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Protective effect of methanol extract of *Annona muricata* (soursop) leaves against bromate-induced kidney and liver damage in Wistar rats

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ABSTRACT

The present study investigated the protective effect of methanol leaf extract of Annona muricata (soursop) against bromate-induced kidney and liver damage in male albino Wistar rats. After acclimatization to laboratory conditions for 7 days, 36 rats were randomly and equally assigned to 6 groups. Five groups received bromate ion at a dose of 60 mg/kg body weight (bwt) via gavage. Bromate-exposed rats in the negative control (group II) were untreated, while 3 groups of bromate-exposed rats (III, IV, and V) were co-administered graded doses of Annona muricata extract (200, 300, and 500 mg/kg bwt, respectively). Rats in the normal control (group I) received saline only, while rats in group VI were given the standard liver-protective drug silymarin at a dose of 100 mg/kg bwt. All treatments were given via orogastric tube, and all groups were permitted ad libitum access to standard rat chow and water. At the end of 21 days, the rats were sacrificed under chloroform anesthesia, and blood samples were collected through cardiothoracic puncture, after which the liver and kidneys were excised. Sections for histology were preserved in formol saline, while the remaining sections were kept deep-frozen before analysis. Serum levels of alanine transaminase (ALT), aspartate transaminase (AST), total and direct bilirubin (Tbil and Dbil), lactate dehydrogenase (LDH), creatinine, blood urea nitrogen (BUN) and electrolytes, were determined using standard biochemical procedures. Glutathione peroxidase and catalase were assayed in liver and kidney tissue homogenates. Histopathological examinations were also carried out on the liver and kidney.

The results obtained showed that bromate ion induced toxicity in the experimental animals, as indicated by significant ($p < 0.05$) elevations in serum levels of ALT, AST, Tbil and Dbil., LDH, creatinine, and BUN, especially in the negative control group (II) which received only bromate ion. Moreover, bromate induced significant decreases in tissue GPx activities. However, these changes were reversed by the leaf extract of Annona muricata to levels largely comparable with those of the standard drug silymarin, although there were no significant changes in serum electrolyte levels.

These results indicate that the methanol extract of Annona muricata exerted a protective effect against bromate-induced kidney and liver lesions in albino rats, most likely through its antioxidant potential.

Keywords: Annona muricata, Antioxidants, Potassium bromate, toxicity, Liver, Kidney, Rats.

INTRODUCTION

Over the years, food additives have been used to optimize food quality, increase shelf-life, and possibly increase the yield of certain commercial products. Potassium bromate is used in the baking industry as a dough improver in bread production, and in commercial products such as hair dyes. It acts at the late phase of the dough-making process by enhancing the viscoelastic property of the dough during the baking process, thereby increasing the volume of bread following the oxidation of sulfhydryl groups present in the gluten of flour. In addition, it is used in beauty care products (cold wave hair solutions) for oxidation of sulfur and vat dyes, and for addition to cleaning of boilers in pharmaceutical industries [1]. Moreover, it is used in cheddar making and malting, and as a component of Japanese fish paste [2, 3, 4] Oloyede and Sunmonu, 2009). Classified under group 2B probable human carcinogen, potassium bromate has been banned in many countries, including Nigeria. However, recent studies [5, 6] reveal that it is still being applied in the bakery industry at concentrations above the Food and Drug Agency (FDA) recommended level of 0.02 mg/kg. Indeed, samples of bread from Nigeria have been reported to contain as much as 0.62515 mg/kg potassium bromate.

Several studies have shown that various species of the soursop plant Annona muricata, a member of the Annonaceae family, exert various pharmacological effects i.e., cytotoxic, antimicrobial, and wound-healing properties [7]; antidiabetic and hypolipidemic effects [8]; hepatoprotective and bilirubin-lowering potential [9]; anticarcinogenic effects [10, 11]; gastroprotective effect [12]; and anticonvulsant effects [13]. Annona muricata L. is commonly known as soursop due to the sweet and sour taste of its fruit. The plant is known by other names such as Graviola (Portuguese) or guanabana (Latin American Spanish). It is an erect, terrestrial, evergreen tree that

grows to a height of 5-8 meters. Its open, roundish canopy is covered in huge, glossy, dark green leaves that produce fruit with spiky hearts [13, 14].

The leaves of soursop contain different groups of bioactive compounds called annonaceous acetogenins [15]. These substances comprise murihexocin and annocuricin [16]; annopentocin A, B and C, (2,4-cis)-annomuricin-D-one, murihexoocin A and B (2,4-trans)- annomuricin-D-one, 4-acetyl gigantetrionin, cis-gigantrionin [17]; muricatocin A, B and C [18], and annohexocin [19]. Phytochemical studies have also shown the presence of alkaloids, flavonoids, carbohydrates, proteins, tannins, saponins, phytosterols, terpenoids, and essential oils [20]. Studies have shown that extracts of the leaves are gastroprotective [21], antidiabetic and hepatoprotective [22], and antibacterial [23]. This study was carried out to investigate the protective effect of the leaf extract of the soursop plant against bromate-induced toxicity in Wistar rats.

MATERIALS AND METHODS

Experimental Rats

Sixty healthy adult male albino Wistar rats weighing between 100 and 150g were obtained from a breeder in the Department of Biochemistry, University of Benin, Benin City. The rats were housed in the Animal House of the department, and were acclimatized for 7 days before the commencement of the experiment. The rats were permitted ad libitum access to rat chow and water.

Chemicals and Kits

Potassium bromate (KBrO3), standard hepatoprotective drug (silymarin), and kits for ALT, AST, total and direct bilirubin, urea nitrogen, creatinine, and LDH (products of RANDOX, UK) were purchased from Pyrex Scientific, Benin City, Edo State. Methanol was purchased from O.G. Medicals, Warri, Delta State.

Preparation of Extract and Reagents

Preparation of Crude extract of A. Muricata

Large quantities of fresh leaves of the plant were collected from trees in household gardens in Benin City and around the University of Benin, Edo State, Nigeria. The leaves were washed and air-dried for four (4) weeks. Thereafter, the leaves were pulverized, and the resultant material (840g) was macerated in 99.5% methanol for 24 h, followed by filtration using cheesecloth. The extract obtained was concentrated in vacuo in a rotary evaporator, resulting in a viscous gel that was air-dried. The rats were weighed after acclimatization, and the gel-like crude methanolic extract obtained was weighed. A 10% Tween-80 in normal saline served as a vehicle for the extract. The weighed crude extract of the different groups was reconstituted in the Tween-80-saline solution and kept in small-capped plastic containers in a refrigerator at -4oC until used.

The rats were exposed to bromate in drinking water containing the toxin at a concentration of 0.014 g/mL in physiological saline.

Administration of extract

Six groups of rats (groups I to VI) were used in this study, with 6 rats per group. Rats in groups II to VI were given bromate at a dose of 60 mg/kg daily via oral gavage, while rats in group I served as normal controls. At the same time, groups III. IV and V were given the extract at doses of 200, 300, and 500 mg/kg via oral gavage, respectively, while rats in group VI were given silymarin via the same route. Rats in group II received bromate only, while rats in group I were untreated and served as normal controls. The treatment lasted for 21 days, and rats in each group were weighed weekly.

Biochemical Assays

At the end of treatment, the rats were sacrificed through cervical dislocation, and blood samples were collected through the retro-orbital axis. Serum total and direct bilirubin, serum transaminases (ALT and AST), serum lactate dehydrogenase, serum urea nitrogen, and serum creatinine were determined using RANDOX Kits in line with the manufacturers' instructions. Catalase activity was determined as described in [24]. Glutathione peroxidase (GPx) was determined as described in [25].

Histopathological Examinations

After the rats were sacrificed, liver and kidney tissues were excised. The fresh tissue samples were fixed in 8% formaldehyde solution in phosphate-buffered saline (PBS). After washing in PBS and dehydration in a series of alcohol dilutions and embedding in paraffin, microtome sections were cut and subjected to routine histological analysis using hematoxylin and eosin staining. The stained slides were examined under a light microscope, and photomicrographs were obtained using a Nikon research microscope (Novex, Holland) connected to the microscope.

Statistical Analysis

Data are expressed as mean ± standard deviation. Differences amongst multiple groups were analyzed using one-way analysis of variance (ANOVA). Post-hoc comparative analysis was performed using multiple comparison tests. Statistical significance was set at $p < 0.05$. All statistics were done using SPSS for Windows (version 28).

RESULTS

Effect of selected doses of AME on weight of Wistar rats given bromate

In rats given bromate only, there was a significant decrease in body weight, when compared to control rats given saline only. However, the body weights of rats given AME and bromate at doses of 300 and 500 mg/kg, and rats given silymarin were comparable to that of the control rats ($p < 0.05$). These results are shown in Table 1.

Table 1: Effect of bromate and AME on body weights of rats

Group	Mean weight (g)
I (normal saline)	36.5 ± 1.20[a]
II (BrO_3 untreated)	17.9 ± 3.02[b]
III (BrO_3 + 200mg/kg AME)	24.0 ± 1.96[b]
IV (BrO_3 + 300mg/kg AME)	27.2 ± 9.02[a]
V (BrO_3 + 500mg/kg AME)	32.7 ± 12.17[a]
VI (BrO_3 + 100mg/kg SILYMARIN)	36.6 ± 7.24 [a]

Results are mean ± SD of 6 independent measurements. Values with different superscripts differ from the normal control ($p < 0.05$).

Effect of graded doses of AME on liver function parameters

The results on effect of Annona muricata on some liver parameters are shown in 2 below, with significant increases seen in rats given only bromate ion (II), when compared to the normal control. Rats that received the toxin as well as the extract (III-V) had significantly reduced ($p<0.05$) values, when compared to the bromate-only group, thereby
indicating the protective effect of the extract against the toxin. Rats given the bromate ion as well as silymarin (VI) had results in close-range with the groups administered AME.

Table 2: Effect of methanol extract of Annona muricata on liver function parameters

	GROUPS					
Parameter	**Control**	**II**	**III**	**IV**	**V**	**VI**
ALT (mg/dL)	13.16 ±3.50[a]	32.53 ± 2.46 [b]	11.65 ±0.89[a]	5.55 ± 4.34 [a]	12.91± 10.33 [a]	5.80 ± 1.07[a]
AST (mg/dL)	7.89 ± 4.17[a]	38.77 ±14.75[b]	19.64 ± 4.89 [a]	13.33± 2.89[a]	32.28 ± 2.37 [b]	27.01± 2.64 [b]
TBIL (µmol/L)	0.35 ± 0.06[a]	0.81 ± 0.55[b]	0.72± 0.46[a]	0.71 ± 0.46[a]	0.67 ± 0.20[a]	0.54 ± 0.37[a]
DBIL (µmol/L)	0.77± 0.23[a]	0.95± 0.09[b]	0.54± 0.14[a]	0.41± 0.11[a]	0.40± 0.13[a]	0.37± 0.04[a]
LDH (U/L)	207.73± 10.7 [a]	229.74± 32.9[b]	187.09± 61.5[a]	171.96± 73.8[a]	163.70± 82.4[a]	147.19± 93.6[a]

Results are mean ± SD of six independent measurements. Values with different superscripts differ significantly ($p<0.05$) from the normal control.

Effect of graded doses of AME on kidney parameters

The results shown in Table 3 are indicative of the protective effect of Annona muricata on some kidney function panel, with significant increases observed in rats administered bromate ion only (II), relative to the normal control. Rats that received the toxin along with the extract (III-V) had significantly reduced ($p<0.05$) values when compared to the bromate-only group.

Table 3: Effect of methanol extract of Annona muricata on kidney parameters

	Groups					
Parameter	**Control**	**II**	**III**	**IV**	**V**	**VI**
BUN (mg/dL)	4.17± 0.51[a]	6.94± 1.22[b]	5.23± 0.62[a]	4.28± 1.33[a]	3.72± 0.09[a]	3.49 ± 0.42[a]
Creatinine (mg/dL)	20.56± 4.35[a]	35.16± 1.33[b]	32.50± 0.66[a]	33.17± 4.33[a]	37.18± 1.78[a]	36.58± 2.65[a]
Na^+ (mmol/L)	147.3±11.7[a]	75.2±22.4[b]	72.0±16.4[b]	76.7±23.8[b]	113.4±24.2[a]	119.3±22.9[a]
Cl^- (mmol/L)	58.7±9.7[a]	66.1±11.0[b]	62.3±2.3[b]	55.6±3.3[a]	63.0±3.4[b]	60.0±3.1[b]
K^+ (mmol/L)	5.2±1.7[a]	10.0±1.6[b]	4.9±1.5[a]	6.4±0.9[a]	8.1±1.0[b]	7.5±1.1[a]
HCO_3- (mmol/L)	37.7±5.6[a]	16.9±1.8[b]	28.7±2.0[a]	35.3±2.0[a]	70.3±1.4[b]	89.0±9.0[b]

Results are mean ± SD of six independent measurements. Values with different superscripts differ significantly ($p<0.05$) from the normal control.

Effect of graded doses of AME on antioxidant parameters of Wistar rats given bromate

Decreased activity of glutathione peroxidase (GPx) was seen in rats given bromate only (II). However, in rats administered bromate as well as the extract, the activities of GPx in liver and kidney homogenates were comparable to control values. There were no significant differences in catalase activities in liver homogenate between the bromate-treated groups and control. However, bromate caused significant decreases in kidney tissue homogenate, when compared to the control group. These results are shown in Table 4.

Table 4: Effect of methanol extract of Annona muricata on antioxidant parameters

Group	GPx		Catalase	
	Liver (U/mg protein)	Kidney (U/mg protein)	Liver (U/mg protein)	Kidney (U/mg protein)
Control	2.80±0.045[a]	2.62±0.026[a]	0.131±0.001[a]	0.137±0.006[a]
II	1.46±0.045[b]	1.45±0.006[b]	0.141±0.002[b]	0.136±0.006[a]
III	1.48±0.021[b]	1.66±0.026[b]	0.134±0.004[a]	0.132±0.006[b]
IV	1.98±0.188[a]	1.801±0.03[a]	0.122±0.003[a]	0.128±0.004[b]
V	2.45±0.055[a]	2.14±0.135[a]	0.129±0.004[a]	0.124±0.004[b]
VI	2.04±0.474[a]	2.11±0.135[a]	0.133±0.004[a]	0.127±0.002[b]

Results are mean ± SD of six different groups (p < 0.05). Values with different superscripts differ significantly (p<0.05) from the normal control.

Results of histopathology

Figure 1 shows photomicrographs of liver sections in all six groups (I-VI). The bromate exposure caused kidney and liver lesions. In the liver, there was evidence of congestion, inflammation, and fatty changes. The kidney tissue (Figure 2) had atrophied renal corpuscle and reactive glomerulus. However, these lesions were reversed dose-dependently by the *Annona muricata* extract

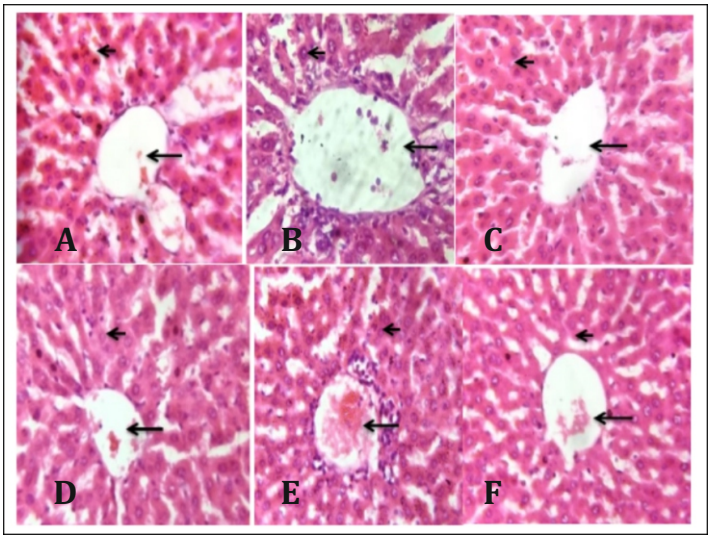

Figure 1: Photomicrographs of liver sections from all groups. (A) Liver section from control showing normal architecture, with visible centriole (long arrow) and well-fenestrated sinusoids and hepatocytes (short arrow). (B) Liver section from rats given bromate only, revealing centriole with thickened wall surrounded by mononuclear and inflammatory cells (long arrow), as well as sinusoids and hepatocytes with mild fatty changes (short arrow). (C) Liver section from rats given 200 mg/kg extract plus bromate, showing visible centriole (long arrow) and mldly dilated sinusoids. The hepatocytes reveal pyknotic nucleus (short arrow). (D) Liver section from rats given 300 mg/kg extract plus bromate showing visible centriole (long arrow) and well-fenestrated sinusoids and hepatocytes with vacuolated nucleus (short arrow). (E) Liver section from rats given 500 mg/kg extract plus bromate. There are visibly congested centrioles surrounded with inflammatory and mononuclear cells (long arrow), as well as sinusoids and hepatocytes with (short arrow) with vacuolated nucleus. (F) Liver section from rats given silymarin plus bromate. There are visible centrioles (long arrow) and well-fenestrated sinusoids and hepatocytes with vacuolated nucleus and mild fatty changes (short arrow). H & E; x400).

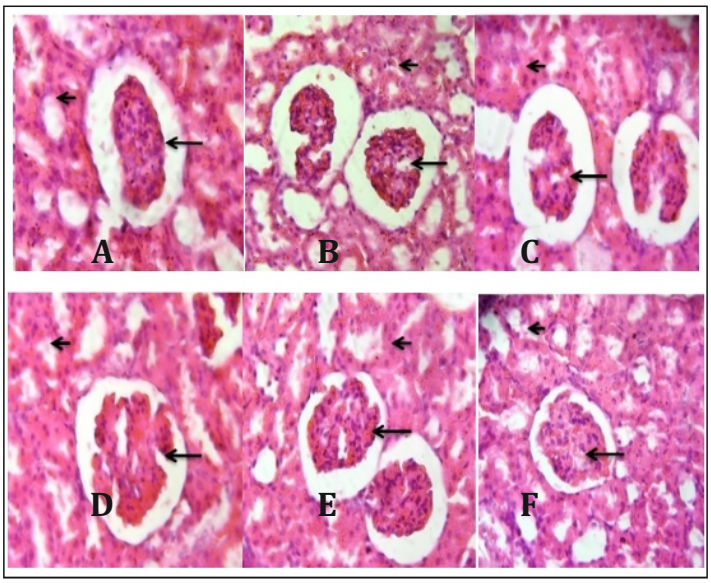

Figure 2: Photomicrographs of kidney sections from rats in all groups. (A) Kidney section from control rats showing normal features, with prominent renal corpuscle with glomerulus (long arrow) with tubules (short arrow) and interstitial. (B) Kidney section from bromate-only group revealing visible atrophied renal corpuscle and reactive glomerulus (long arrow) with dilated tubules (short arrow) and distorted interstitial cells. (C) Kidney section from rats given extract at a dose of 200 mg/kg plus bromate revealing visible renal corpuscle with mildly reactive glomerulus (long arrow) tubules (short arrow) and interstitial. (D) Kidney section from rats exposed to 300 mg extract along with bromate. There are visible renal corpuscles with mildly reactive glomerulus (long arrow) tubules (short arrow) and interstitial cells. (E) Kidney section from rats given 500 mg/kg extract plus bromate revealed visible renal corpuscle and mildly reactive glomerulus (long arrow) with tubules (short arrow) and interstitial cells.

(F) Kidney sections from rats given silymarin plus bromate showed visible renal corpuscle with glomerulus (long arrow) tubules (short arrow) and interstitial cells (H & E; x400).

DISCUSSION

The present study showed significant differences in body weight gain among the experimental groups I-VI. The rats in the normal control group (I) administered only saline increased in weight during the twenty-one (21) days of treatment. However, all other groups (II-VI) which received 60 mg/kg body weight bromate ion had significant weight losses; most especially the negative control group. However, simultaneous administration of the extract to three groups (III-V) triggered a quick recovery from the deleterious effect of bromate as a result of lipid peroxidation and membrane damage due to the generation of ROS. Group VI which was also exposed to the same amount of the bromate ion but treated with silymarin, a standard liver protection drug, was comparable with the normal control group. This indicates that silymarin protected the liver from bromate-induced damage. The bromate administration induced a significant decrease in body weight as seen in group II, when compared to the normal control group. However, following the administration of graded doses of the *Annona muricata* leaf extract, there was a marked increase in body weight, which is a demonstration of the antioxidant properties of the plant. In a similar study, [26] reported decreased body weight for the DMN-administered rats when compared with the control group, but a significant increase in the body weight of the rats was seen when treated with 400mg/kg of methanolic leaf extract of *Annona muricata*. In essence, it can be inferred that the methanolic leaf extract of *Annona muricata* reversed the toxic effect of bromate, with respect to weight loss in the rats. This is in agreement with the hepato-protective activity of *Annona muricata* leaf extract which was reflected in significant reductions in bromate-induced increases in serum transaminases (ALT and AST). Thus, the bromate-induced hepatocyte damage was mitigated which was evident from histology, was mitigated by the extract. Relative to the negative control rats which received only bromate ion, the simultaneous administration of graded doses of AME significantly reduced levels of ALT and AST in groups III, IV, and V. The reversal of increased serum enzymes in $KBrO_3$-induced hepatic injury by the extract may be due to the halting of leakage of intracellular enzymes by the membrane-stabilizing anti-oxidant property of the extract. The hepato-protective activity of *Annona muricata* may be attributed to the presence of phytochemicals such flavonoids, saponins, ascorbic acid, alkaloids, triterpenoids, tannins as well as other bioactive agents called annonaceous acetogenins which are peculiar to *Annona muricata*. Thus, the effect of the extract is made obvious as there was a restoration of membrane integrity and regeneration of hepatocytes [27]. The presence of flavonoids in the extract may be responsible for the antioxidant effects and its hepatoprotective activity. Increased serum levels of aspartate aminotransferase (AST) are seen in disorders such as viral hepatitis and cardiac infarction as well as in myocyte damage. The side-by-side administration of the plant extract at three graded- doses (200, 300 and 500 mg/kg) along with bromate resulted in the protection of hepatocytes against bromate-induced damage. The decreases in serum transaminase levels were higher in group IV treated with 300 mg/kg body weight of the extract than in groups III and V which received 200 and 500 mg/kg body weight AME, respectively, indicating a partly dose-dependent effect of the extract. An earlier study by [28] showed bromate-induced increases in serum transaminases were reduced by administration of methanolic extract of *Portulaca oleracea.* Decreased levels of serum transaminases suggests stabilization of plasma membrane integrity and protection of hepatocytes against damage caused by the hepatotoxin. Raised levels of serum enzymes are indicative of cellular damage and loss of the functional integrity of the hepatocyte membrane. Moreover, a study [29] revealed that *Annona muricata* ameliorated toxicity induced by carboplatin in male albino Wistar rats. The present study also investigated the bilirubin clearance levels of the extract. The results showed that bromate induced significant increases in bilirubin levels, relative to the normal control group. However, the extract, brought about dose-dependent decreases in bilirubin levels. Moreover, group VI treated with silymarin recorded low bilirubin levels, thereby confirming its efficacy as a hepatoprotective agent. The dose-dependent decrease in bilirubin is in agreement with the decreases in activities of serum transaminases, as well as liver histology. These changes could be ascribed to the antioxidant activity of the plant. Previous studies have reported that Annona muricata lowered bilirubin levels in carboplatin-induced oxidative damage in DMN-treated rats [26, 29], and in bromate-exposed rats treated with Portula oleracea [29]. Thus, the antioxidants and other components present in the leaf extract neutralized the ROS generated by the bromate toxin, but not as much as the standard drug. Significant increases were observed in the serum levels of lactate dehydrogenase in the groups (II-VI) administered bromate ion, when compared to the normal control group. This is a consequence of the oxidative stress induced by bromate ion. However, side-by-side treatment with the extract significantly reduced the hitherto elevated levels of lactate dehydrogenase in a dose-dependent pattern thus, bringing the levels of lactate dehydrogenase to near normal. Increased activity of lactate dehydrogenase is indicative of conditions such as kidney damage and liver inflammation which were seen in the results from histology.

Serum levels of blood urea nitrogen (BUN) were significantly elevated in bromate-treated groups, when compared to the normal control group. This is consistent with the findings of an earlier study which showed that bromate induced significant increases in BUN [30]. In this study, treatment with graded doses of the extract effectively protected the rats against BrO_3-induced nephrotoxicity as seen by the significant decreases in BUN levels, when compared to rats treated with $KBrO_3$ alone. This corroborates the report in a previous investigation which showed that the significant rise in serum urea may be a sign of renal impairment since the kidneys are unable to eliminate them [31]. Thus, treatment with the extract drastically reduced serum urea, thereby improving renal function. This opens up the possibility that *A. muricata* extracts might offer tissues a form of protection, consistent with a previous finding [32]. Moreover, administration of $KBrO_3$ resulted in a significant increase in serum levels of creatinine (Cr) when compared to the normal control group. A similar result was also obtained in a previous study which reported the combined toxic effects of potassium bromate and sodium nitrite in some key renal markers in male Wistar rats [33]. However, side-by-side treatment with extract produced no significant differences in levels of serum creatinine among the treatment groups (II-VI), when compared to the negative control group. This could most

probably be a consequence of irreversible damage on the nephrons of the kidney. Moreover, blood electrolyte (Cl and K) levels were significantly elevated in treatment groups when compared to the normal control group. In contrast, sodium and bicarbonate levels were decreased in the group administered bromate alone, when compared to the normal control group. Administration of graded doses of *Annona muricata* had no significant effect on the levels of Cl, HCO_3, and Na^+. These results are in agreement with the lesions seen in the kidney sections of the bromate-treated rats, and their mitigation by extract.

There was a significant decrease in GPx activity in group II which received only bromate. This was probably a reflection of increased levels of ROS and oxidative stress induced by bromate. However, following the administration of the extract in graded doses, GPx levels increased significantly in the treatment groups in a dose-dependent pattern, indicating that the extract increased levels of antioxidants in the treated groups. No significant change was observed in catalase activity.

Histopathological examinations of the liver revealed that the administration of potassium bromate caused intense damage to liver tissue architecture, as seen in the photomicrograph of the sections. Distortion in tissue architecture, congestion of the central vein, and sinusoidal dilatation as well as cell necrosis were recorded in the treatment groups (II-VI) These findings are in agreement with reports of congestion, hemorrhage, and degenerative changes in liver of Wistar rats exposed to potassium bromate [34], as well as potassium bromate-induced congestion of the central vein in the hepatocytes and infiltration of the interstitial cells [35]. In the present study, the lower degree of liver damage seen in the photomicrograph of the sections from groups III-V may be related to the protective effect of the extract against bromate-induced free radical damage due to its antioxidant- properties. This agrees with the earlier report in which it was demonstrated that *Annona muricata* leaf and fruit extracts exerted hepatic and renal protective effects on Ehrlich Ascites carcinoma in mice [36]. In the present investigation, the results of histological examination of the liver tissue were consistent with the pattern of changes in serum levels of liver marker enzymes.

In kidney photomicrographs, the extract-treated groups (III-V) showed mildly reactive glomerulus, when compared to the negative control group (II) which received bromate only. This result is consistent with the levels of kidney damage markers such as creatinine and urea nitrogen which were observed to be significantly elevated in the negative control group, when compared to the normal control group (1). This indicates that the *Annona muricata* leaf extract protected the kidney from bromate-induced damage, due probably to the presence of tannins, saponins, flavonoids, and glycosides which have been shown to aid in the treatment of kidney diseases [36].

CONCLUSION

The results obtained from this investigation indicate that methanolic leaf extract of Annona muricata exerted a fairly dose-dependent protective effect against bromate-induced kidney and liver damage in Wistar rats through its antioxidant properties. In some cases, the protective effect was comparable to that produced by the standard hepatoprotective drug, silymarin. These findings underscore the potential benefits of Annona muricata in protecting the kidney and liver from the toxic effects of bromate.

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