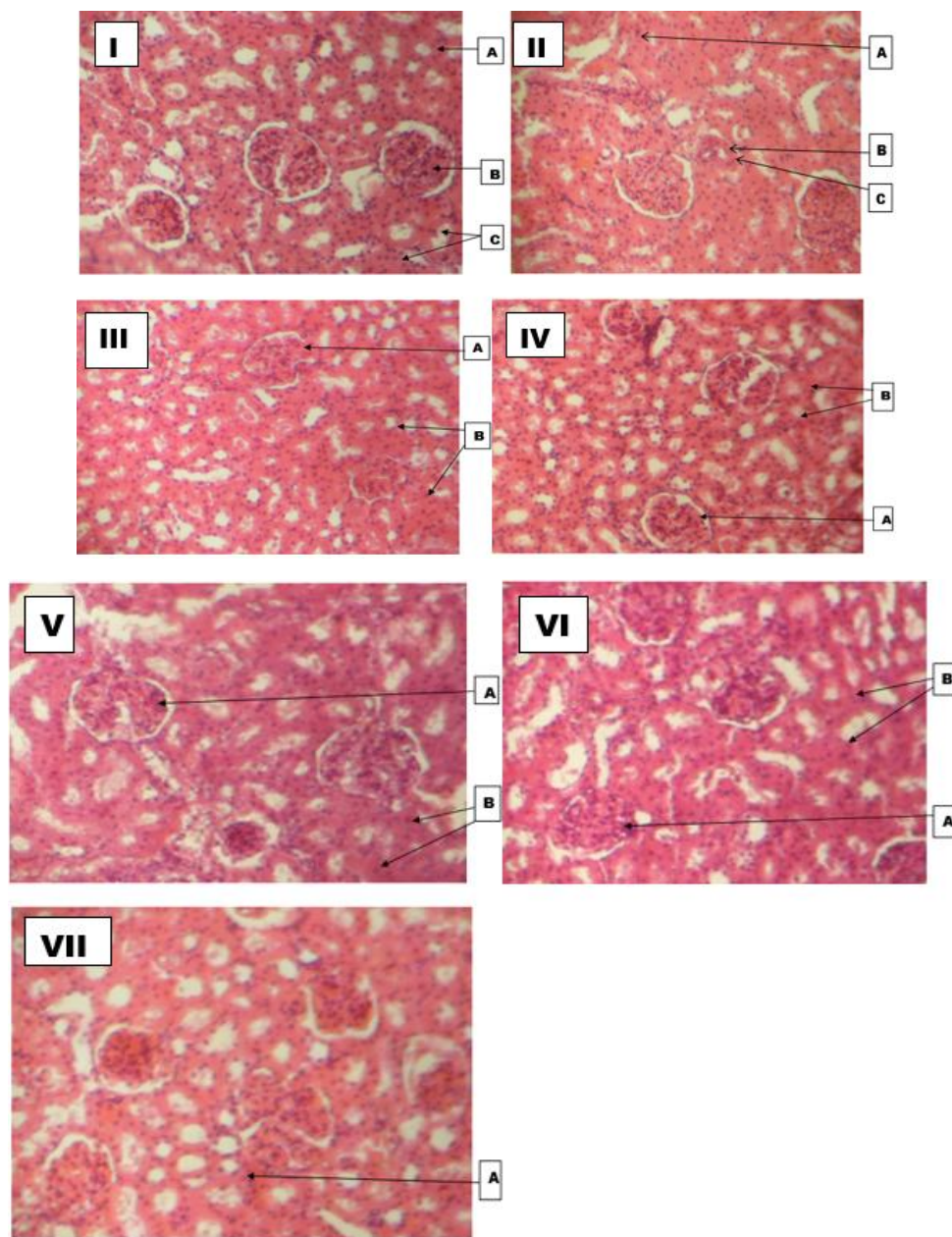


PLATE 1: Groups I-VII (H&E x 100): (I) Interstitial space (A), Normal, glomeruli (B), and tubules (C); (II) Focal tubular cloudy swelling (A), arteriolar luminal obstruction (B) and intimal ulceration; (III) Fairly normal glomeruli (A) and tubules (B); (IV) Fairly normal glomeruli (A) and tubules (B); (V) Fairly normal glomeruli (A) and tubules (B); (VI) Fairly normal glomeruli (A) and tubules (B); (VII) Mild interstitial congestion (A)

Discussion

Chronic kidney disease has been categorized as a public health concern and, in recent times, has led to the death of many people, especially in low and middle-income countries. Potassium bromate (KBrO_3), which is commonly used to enhance bread dough and size, is a known kidney toxicant

[7]. Prolonged exposure to bromate results in chronic kidney disease as well as renal cell and thyroid carcinomas in rats, hamsters and mice during chronic exposures [53].

In this study, KBrO_3 administration significantly ($P < 0.05$) elevated serum protein concentrations (Table 2) at the end of the treatment

period. This finding is consistent with those observed by Josiah *et al.* [54]. This marked increase was, however, reversed dose dependently when *Annona muricata* leaf extract was administered. In the groups treated with extract alone, no significant change was observed when compared with the control. Zubaidi *et al.* [55] have reported a similar finding. The increased protein concentration observed in the bromate alone-treated group may be due to increased protein synthesis, which is a result of the induction of detoxifying protein synthesis [56]. The protective function of protein is dual; they protect the cells by ensuring that cellular integrity is maintained (cytoskeletal proteins), while some shield the cells from free radicals, chemical injury and xenobiotic agents [56, 57].

The major end product of protein breakdown is urea. It is primarily produced in the liver and secreted by the kidneys. It serves as the vehicle for getting rid of ammonia from the body. Urea determination is very useful in medical investigations to ascertain kidney function [58]. Generally, increased urea levels are associated with renal ischemia, nephritis, urinary tract obstruction, and some extrarenal diseases. The observed nephrotoxicity induced by potassium bromate in this research is similar to earlier observations [7, 59, 60].

Urea concentration in the serum increased significantly in the bromate alone-treated group, thus indicating renal damage and impairment of renal function (Table 1). Adewole and Ojewole [31] also reported similar findings with potassium bromate. Treatment with 150mg/kg, 300mg/kg and 450mg/kg *Annona muricata* leaf extract together with bromate showed significant reduction in serum urea levels, indicative of protection by the extract. This protection was observed to be dose-dependent, with the high dose showing the most effective protection. Administration of the extract alone showed a reduction in urea concentration, thus enhancing kidney function. This implies that chronic exposure to bromate results in severe kidney damage. The present result is similar to a previous study reported [7, 61].

Creatinine, a by-product of creatine phosphate metabolism in muscle, remains a widely used biomarker for assessing renal function mainly

because its rate of production is relatively stable and its elimination depends heavily on glomerular filtration. Elevations in serum creatinine therefore indicate reduced kidney filtration capacity, i.e., a decreased glomerular filtration rate (GFR), and serves as a signal for renal parenchyma injury [62]. Notably, persistently high creatinine may also accompany conditions such as glomerulonephritis, nephrotoxic exposures, or progressive chronic kidney disease, and therefore is important both diagnostically and prognostically. The results of this study (Table 1) have revealed marked increase in serum creatinine following bromate toxicity, which is indicative of kidney injury. *Annona muricata* methanol leaf extract significantly restored kidney function as seen in the reduction of serum creatinine levels in the extract treated groups. These findings are in agreement with that reported in previous studies [61, 73].

Oxidative stress is a cellular condition in which there is an imbalance in the concentrations of oxidants and antioxidants. Antioxidant enzymes are an important part of the cell's defence mechanism against oxidative stress. They scavenge and neutralize free radicals, which are highly reactive molecules that can damage cellular architecture and biomolecules, including lipids, proteins and DNA. Lipid peroxidation is the oxidative degradation of polyunsaturated fatty acids, involving the formation of lipid radicals and eventually membrane damage [63] and leakage of cellular enzymes into the bloodstream. Potassium bromate has been reported to induce lipid peroxidation and disrupt membrane architecture via the production of oxygen radicals during its metabolism, giving rise to oxidative stress and DNA damage [11, 64, 65]. In this study (Table 2), significant increases were observed in the levels of malondialdehyde in the kidneys in the bromate alone group, while antioxidant enzymes, catalase, superoxide dismutase glutathione peroxidase activities were reduced. Reduced glutathione concentration was also reduced.

Malondialdehyde (MDA) levels were significantly elevated in the bromate alone group when compared with the control. Administration of graded doses of *Annona muricata* leaf extract conferred enhanced function of the nephron as indicated by the significant decrease in MDA levels. However, administration of graded doses of



Annona muricata leaf extract remarkably protected the kidneys from the deleterious effects of KBrO_3 by inhibiting lipid peroxidation. This effect is in agreement with reports by Oladele *et al.* [66] and Romaidi *et al.* [67].

Endogenous antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD), also showed significant decreased activities. Treatment with *Annona muricata* leaf extract significantly increased SOD and catalase activities. Although GPX activity was observed to increase, the increase was not significant when compared with the bromate alone-treated group. This may be due to the effect of bromate on the activity of glutathione peroxidase in the kidney. Increased enzyme activity can be attributed to increased enzyme induction [68].

Reduced glutathione (GSH) concentration in the bromate alone-treated group was significantly reduced after ninety days. Administration of 300mg/kg and 450mg/kg *Annona muricata* leaf extract and bromate resulted in a significant increase in GSH concentration. The antioxidant effect of *Annona muricata* leaf extract has been reported [33, 61, 69]. *Annona muricata* leaf extract could modulate the synthesis of GSH for the detoxification of bromate radicals, due to the presence of flavonoids [25]. This is observable because flavonoids from vegetables have been demonstrated to increase intracellular concentrations of GSH for detoxification. Farombi *et al.* [64] and Myhrstads *et al.* [70] reported that KBrO_3 depleted glutathione (GSH) content in various tissues, which caused a decrease in phase II metabolizing enzymes like glutathione peroxidase (GPX) and glutathione reductase (GSR). They reported that bromate increased thiobarbituric acid reactive substances (TBARS) in tissues, caused lipid peroxidation and disrupted protein concentration. The significant decrease in endogenous antioxidants and increased MDA by bromate indicates its pro-oxidant role in rat kidneys. CAT, GPX, GST, SOD, and GSH are an armory of endogenous antioxidants that 'mop up' free radicals which are harmful to cells, hence serve as a defense against oxidative stress. The ability of the body to produce these antioxidants is regulated by genes and altered by exposure to environmental factors like chemicals and diet [71]. *Annona muricata* leaves contain a plethora of phytochemicals [72] that are potent antioxidants,

which may have contributed to the improved antioxidant activity of the extract against bromate-induced kidney oxidative stress.

Bromate has been reported to alter nephron architecture in its toxic effect. Histopathology results supported enzymatic evidence of kidney damage as revealed by the focal tubular cloudy swelling, arteriolar luminal obstruction, intimal ulceration and hyperplasia. Treatment with the graded doses of *Annona muricata* leaf extract showed fairly patent tubules with mild interstitial congestion. Findings from this study are consistent with research done by [67] who reported the efficacy of *Annona muricata* leaf extract on breast cancer cell lines. Several studies have reported the protective role of phytochemicals on kidney cells. [7] reported similar findings in this study. Other studies have also proven the protective effect of *Annona muricata* methanol leaf extract on kidney morphology in carbon tetrachloride-induced toxicity [73]. Goffart *et al.* [61] reported a similar protective effect in 7,12-dimethylbenz (a) anthracene (DMBA) intoxicated rats, while Usunobun and Okolie [74] reported positive findings in dimethylnitrosamine administered rats. *Annona muricata* leaves possess phenolics, acetogenins, catechin, epicatechin, rutin, other flavonoid glycosides, and chlorogenic acid, which have antioxidant effects. Handayani *et al.* [75] revealed renal protection by *Annona muricata* in alloxan-induced diabetic mice. In another study, the protective role of *Annona muricata* in the blood of rats challenged with 1,2-dimethylhydrazine-induced colorectal carcinogenesis was reported [76].

Haematology results in this study (Table 3) showed no significant ($p < 0.05$) difference between the control and treated groups. Although there were disturbances in the values of WBC, RBC, Hgb, MCV, PCT, Leukocytes and Granulocytes in the bromate only group, which were reversed by the extract, they were slight changes which were not statistically significant. Bromate may not have a direct effect on haematology indices; however, *Annona muricata* methanol leaf extract preserved the haematology conditions as compared with the control.

Histopathology results (Plate 1) establishes marked damage of the kidney architecture in the group treated with bromate alone. On treatment with the extract, the kidney micrograph showed mild interstitial congestion and fairly patent



tubules, indicating the protective role of *Annona muricata* methanol leaf extract. These results thus, validates the biochemical findings on the protective effect of *Annona muricata* methanol leaf extract in this study.

Conclusion

In conclusion, bromate induced impaired renal function, increased lipid peroxidation and inhibited antioxidant enzyme activities, thus increasing renal oxidative stress. *Annona muricata* leaf extract protected the kidneys from these deleterious effects of bromate by enhancing kidney function, increasing renal antioxidant enzyme activities, inhibiting lipid peroxidation and enhancing glomeruli and tubular morphology. The protection may be attributed to the numerous potent phytochemical constituents of the leaves. This study has therefore corroborated the protective role of *Annona muricata* leaf extract in the kidneys, serving as a safe, natural, affordable alternative for the management of kidney diseases. It may be harnessed as a source of natural products for drug design.

Acknowledgement

The authors acknowledge the provision of laboratory work space by Benson Idahosa University and the technical support of Mr. Derek Ahamioje, the Laboratory Technologist.

Conflict of Interest

The authors declare no conflict of interest.

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HOW TO CITE.

Okungbowa, A., & Okolie, N. (2025). *Annona muricata* Mitigates Kidney Damage in Rats following Chronic Exposure to Bromate. *KolaDaisi University Journal of Applied Sciences*, 2, 7-18. <https://doi.org/10.5281/zenodo.17428608>



