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Methanol extract of *Euphorbia heterophylla* leaf ameliorate liver and kidney damage in rats administered with potassium bromate

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ABSTRACT

The study aimed to investigate the ameliorative capacity of methanol extract of Euphorbia heterophylla leaves (ELE) on liver and kidney functionality in the presence of potassium bromate ($KBrO_3$). Twenty (20) male albino rats weighing between 180 – 200 g were assigned randomly into four groups. Group 1 served as the control and received only distilled water. Group 2 was administered 20 mg $KBrO_3$/kg body weight. Group 3 received 100 mg ELE/kg body weight, while group 4 rats were administered concurrently; 20 mg $KBrO_3$ and 100mg ELE/kg body weight. The administration lasted for 21 days. Animals were allowed free access to water and feed ad-libitum. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) tests were employed for antioxidant assay. Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), and Alanine Transaminase (ALT) activities were assayed alongside albumin, bilirubin (T and D) as a measure of liver function. Catalase and Malondialdehyde (MDA) concentrations were monitored in the liver for possible peroxidation. Creatinine, urea, uric acid and the electrolytes (Na^+, K^+, Cl^-) concentrations were determined to measure kidney function. The extract demonstrated significant antioxidant activity with DPPH and FRAP assays. IC_{50} for DPPH was 255.63 µg/mL and 129.69 µg/mL for vitamin C with FRAP value of 1.58 ± 0.26 against 2.00 ± 0.00 for Vitamin C. Generally, the assay results showed significant increases in all parameters except catalase, when bromate was administered and results compared with the control. Following the co-administration of bromate and the extract, the enzyme activities (ALP, AST, and ALT) were significantly lowered. However, a significant increase in catalase activities in the liver, MDA concentration was significantly lowered in the tissue. The significantly high concentration of albumin, bilirubin (D and T), urea, creatinine and uric acid were ameliorated when bromate and the extract were administered. The changes in the electrolytes (Na^+, K^+, Cl^-) concentrations were also reversed. ELE exhibited significant ameliorative effects in bromate-induced damage in rats' liver and kidney tissues.

Keywords: *Euphorbia heterophylla, Methanol extract, Liver Damage, Potassium bromate, Administration*

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INTRODUCTION

Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, is a crucial factor in the pathogenesis of various diseases, including liver and kidney damage. ROS, such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are naturally produced during normal metabolic processes. However, when produced in excess, ROS can lead to cellular damage by reacting with lipids, proteins, and DNA, resulting in lipid peroxidation, enzyme inactivation, and genetic mutations (Maes *et al.*, 2018).

Potassium bromate ($KBrO_3$) is a potent oxidizing agent widely used as a food additive and in water disinfection. However, its toxicological profile indicates that it can cause severe oxidative damage, particularly to the liver and kidneys, when ingested (Oseni *et al*., 2015). Studies have shown that $KBrO_3$ induces nephrotoxicity and hepatotoxicity by enhancing lipid peroxidation, depleting antioxidant enzymes, and causing DNA damage, leading to cell death (Dimkpa *et al*., 2015). The oxidative damage mediated by $KBrO_3$ is linked to the generation of free radicals that overwhelm the body's natural antioxidant defenses, resulting in significant cellular and tissue injury (Andrzej and Beata, 2016; Venu-Shanmu *et al*., 2019).
Bromate is a cytotoxic agent whose molecular and cellular mechanism of toxicity was investigated in normal rat kidneys (NRK) cells and human embryonic kidney (HEK) cells. The study revealed that cellular responses to bromate occur at a dosage of 2.4mM and 4.8mM for 24hr and 48hr (Zhang, 2010). This event resulted in the formation of chromatin and nuclear condensation that led to necrosis (Zhang, 2010).

Given the widespread use of potassium bromate and its potential health risks, there is a critical need to identify effective strategies to mitigate its toxic effects, particularly through the use of natural antioxidants. *Euphorbia heterophylla*, a plant widely distributed in tropical and temperate regions is commonly known as spurge weed or desert milkweed, and belongs to the family *Euphorbiaceae*. It's been traditionally used for the treatment of various ailments. The plant possesses a characteristic milky latex, which is utilized in traditional medicine as a purgative, diuretic, and treatment for conditions such as constipation, gonorrhea, and malaria (Unekwe *et al*., 2006). The leaves of *E. heterophylla* are rich in phytochemicals, including flavonoids, alkaloids, tannins, and sterols, which contribute to its pharmacological activities (Okeniyi *et al*., 2013; Ughachukwu *et al*., 2014).

Several studies have reported the antioxidant, anti-inflammatory, and antibacterial properties of *E. heterophylla*, attributing these effects to its diverse phytochemical composition. The plant's antioxidant properties are particularly noteworthy, as they play a significant role in scavenging free radicals, thereby protecting cells from oxidative damage (Falodun and Agbakwuru, 2004). These properties make *E. heterophylla* a potential candidate for mitigating oxidative stress-related damage in vital organs such as the liver and kidneys. The increasing concern over the toxic effects of potassium bromate, resulting from its ability to induce oxidative stress leading to liver and kidney damage, necessitates the exploration of natural remedies with antioxidant potential. While chemical agents have been studied for their capacity to counteract bromate-induced oxidative damage, there remains a significant gap in the use of medicinal plants for this purpose (Olajide *et al*., 2016). However some medical plants have been reported to exhibit an inhibitory effect on the inducement of oxidative stress on animal tissues (Andrzej and Beata, 2016). Both the liver and kidney are organs involved in secretory and excretory functions to which bromate has been reported to cause damage. Therefore, the reported toxic effects of bromate on both organs and the growing evidence of its pharmacological benefits have aroused the interest of the authors in the study on *E. heteropylla* leaf extract to explore its contribution to the understanding of how the plant could be harnessed as a therapeutic agent to mitigate the harmful effect of oxidative stress particularly in the context of exposure to environmental and dietary sources of toxins such as Potassium bromate.

Euphorbia heterophylla like other species such as *E. hirta*, been reported to be a natural source of antioxidants, capable of neutralizing free radicals and protecting tissues from oxidative injury (Ghosh *et al*., 2019). Given its traditional use and the growing evidence of its pharmacological benefits, it is imperative to investigate its protective effects of *E. heterophylla* against potassium bromate-induced toxicity in rats. This study aims to contribute to understanding how *E. heterophylla* can be harnessed as a therapeutic agent to mitigate the harmful effects of oxidative stress, particularly in the context of exposure to environmental and dietary toxins like potassium bromate.

MATERIALS AND METHODS

Plant material

Matured leaves of *E. heterophylla* was collected from the Prince Abubakar Audu University, campus located in Anyigba, Dekina Local Government, Kogi State, Latitude 7º 48' 59" N and Longitude 7º 18' 74". The plant was identified and authenticated based on its morphological characteristics as described by Burkill (1994, 2002).

Experimental animals

A total of twenty (20) male albino wistar rats (*Rattus norvegicus*) of average weight 200 ± 4.5 g were obtained from the University of Nigeria, Nsukka, Nigeria. They were kept in aluminum metabolic cages at the animal care unit of the Department of Biochemistry, Prince Abubakar Audu University, Anyigba for two weeks for acclimatization in line with the institution's Animal Ethics Committee on the care and use of animals. The environmental specifics (ventilated room, 12hr light/12hr darkness) with relative humidity 45 – 60 % at 26 ± 3 ºC were strictly observed.

Assay Kits

Analytical kits for alkaline phosphatase, ALP EC.3.1.3.1, Aspartate amino transferase, ALT EC.2.6.1.1 and Alanine amino transferase, EC.2.6.1.2 were purchased from Randox Laboratories Ltd, United Kingdom. Assay kits for organ functional tests (Urea, Creatinine, Uric acid, the

electrolytes albumin and bilirubin (total and direct) were products of Agape Diagnostics, Switzerland.

Bromate

Labtech Chemical Nigeria, Lagos supplied the potassium bromate, CAS No. 7758 – 01 – 2.
Methanol: Methanol used for the extraction was a product of Sasol Chemical Industries Ltd, 50 Katherine Street, Johannesburg, 2090, South Africa. All other reagents are of analytical grade and were prepared in glassware with distilled water, and then stored in reagent bottles until required.

Methods

Preparation of plant extract

Matured leaves of *E. heterophylla* were washed and air-dried. When fully dried, the leaves were pulverized into powder using Warring blender. The pulverized leaves were soaked in high grade 95% methanol in ratio 1:3 w/v for 36 hours. The mixture was filtered using Whatman filter paper, of grade 1. The residue was collected and the filtrate evaporated in a rotary evaporator followed by drying using a water bath. The extract was collected and further dried between filter paper and was stored in a sealed, clean tube until required for other analysis (Wadankar *et al*., 2022).

***In-vitro* Analysis**

Determination of Antioxidant Property of *E. heterophylla*

The free radical scavenging properties of the leaf extract was determined using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazylhydrate) according to the method of (Arogba and Omede, 2012), and the Ferric Reducing Antioxidant Power, (FRAP) method as described by (Arogba, 2015).

Bioassay

Animal grouping and administration of extract

The twenty rats used for the study were divided into four groups randomly such that each group consisted five rats. Potassium bromate ($KBrO_3$) and *Euphorbia heterophylla* extract (Eu-hp) solutions were prepared with distilled water to provide the required concentrations. The solutions were administered orally to the rats in a single daily dose as indicated below. Rats in group 1 received distilled water only and served as control. Group 2 rats were administered 20 mg $KBrO_3$/kg body weight of rats. Group 3 rats were administered with 100mg extract of Eu-hp/kg body weight of rat, while group 4 rats received 20 mg $KBrO_3$/kg body weight and 100 mg extract of Eu-hp/kg body weight of rats concurrently. The administration of the substances lasted 21 days.

Preparation of tissue homogenate and serum sample

Twenty-four (24) hours after the final dose (day 21), the rats were fasted overnight. Thereafter they were subjected to ether anaesthesia. At a subconscious state, the rats were brought out of the desiccator for blood collection. Blood was collected into clean, dry sample bottles, by cardiac puncture. The blood was left for 10 minutes at room temperature to clot. This was centrifuged at 4000 rpm for 30 minutes (Akanji *et al*., 2008) using refrigerated centrifuge (Heraus – Christ GMBH Osterode). Using Pasteur pipettes, sera were collected by aspiration into clean, dry sample bottles. The sera were stored in the refrigerator until required for use, which did not exceed 12 hours after the collection of sera (Yakubu and Musa, 2012). A portion of the liver was cut, washed and weighed. 1 g of the liver tissue was suspended in 10 mL, 0.1 M phosphate buffer (pH 7.4), and homogenized using Tissue homogenizer. The homogenate was centrifuged in order to collect the post mitochondrial supernatant for the determination of enzymic antioxidant.

Determination of biochemical parameters

The biochemical parameters and their methods of determination are as follows;
Alanine and Aspartate aminotransferases (Reitman and Frankel, 1957), Alkaline phosphatase (Tiez *et al*., 1983), Urea (Fawcett and Scout, 1960), Creatinine (Bartel and Bohmer, 1973), Uric acid (Shaoshi *et al*., 2019), while bilirubin total and direct (Malloy, 1938) and the electrolytes by Trinder (1951) and Schales and Schales (1941). Catalase activity was measured by the method (Beers and Sizer, 1952).

Statistical analysis

The statistical analysis was conducted using the statistical package for social sciences (SPSS) IBM version 20. Results were presented as mean ± standard deviation. Separation of mean was conducted for test of significance at $p < 0.05$. Values with $p < 0.05$ were considered statistically significant and are denoted by different alphabets (Yakubu and Musa, 2012).

RESULTS

Yield of extract (%)

The methanol leaf extract of *E. heterophylla* yielded 9.35 %

GC-MS fingerprinting:

The GC-MS results showed that the methanol extract of *E.*

Table 1: Gas Chromatography tandem Mass Spectrometry (GC-MS) fingerprinting of methanol extract of *Euphorbia heterophylla* leaf.

Peak no	RT (mins)	Name of compound	Class of compound	Molecular formula	Molecular weight
1	5798	Isothiazole	Aromatic	$C_3H_3N_5$	85.12 g/mol
2	18:437	Phenol	Aromatic	C_6H_5OH	94.11 g/mol
3	29.756	Butanamide	Fatty amide	$C_3H_7CONH_2$	87.02 g/mol
4	32.750	Propiolic acid, succinic	Carboxylic acids	$C_3H_6O_2$, $C_4H_6O_4$	74.05, 118.09 g/mol
5	39.70	Methyl ester	Fatty acid	$C_{14}H_{28}O_2$	228.37 g/mol
6	40.922	Hexenedithioic aicd	Carboxylic acid	$C_6H_{10}O_4$	146.12 g/mol
7	42.597	2,5-dichloro-1- cyclohexanol	Alkanol	$C_6H_{10}Cl_2O$	169.05 g/mol
8	42,800	1 H-phenalen-1-one	Aromatic	$C_{13}H_{10}$	166.22 g/mol
9	43.206	Butyric acid	Carboxylic acid	C_3H_7COOH	88.11 g/mol
10	44.577	Acetamiprid	Aromatic	$C_{10}H_{11}ClN_4$	222.68 g/mol
11	61.428	Phenanthrene	Aromatic	$C_{14}H_{10}$	173.23 g/mol

Table 2: Antioxidant activity of the extract using DPPH technique.

Conc. (µg/mL)	Extract (% inhibition)	Vitamin C (% inhibition)
3,200	88.0 ± 3.07	96.6 ± 4.01
1600	76.0 ± 2.50	87.0 ± 2.35
800	65.1 ± 1.64	75.0 ± 4.70
400	60.2 ± 3.01	71.1 ± 4.06
200	47.2 ± 0.48	64.0 ± 3.02
100	43.0 ± 1.70	47.0 ± 1.31
50	22.3 ± 1.10	30.2 ± 1.20
25	10.2 ± 0.24	21.1 ± 020
IC_{50}	255.63^b	129.69^a

Values are expressed as the Mean ± standard deviation of three determinations. Values with the same superscriptss on the same row are not significantly different (p > 0.05)

heterophylla leaves contained more of aromatic compounds than amide and alkanoic compounds. Six out of the eleven principal compounds identified were aromatic compounds including the phenols (Table 1).

Antioxidant activity measurement

The DPPH technique

The IC_{50} of *E. heterophylla* leaf extracts was significantly high ($p > 0.05$) when compared with the standard (Vitamin C) (Table 2).

The FRAP technique

The result of FRAP of *E. heterophylla* was significantly lower ($p < 0.05$) than that of the standard (Vitamin C) (Table 3). Administration of $KBrO_3$ caused a significant ($p < 0.05$) increase in the activities of ALP, AST and ALT in the serum of the rats compared with the control (Table 4). However, the concurrent administration of $KBrO_3$ and *E. heterophylla* and administration of the extract alone significantly ($p < 0.05$) ameliorated and tended to bring the induced alterations in the enzymes activities towards the control values (Table 4).

The concentrations of albumin, total and direct bilirubin were significantly ($p < 0.05$) elevated under the administration of bromate (Table 5). However, the various concentrations were insignificantly ($p < 0.05$) brought towards the control and even the bromate-alone group (Table 5). Table 6 depict the influence of the extract on the kidney functional parameters following the administration of potassium bromate. Creatinine, urea and uric acid were all significantly ($p < 0.05$) raised following the administration of bromate. There was however, a significant ($p < 0.05$) reduction in the concentration of creatinine but an insignificant ($p < 0.05$) decrease in urea and uric acid concentration.

Table 7 represents the effect of bromate and its co-administration on the antioxidant enzyme, catalase, and malonedialdehyde produced in the liver of the animals. Catalase activity was significantly ($p < 0.05$) decreased in the liver under the administration of bromate but significantly ($p < 0.05$) elevated with the co-administration of bromate and the extracts. However, the level of malondialdehyde was significantly ($p < 0.05$) higher in the bromate group than in other groups. The serum concentrations of the electrolytes (Na^+, K^+, and Cl^-) in rats administered potassium bromate ($KBrO_3$), extract of *Euphorbia heterophylla* leaf, and their combination are presented in (Table 8). Administration of $KBrO_3$ alone resulted in a significant ($p < 0.05$) elevation in the levels of Na^+, K^+, and Cl^- compared to the control group. Although, treatment with the plant extract alone did not significantly alter the electrolyte levels relative to the control. However, co-administration of $KBrO_3$ with the *E. heterophylla* extract resulted in a partial, though statistically insignificant ($p >$

Table 3: Antioxidant activity of the samples using FRAP technique.

Sample	FRAP value
Extract	1.58 ± 0.26[a]
Vitamin C	2.00 ± 0.00[b]

Values are expressed as Mean Standard deviation of three determinations. Values with the different superscript in the same column are significantly different at p > 0.05

Table 4: Serum ALP, AST and ALT activity of rats administered with $KBrO_3$, extract of *E. heterophylla* leaf and their combination (IU/L).

Group	ALP	AST	ALT
Control	52.73 ± 0.08[a]	34.11 ± 0.71[b]	25.23 ± 0.88[a]
$KBrO_3$	60.60 ± 0.08[d]	48.65 ± 0.34[c]	30.70 ± 1.16[b]
Extract	53.05 ± 0.10[b]	30.55 ± 0.75[a]	25.62 ± 0.88[b]
$KBrO_3$ + Extract	55.24 ± 0.22[c]	33.95 ± 0.73[b]	26.10 ± 0.87[c]

Values are expressed as the Mean Standard deviation of three determinations. Values with different superscript in the same column are significantly different at p > 0.05

Table 5: Serum Albumin, total bilirubin and direct bilirubin concentration of rats administered with $KBrO_3$, extract of *E. heterophylla* leaf and their combination.

Group	Albumin g/dL	Total Bil. mg/dL	Direct Bil. mg/dL
Control	3.15 ± 0.31[d]	1.22 ± 0.05[a]	0.97 ± 0.04[a]
$KBrO_3$	3.81 ± 0.24[a]	1.61 ± 0.04[d]	1.42 ± 0.01[c]
Extract	3.05 ± 0.24[c]	1.30 ± 0.04[b]	1.03 ± 0.04[a]
$KBrO_3$ + Extract	3.51 ± 0.09[b]	1.49 ± 0.03[c]	1.29 ± 0.04[b]

Values are expressed as Mean Standard deviation of three determinations. Values with same alphabet on the same column are not significantly different at p > 0.05.

Table 6: Creatinine, urea and uric acid concentration of rats administered with $KBrO_3$, extract of *E. heterophylla* leaf and their combination.

Group	Creatinine (g/dL)	Urea (g/dL)	Uric acid (g/dL)
Control	2.08 ± 0.09[a]	17.38 ± 0.70[a]	3.43 ± 0.51[a]
$KBrO_3$	2.55 ± 0.08[b]	26.10 ± 0.34[d]	7.35 ± 0.81[d]
Extract	1.94 ± 0.15[c]	21.05 ± 0.36[b]	4.03 ± 0.16[b]
$KBrO_3$ + Extract	2.14 ± 0.26[b]	21.85 ± 0.30[c]	5.96 ± 0.14[c]

Values are expressed as the Mean Standard deviation of three determinations. Values with different superscript in the same column are significantly different at p > 0.05

Table 7: Serum catalase and lipid peroxides levels of rats administered with $KBrO_3$, extract of *E. heterophylla* leaf and their combination.

Group	Catalase (U/mg tissue)	Lipid peroxides (nmoles of MDA/g tissue) malondialdehyde
Control	10.20 ± 0.70[a]	1.03 ± 0.30[a]
$KBrO_3$	8.43 ± 0.31[b]	2.63 ± 0.03[d]
Extract	9.22 ± 0.25[a]	1.18 ± 0.31[b]
$KBrO_3$ + Extract	9.47 ± 0.37[a]	1.35 ± 0.27[c]

Values are expressed as Mean Standard deviation of three determinations. Values with different superscripts in the same column are significantly different at p > 0.05

Table 8: Serum Na^+, K^+ and Cl^- of rats administered with $KBrO_3$, extract of *E. heterophylla* leaf and their combination.

	Na^+(mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
Control	152.22 ± 3.80[b]	4.97 ± 0.41[a]	55.08 ± 3.33[a]
$KBrO_3$	198.23 ± 5.01[c]	8.25 ± 0.21[b]	65.02 ± 3.29[c]
Extract	145.36 ± 3.72[a]	4.79 ± 0.23[a]	56.84 ± 3.17[a]
$KBrO_3$ + Extract	148.04 ± 3.22[a]	5.11 ± 0.22[a]	61.62 ± 3.43[b]

Values are expressed as the Mean Standard deviation of three determinations. Values with different superscript in the same column are significantly different at p > 0.05

0.05), restoration of electrolyte levels compared to the $KBrO_3$ group.

DISCUSSION

As a member of the chemical biocides, potassium bromate, like sodium metabisulphite (both food preservatives) is a strong oxidizing agent due to its high oxygen contents. Their high oxidizing power has implicated them as potent free radical generators capable of causing damage to cells. The results of this study like other previous work have proved the capacity of bromate to produce reactive oxygen species which are free radicals responsible for such cellular macromolecular damages. The peroxidation results served as a proof.

The DPPH and FRAP assays conducted in this study on *E. heterophylla* has demonstrated its antioxidant activity. This was expressed in the result of *in vivo* antioxidant assay by monitoring the activity of catalase in the liver of rats. The presence of phytoconstituents such as flavonoids in *E. heterophylla* must be responsible for the ability of the plant to scavenge free radicals. From the DPPH and FRAP analysis between the standard vitamin C and the plant (FRAP: Extract 1.58 ± 0.26; Vit C; 2.00 ± 0.00, IC_{50} Ext 255.63; Vit C 129.69). The results showed that methanolic leaf extracts of *E. hetrophylla* demonstrated a significant antioxidant activity high enough to fight the free radicals produced by the administration of bromate into rats. This is in tandem with Ghosh *et al*. (2019) on *E. heterophylla*, a member of the *Euphorbia* class.

Both the kidneys and liver are soft-tissued organs prone to all forms of metabolically chemical assaults that can negatively impact their regulatory activities. Therefore, enzyme assay, a rapid and fastest diagnostic indicator of cellular damage, was employed in monitoring the effect of bromate and the role of *E. heterophylla* in combating these effects.

The GC-MS analysis was employed to reveal the various classes of compounds in *E. heterophylla* leaf extract. The method is a rapid and reliable means of detecting the chemical constituents of plant parts. The biological property of such compounds dictate their biochemical functions (Karki, 2020). Predominant among the classes of the compounds are the aromatics to which the observed antioxidant potential of the plant may be ascribed e.g the phenolics.

Studies by Olajide et al. (2014, 2016), Akaoji et al. (2008), and Kurokawa et al. (1990) have highlighted bromate's harmful impact on cell membranes, causing enzymes to escape from their normal locations. On the other hand, *Euphorbia heterophylla* leaf extract appears to counteract these effects and restore cell stability. Elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels serve as crucial indicators of liver cell damage. Bromate administration has been linked to significantly increased serum levels of alkaline phosphatase (ALP), ALT, and AST, suggesting membrane damage and necrosis. This could be due to impaired membrane permeability, allowing liver enzymes to enter the serum, as supported by Adefegha et al. (2015).

Administration of the extract concurrently with $KBrO_3$ caused a decrease in the activities of these enzymes an indication that the extract has hepato- and nephron-protective potential; this was in agreement with the report of Igwe *et al*. (2019) and Augustine *et al*. (2013) that *E. heterophylla* is not hepatotoxic. The plant extract probably had been able to redeem the restoration of both the synthetic, regulatory and excretory roles of these organs. The ability of *E. heterophylla* to ameliorate the induced hepatotoxicity could be due to the antioxidant and anti-inflammatory activities of the plant extract (Yakubu *et al*., 2013; Ghosh *et al*., 2019). The significantly high (urea, creatine, uric acid) concentrations of the liver and kidney function markers in serum when bromate was administered confirmed damage to these organs. However, the administration of the extracts reversed the phenomenon leading to a significant reduction in the concentration values. The liver plays significant roles in the synthetic and secretory apparatus for protein metabolism, therefore, a significant concentration increase in albumin under bromate administration compared with the control may be a direct correlation with the effect of bromate on the liver cells.

The concentration of serum urea and creatine are usually employed for the estimation of renal glomerular function (Chawla, 2003). Increased serum urea concentration is an indication of reduced glomerular filtration rate in rats under study (Satarug and More, 2004). Chawla (2003) reported the association between elevated levels of serum urea and creatine and kidney disease. In this study, the methanol leaf extract of *E. heterophylla* showed an ameliorating capacity against the toxic effect of bromate in the kidney. This was evident by the significant decrease in creatinine and urea levels in the co-administration of bromate and *E. heterophylla* compared with the bromate-alone group.

High concentration levels of serum electrolytes (Na^+, K^+ and Cl^-) as shown in Table 8, is an indication of adverse implication on the reabsorption of the electrolytes at the tubules. This will undoubtedly affect the renal blood flow as well as the glomerular filtration rate (Ojo *et al*., 2014). Elevated uric acid concentration indicates kidney disease or cancer. Maybe the kidney could not eliminate the uric acid efficiently. *E. heterophylla* reversed the observation from the bromate group. The free radicals generated by bromate in the system of rats are activated causing them to bind covalently to the macromolecular entities in the cells and induce peroxidation of membrane lipids ER rich in polyunsaturated fatty acids (PUFAs). This results in the formation of lipid peroxides, a major cause of hepatotoxicity by oxidative compounds. Elevated values of lipid peroxides are an indication of lipid peroxidation which results in damage to liver cells and failure of the antioxidant defense system to inhibit the formation of excessive free

radicals (Achiliya *et al*., 2004; Ayeni *et al*., 2016). Results from this study shows the *E. heterophylla* was able to curtail or prevent this mechanism of peroxidation from its antioxidant properties. This is also evident in the levels of catalase as shown in Table 7.

The wide range of phytochemical constituents e.g flavonoids of *E. spp* has associated many properties, such as antioxidant, anti-inflammatory, anti-tumor, anti-microbial and anti-fungal properties on this class of plants, to which *E. heterophylla* belongs. The results of this study have supported the antioxidant claim. *E. heterophylla* was able to ameliorate the damaging effect of bromate, through its antioxidant and hepato-protective activities. The protective effects of the extracts on liver and kidney functions were significant as evidenced by the reduction in serum enzyme levels caused by bromate and the improvement in other biochemical markers. Therefore, the findings showed that *E. heterophylla* has the potential to remedy oxidative stress caused by the administration of bromate into rats.

Conclusion

This study has demonstrated that *Euphorbia heterophylla* methanolic leaf extract possesses significant antioxidant and protective properties capable of mitigating bromate-induced oxidative stress and tissue damage in rats. The extract exhibited strong radical-scavenging activities as shown in both DPPH and FRAP assays and enhanced catalase activity *in vivo*, supporting its antioxidant potential. GC-MS analysis confirmed the presence of biologically active compounds, particularly phenolics and flavonoids, which likely contributed to the observed effects. Furthermore, co-administration of the extract with potassium bromate significantly reduced the elevated levels of biochemical markers associated with liver and kidney dysfunction, including ALT, AST, ALP, urea, creatinine, and uric acid, as well as lipid peroxidation levels. This suggests that *E. heterophylla* offers both hepatoprotective and nephroprotective benefits. The results support the ethnopharmacological use of this plant and provide scientific evidence for its therapeutic potential in the management of oxidative stress-related disorders.

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