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Theobroma cacao fortified-feed ameliorates potassium bromate-induced oxidative damage in male Wistar rat

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ABSTRACT

Some therapeutic and beneficial health properties of the *Theobroma cacao* leaf have been documented. This study evaluated the ameliorative effect of *Theobroma cacao-fortified* feed against potassium bromate-induced oxidative damage in male Wistar rats. Thirty rats were randomly grouped into A-E. Except for E (the negative control), the rats in the other groups were administered 0.5 ml of 10 mg/kg body weight of potassium bromate daily using oral gavage and then allowed access to feed and water ad libitum. Groups B, C, and D were fed with 10 %, 20 %, and 30 % leaf-fortified feed respectively, while the negative and positive control (A) was fed with commercial feed. The treatment was carried out consecutively for fourteen days. In the liver and kidney, there was a significant increase ($p < 0.05$) in total protein concentration, a significant decrease ($P < 0.05$) in MDA level, and SOD activity in the fortified feed group compared to the positive control. Furthermore, in the serum, there was a significant increase ($p < 0.05$) in the albumin concentration, and ALT activity, and a significant decrease ($p < 0.05$) in urea concentration in the fortified feed groups compared to the positive control. The histopathology of the liver and kidney in the treated groups showed moderate cell degeneration compared to the positive control group. Antioxidant activity due to the presence of flavonoids and metal chelating activity of fiber in *Theobroma cacao* leaf could be responsible for the ameliorative effect of the fortified feed against potassium bromate-induced oxidative damage.

1. Introduction

The deleterious effect of potassium bromate (PB) lead to a ban on its use in some countries across the globe, especially in Nigeria where it is used mainly in bakery industries. Potassium bromate is crystalline salt that has no color or odor and is soluble in polar solvents. The use of potassium bromate has been reported in food processing, cosmetic, and pharmaceutical products due to its beneficial oxidizing property [1,2]. Despite its ban by FAO/WHO as an additive to flour, it is popularly found in many bakery products, cheese, beer, and fish paste processing factories. Among global producers of potassium bromate, China came ahead of the other known nations which include Italy, Israel, Germany, Spain, Brazil, Argentina, India, and Japan. The permitted doses vary due to varying restrictions in various countries. However, the FDA advised that permissible levels be less than 75 mg/kg or 50 mg/kg [3,4]. Acute health issues such as diarrhea, vomiting, and abdominal pain have been related to the effects of potassium bromate. Several reports have evaluated the toxicity induced by potassium bromate which includes genotoxic, neurotoxic, hepatotoxicity, nephrotoxic, endocrinological, and a carcinogenic agent [5–7]. Nausea, vomiting, diarrhea, and abdominal discomfort are the immediate consequences of potassium bromate poisoning in humans. Oliguria, anuria, deafness, vertigo, hypotension,

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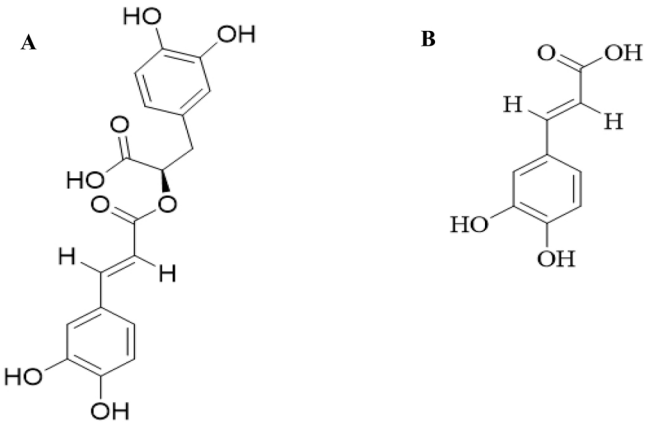

Fig. 1. Chemical structures of rosmarinic acid (A) and caffeic acid (B).

Table 1
Quantitative phytochemical compositions of *Theobroma cacao* leaf.

S/N	Phytochemical	Quantity (mg/100 g)
1	Alkaloids	175.80 ± 0.00
2	Flavonoids	127.88 ±0.11
3	Steroids	15.18 ±0.39
4	Triterpenes	5.40 ±0.01
5	Phenolics	3.12 ±0.01
6	Coumarin	1.20 ±0.00
7	Tannins	0.17 ±0.00
8	Terpenoids	0.07 ±0.00
9	Glycoside	0.08 ±0.00
10	Saponins	0.001 ±0.00

Values are presented as mean ± SEM (n = 3)

central nervous system depression, hearing loss, renal failure, and thrombocytopenia are some of its chronic side effects [8,9]. The use of plant products for medicinal purposes due to their constituent bioactive ingredients dated back centuries. *Theobroma cacao* which has been used extensively in the food industry has been recognized as an important source of polyphenols, flavonoids, catechins, procyanidins, amino acids, polypeptides, and oligopeptides [10,11]. *T. cacao* was classified in the family *Sterculiaceae* together with the genera *Cola, Guazuma*, and *Herrania*. *T. cacao* initially originated in ancient Central America, especially among the Maya and Aztecs. It was cultivated basically for its seeds that could be used for the production of chocolate [12]. The scientific name, "*Theobroma*" is coined from '*Theo*' which means gods while '*broma*' which means food, is noted to be "food of the gods" by the Olmec and Mayan tribes [13]. The sequence of the cacao genome identified 28,798 genomic sequences were identified to code for protein, about 20 % of which are also transposable genes element. This genome is responsible for the coding of Phyto-compounds which include flavonoids, aromatic terpenoids, theobromine, and many other metabolites [14]. Cocoa (*Theobroma cacao* L.) has been noted for its several medicinal uses for many years now and some are documented in the literature. Cocoa has been used to treat poor sexual appetite, anemia, fever, tuberculosis, gout, mental fatigue, and even kidney stones [15]. The polyphenols in cocoa have several positive health impacts, including better heart, kidney, and intestine function as well as nervous system stimulation and eased digestion. When compared to individuals who don't consume much cocoa, Panama's Kuna people report lower rates of diabetes, cancer, and heart disease [15,16]. This implies that cocoa is effective at warding off some diseases. The two main cinnamic acid derivatives found in plant leaves, rosmarinic acid and caffeic acid (Fig. 1 A&B) are crucial for the control of physiological processes [17,18]. One of the many phenolic acids contained in fruit is caffeine (3,4-hydroxycinnamic acid), which has a variety of chemical and pharmacological effects [19]. Due to its antioxidant activity, caffeine has therapeutic benefits and can considerably lessen DNA damage caused by free radicals [20,21]. By using H-abstraction processes, phenolic antioxidants can prevent oxidative damage caused by free radicals [22–24]. Numerous biological functions of rosmarinic acid include the prevention of HIV-1, tumor growth, hepatitis, liver protection, blood clot prevention, and anti-inflammation [25]. By triggering the transcription factor for antioxidant enzymes, nuclear factor erythroid-derived 2-related factor 2 (Nrf2), and by scavenging free radicals (oxygen and nitrogen reactive species) or broken-down prooxidant substances, rosmarinic acid mediates the antioxidant action [26]. However, this current study was to evaluate the ameliorative effect of *Theobroma cacao* leaf fortified against potassium bromate-induced oxidative damage in male Wistar rats.

Table 2
Proximate (A) and amino acid (B) composition of *Theobroma cacao* fortified feed.

A			
Parameters	**Feed (%)**	**Fortified feed (%)**	**Leaf (%)**
Moisture	10.33	11.29	8.8
Protein	13.73	8.98	13.87
Fat	5.86	4.54	6.35
Fiber	5.55	38.62	48.73
Starch	37.84	20.21	28.34
Ash	8.92	12.53	31.07
Sugars	4.67	2.81	2.21
Calcium	1.07	0.29	0.35
Phosphorus	0.66	0.2	0.14
B			
Amino acid	**Feed (%)**	**Fortified feed (%)**	**Leaf (%)**
Arginine	2.21	1.53	1.35
Cysteine	0.49	0.75	0.56
Isoleucine	1.04	1.05	1.09
Leucine	1.37	1.18	0.69
Lysine	3.82	2.56	1.41
Methionine	0.46	0.74	1.12
Threonine	0.74	0.97	1.15
Tryptophan	0.10	0	0
Valine	0.96	0.91	0.63

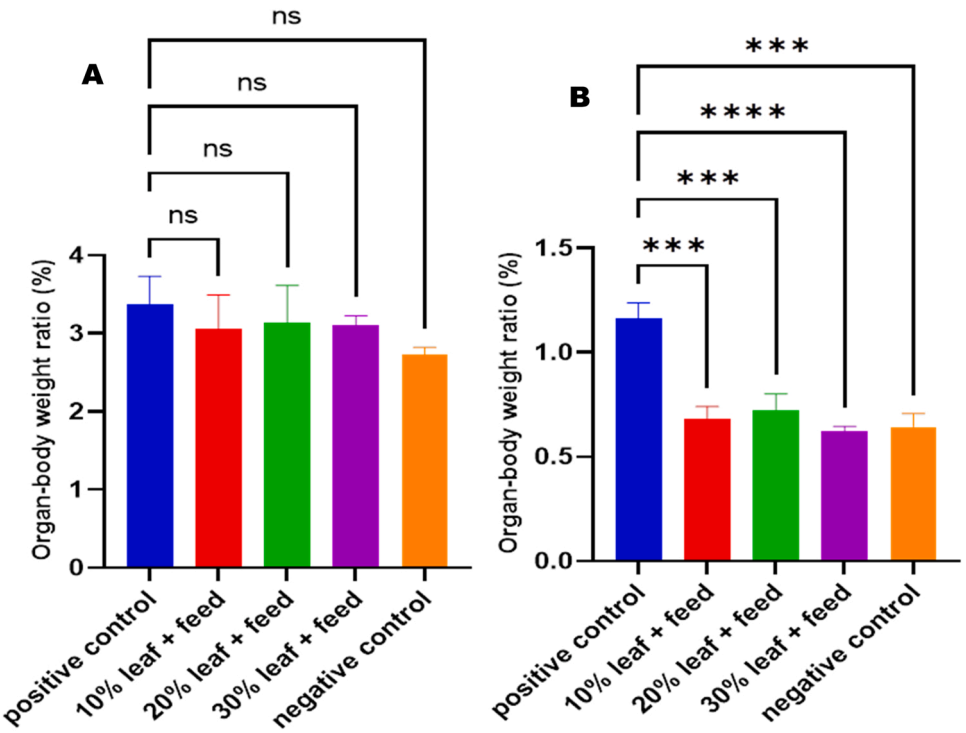

Fig. 2. Effect of *Theobroma cacao* fortified feed on the organ-body weight ratio of the liver (A) and kidney (B) in rats administered potassium bromate for a 14-day treatment. Values are presented as mean ± SEM (n = 4). Bars with different superscripts are significantly different ($p < 0.05$).

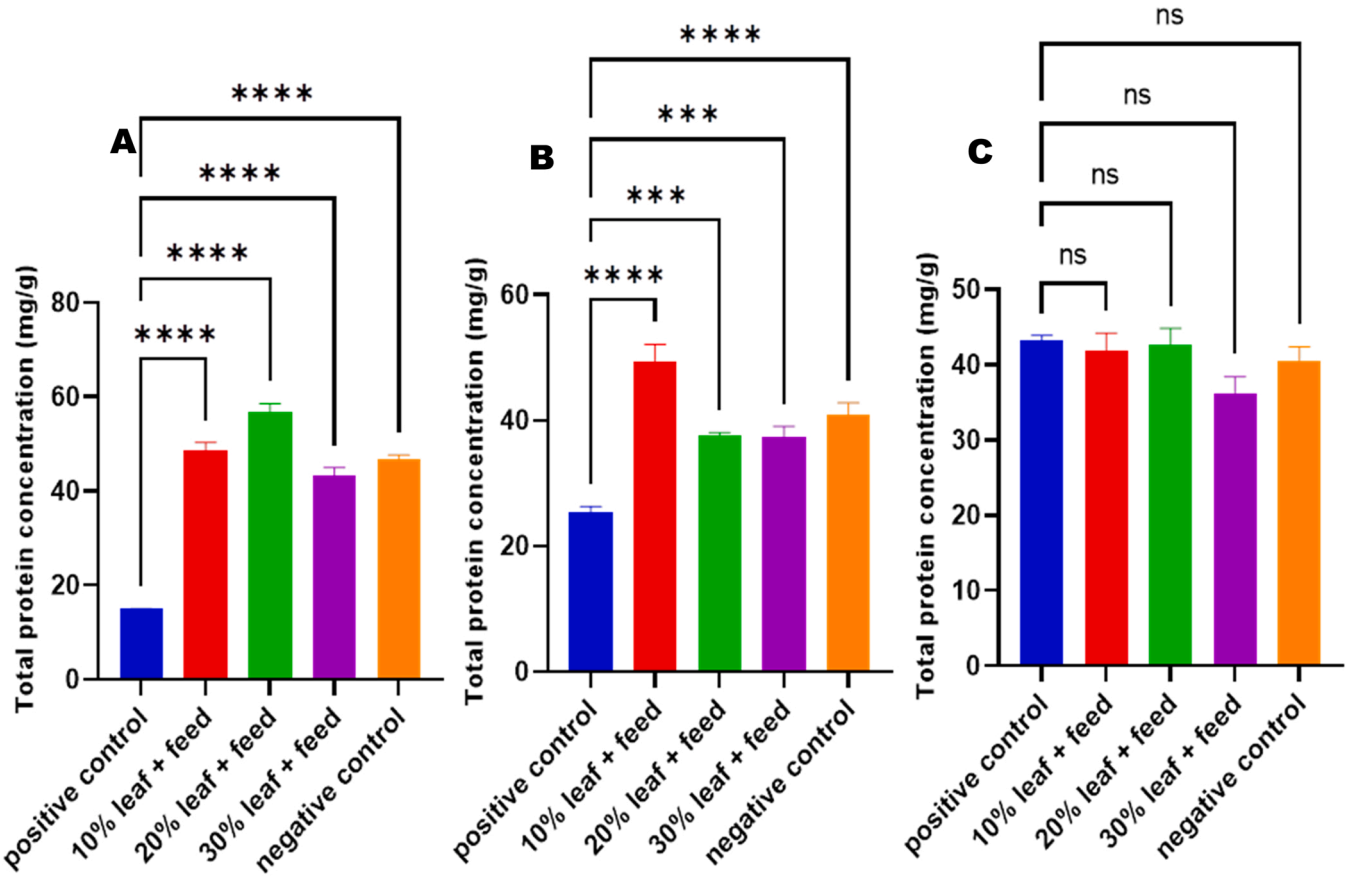

Fig. 3. Effect of *Theobroma cacao* fortified feed on total protein concentration in liver (A), kidney (B), and serum (C) in rats administered potassium bromate for a 14-day treatment. Values are presented as mean ± SEM (n = 4). Bars with different superscripts are significantly different ($p < 0.05$).

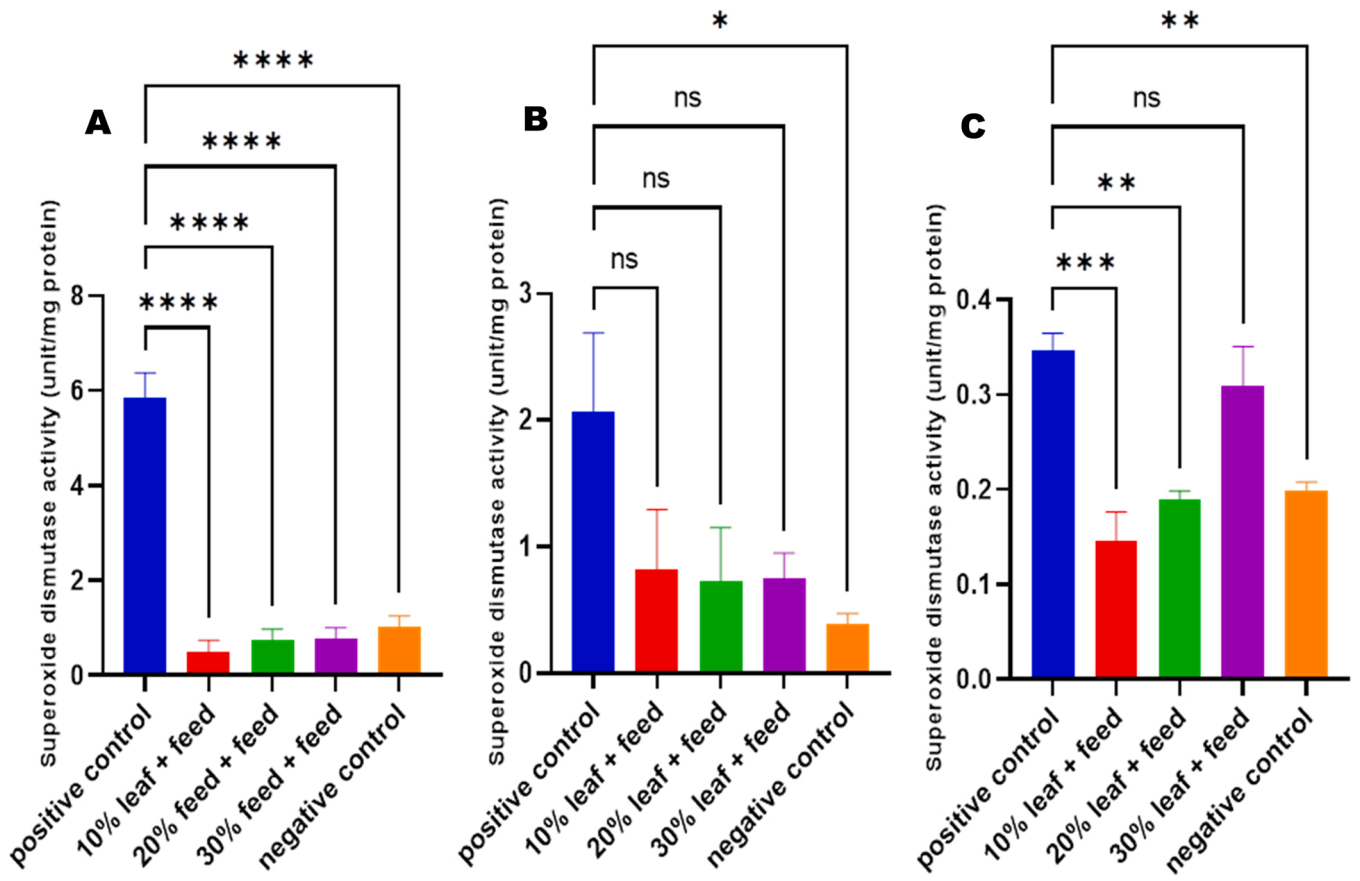

Fig. 4. Effect of *Theobroma cacao* fortified feed on superoxide dismutase activity in liver (A), kidney (B), and serum (C) in rats administered potassium bromate for a 14-day treatment. Values are presented as mean ± SEM (n = 4). Bars with different superscripts are significantly different ($p < 0.05$).

2. Materials and methods

2.1. Procurement of Theobroma cacao leaf and feed fortification

The fresh leaves of *Theobroma cacao* were collected from a cocoa plantation at Landmark University Cocao Plantation in Omu-Aran, Nigeria. The leaf authentication was done at the Department of Plant Biology Herbarium, University of Ilorin in Nigeria. The air-dried pulverized leaves were used for the feed fortification by substituting the daily ratio with pulverized leaf (w/w) as follows: 10 % fortification (10 % *Theobroma cacao* leaf + 90 % normal feed), 20 % fortification (20 % *Theobroma cacao* leaf + 80 % normal feed), and 30 % fortification (30 % *Theobroma cacao* leaf + 70 % normal feed).

2.2. Potassium bromate procurement

Potassium bromate was purchased from Guangdong Guanghua Sci-Tech Co., Ltd, China.

2.3. Phytochemical analysis

The secondary metabolites screening procedure involved extracting one gram of powdered *Theobroma cacao* L. leaves with 100 ml of distilled water (1 % w/v). A conventional approach unique for each phytochemical, including phenolics [27]; flavonoids, and tannins [28]; alkaloids, terpenoids, and cardiac glycosides [29] and saponins [30] was used to determine the presence of a phytochemical in a tiny amount of *Theobroma cacao* leaf extract.

2.4. Evaluation of proximate and amino acids composition

An amino acid analyzer (Jenway, Germany) was used to estimate the amino acid composition, and an automated perfect analyzer (Idexx, Sweden) was employed to measure the proximate composition.

2.5. Animal handling and ethical approval for animal use

Thirty male Wistar rats with an average weight of 150 ± 0.1 g were purchased from the animal holding unit, at the University of Ilorin, Kwara State, Nigeria. The rats were randomly divided and placed in plastic animal cages and kept in a well-ventilated room with alternate dark and light (12 h) cycles. Before the commencement of the experiment, the rats were acclimatized for 7 days with ad libitum access to standard rat feed and clean water. The ethical and animal handling permission, LUAC-0030B, was obtained by Landmark University in Omu-Aran, Nigeria.

2.6. Animal grouping and experimental design

Thirty male Wistar rats with an average weight of 200 ± 0.5 g were randomly distributed into treatment groups: the negative control was administered 0.2 ml distilled water (E) while potassium bromate group (A) was administered potassium bromate orally and then allowed to feed on commercial feed. The treatment groups (B, C, and D) were given potassium bromate orally along with 10 %, 20 %, and 30 % of fortified feed respectively. Each rat received 10 mg/kg body weight of potassium bromate [31].

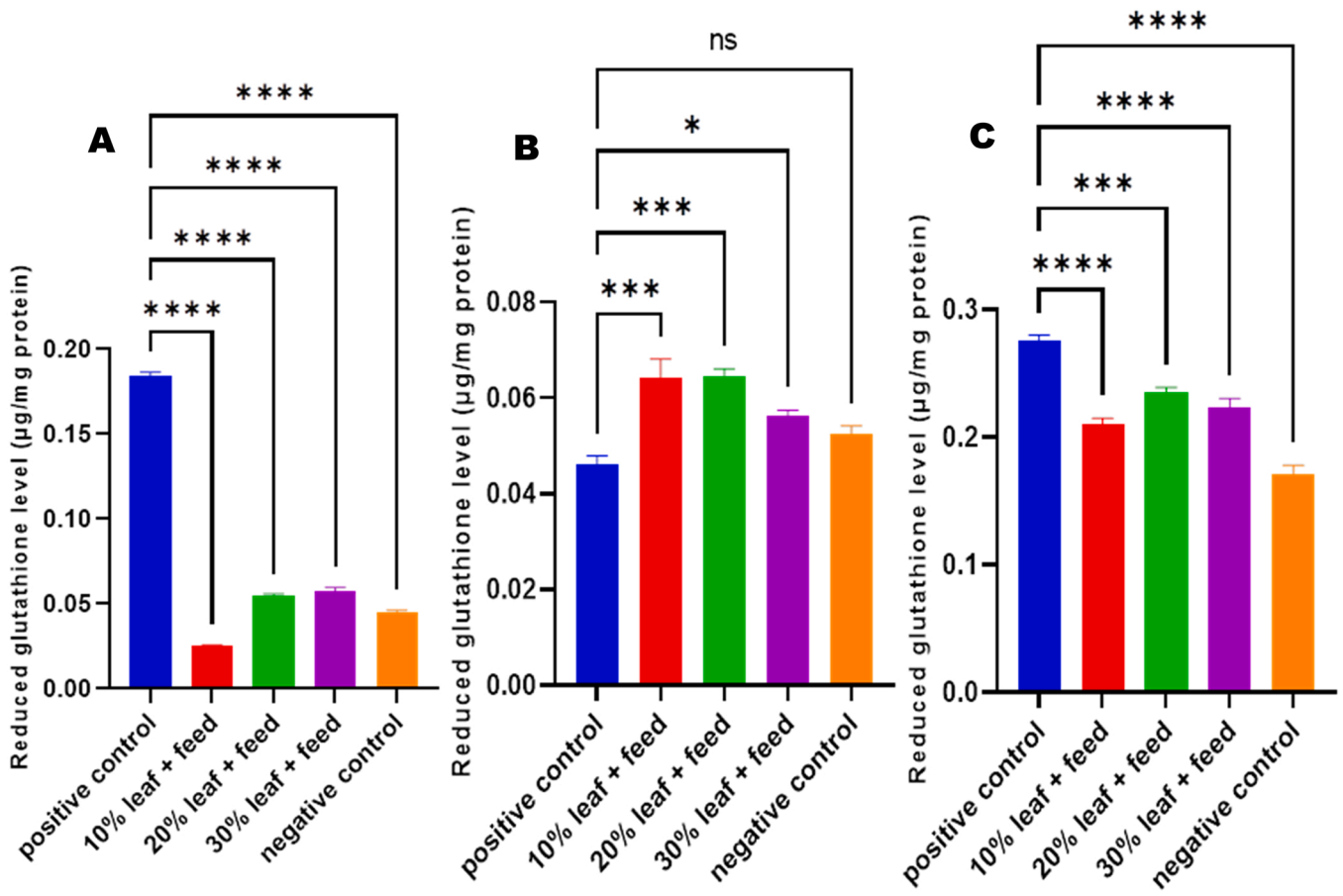

Fig. 5. Effect of *Theobroma cacao* fortified feed on reduced glutathione level in the liver (A), kidney (B), and serum (C) in rats administered potassium bromate for a 14-day treatment. Values are presented as mean ± SEM (n = 4). Bars with different superscripts are significantly different ($p < 0.05$).

2.7. Serum and tissue homogenate preparation

The rats were anesthetized with halothane and subsequently euthanized by cutting open the jugular vein. A small volume of the blood was collected in a sample bottle with an anticoagulant EDTA and was used for hematology assessment while the serum removed from the remaining volume of the blood was used for biochemical assays. The blood collected was centrifuged at 5000 rpm for 10 min using a refrigerated centrifuge (Anke TDL-5000B, Shanghai, China) to obtain the serum. The rat liver and kidney were removed cleaned of blood stains and then weighed for the estimation of the organ-body ratio [32]. The extracted liver and kidney were homogenized with Teflon homogenizer under an ice-cold solution of 0.25 M sucrose [1:5 w/v]. The supernatant fractions of the tissue homogenates after centrifugation at 3000 rpm for 15 mins were collected and stored in a sterile sample bottle. The serum and the homogenates were kept frozen at -4^{o} C to be used for biochemical assays.

2.8. Biochemical analysis

The procedure for protein estimation was estimated according to the method of Gornall, Bardawill [33]. Wright, Leathwood [34] described the method used to describe alkaline phosphate (ALP). Malondialdehyde (MDA), a product of lipid peroxidation was assayed using the method described by Satoh [35]. Misra and Fridovich [36] method was used for the determination of superoxide dismutase activity. Reduced glutathione concentration was estimated by the method of Jollow, Mitchell [37]. Alanine transferase activity (ALT), aspartate transferase activity (AST), creatinine, bilirubin, albumin, and urea concentrations were assayed by using commercial kits.

2.9. Histopathology

Tissues fixation for histological assessment was carried out using the standard method [38]. Representation slices of the liver and kidney for each treatment group were fixed in 10 % formal saline before being processed as usual in preparation for microscopic analysis. The examination will look for cell necrosis, degraded cells, cell detachment, and lymphocyte infiltration in the damaged tissues [39].

2.10. Data analysis

The data were analyzed using one-way ANOVA, followed by the Dunnette post hoc mean comparison test. The results were presented as the mean of four replicates ± standard error of the mean (SEM). $p < 0.05$ were deemed statistically significant between means. Graph pad Prism (version 9, Graph Pad Software Inc., La Jolla, CA) was used for both statistical analysis and graph charting.

3. Results

The phytochemical constituent of the *Theobroma cacao* leaf include

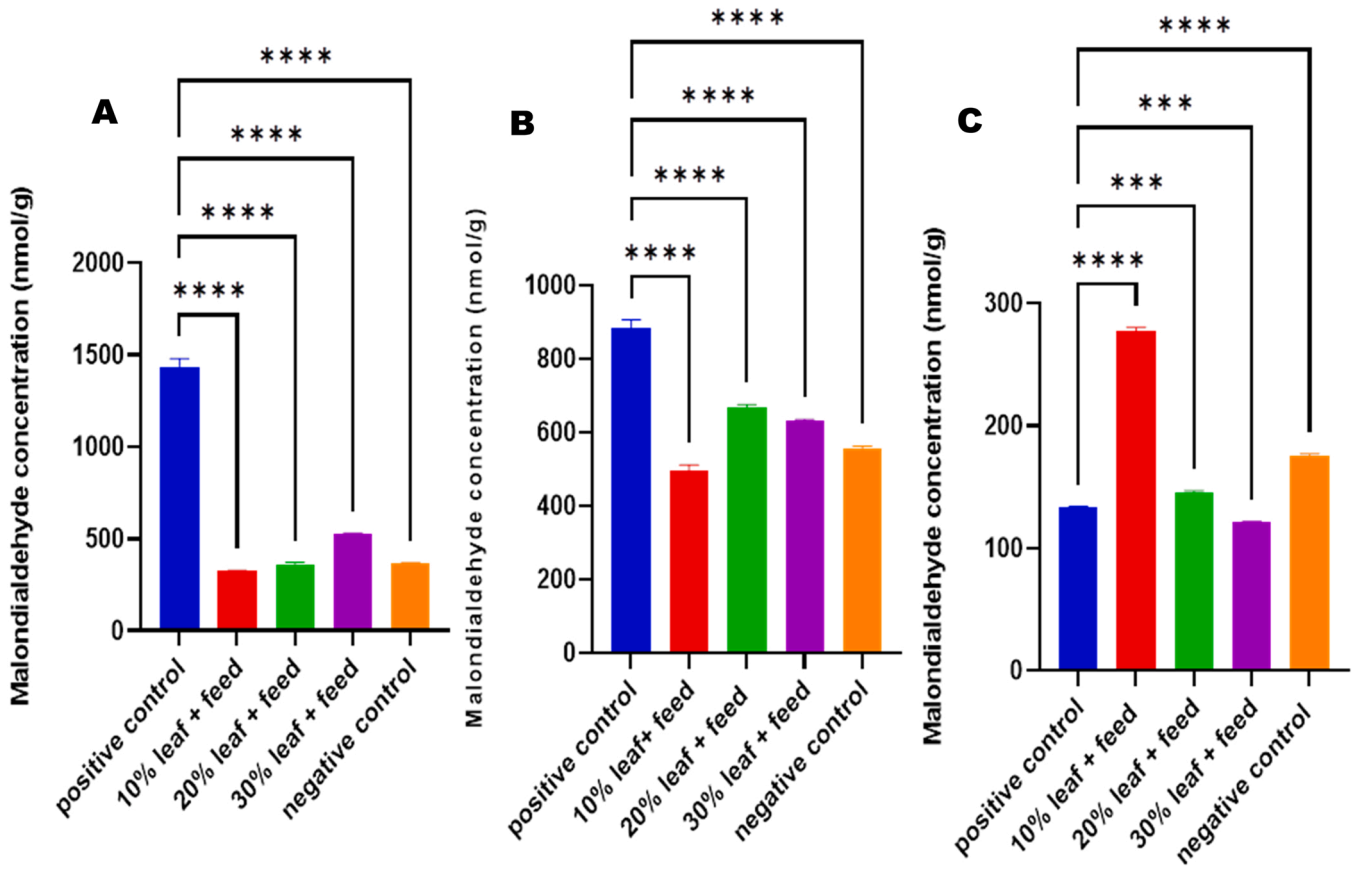

Fig. 6. Effect of Theobroma cacao fortified feed on malondialdehyde concentration of liver (A), kidney (B), and serum (C) in rats administered potassium bromate for a 14-day treatment. Values are presented as mean ± SEM (n = 4). Bars with different superscripts are significantly different ($p < 0.05$).

steroids, glycosides, coumarin, triterpenes, terpenoids, phenolics, saponins, tannins, alkaloids, and flavonoids (Table 1). The proximate composition of fiber in the fortified feed increased compared to the feed (Table 2A). Similarly, the fortified feed had more methionine, cysteine, and threonine content compared to the feed (Table 2B). There was no significant change ($p > 0.05$) in the organ-body weight of the liver for the groups fed fortified feed compared to the positive control (Fig. 2A) contrary to the liver, the kidney organ-body weight ratio of the group administered only potassium bromate showed a significant increase while the treatment groups showed no significant change compared to the negative control (Fig. 2B). There was a significant increase ($p < 0.05$) across the treatment groups compared to the positive control in both the liver and kidney (Fig. 3 A&B). However no significant change ($p > 0.05$) was observed across the treatment groups compared to the negative control in the serum (Fig. 3C). There was a significant decrease ($p < 0.05$) in SOD activity across the treatment groups compared to the positive control in the liver while in the kidney the decrease in SOD activity is not significant ($p > 0.05$) compared to the positive control (Fig. 4 A&B). There was a significant decrease ($p < 0.05$) in SOD activity across treatment groups except the group fed 30 % fortified feed which showed no significant decrease ($p > 0.05$) compared to the positive control in the serum (Fig. 4 C). Reduced glutathione levels decreased significantly ($p < 0.05$) across treatment groups compared to the positive control in the liver and the serum (Fig. 5 A&C). Meanwhile, in the kidney, there was a significant increase ($p < 0.05$) in GSH levels across treatment groups compared to the positive control (Fig. 5 B). There was a significant decrease ($p < 0.05$) in MDA concentration across treatment groups compared to the positive control in the liver and kidney (Fig. 6 A&B). However in the serum there was a significant decrease ($p < 0.05$) in MDA level in the group fed 30 % fortified feed and increased significantly ($p < 0.05$) in 10 % and 20 % fortified feed compared to the negative control (Fig. 6 C). There was a significant decrease($p < 0.05$) in ALP activity across treatment groups compared to positive control in the liver but in the kidney, ALP activity increased significantly ($p < 0.05$) across treatment groups compared to the positive control (Fig. 7 A&B). In the serum, ALP activity increased significantly ($p < 0.05$) in the group administered 10 % fortified feed and with no significant change ($p > 0.05$) in the 20 % and 30 % fortified feed groups compared to the positive control (Fig. 7 C). There was a significant increase in the ALT activity across treatment groups compared to the positive control while the AST activity increased significantly in the groups fed 10 % and 20 % while there was a significant decrease in the group fed 30 % feed compared to the positive control (Fig. 8 A&B). There was a significant decrease ($p < 0.05$) in bilirubin concentration in the groups fed 20 % and 30 % feed and a none significant change ($p > 0.05$) compared to the positive control while the concentration of albumin across treatment groups increased significantly ($p < 0.05$) compared to the positive control (Fig. 8 C&D). There was no significant increase ($p > 0.05$) in the creatinine concentration in the groups administered potassium bromate and fed fortified feed compared with the negative control (Fig. 9A), however for the urea concentration there was a significant increase ($P < 0.05$) compared to the negative control (Fig. 9B). The hematological assessment showed no significant change in the RBC, WBC, HCT, and other hematological parameters considered, in the test groups (Table 3). The photomicrograph of the kidney and liver showed normal cells in the groups fed with 20 % and 30 % *Theobroma cacao* fortified feed compared to the positive control (Figs. 10&11).

4. Discussion

According to Öztürk, Çavuşoğlu [40], the oxidizing characteristic of potassium bromate (KBrO3) has been linked to thyroid, hepatotoxic, and

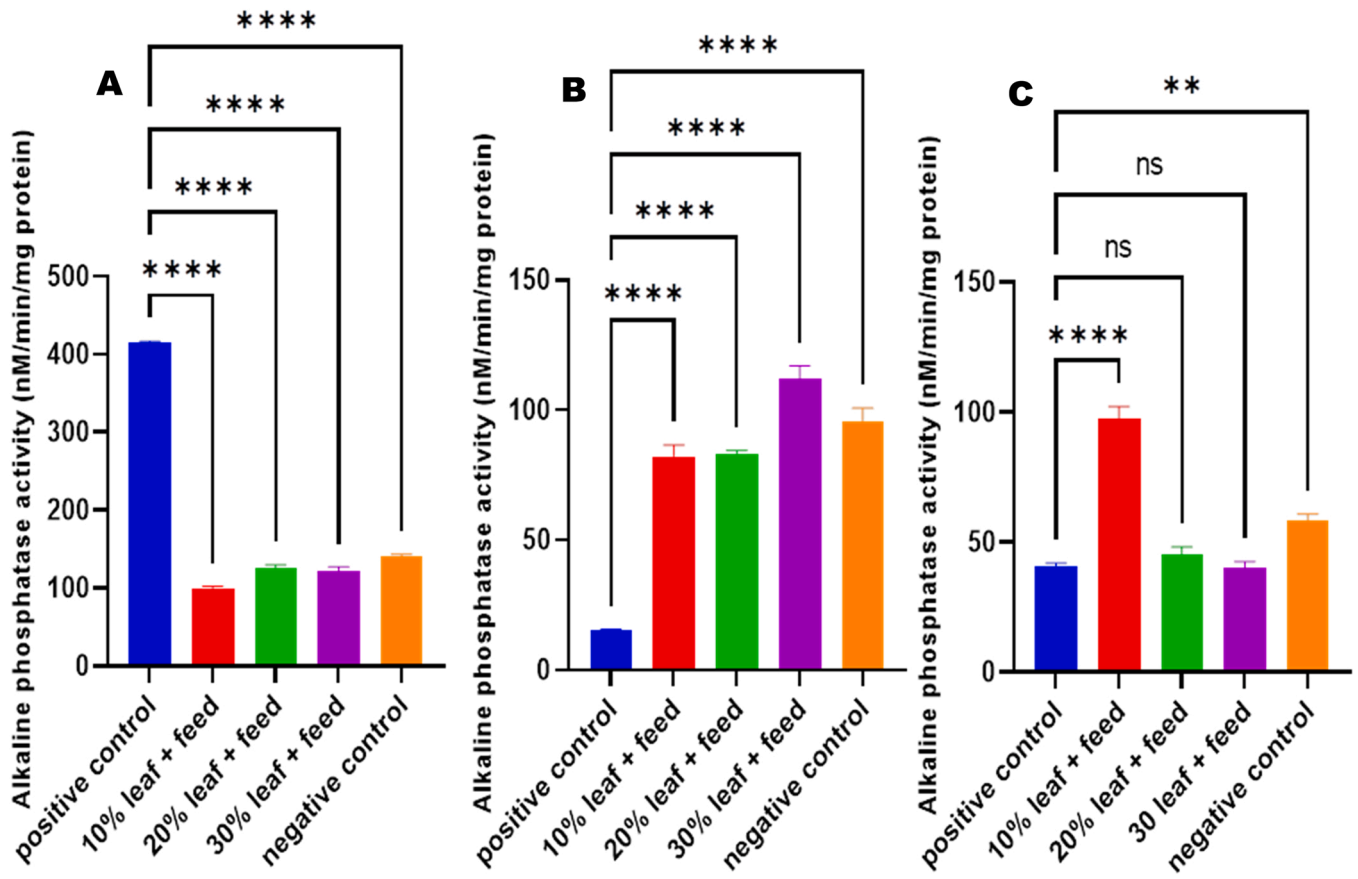

Fig. 7. Effect of *Theobroma cacao* fortified feed on alkaline phosphatase activity of liver (A), kidney (B), and serum (C) in rats administered potassium bromate for a 14-day treatment. Values are presented as mean ± SEM (n = 4). Bars with different superscripts are significantly different ($p < 0.05$).

neurotoxic side effects. The kidney was affected by potassium bromate more severely than the liver. This assertion is corroborated by the increased organ body weight ratio in the current investigation, and the change in organ weight ratio may thus be attributed to kidney cell hypertrophy. Furthermore, the activity of the *Theobroma cacao* leaf in the fortified feed prevented potassium bromate's detrimental effects on the kidney. The presence of flavonoids and phenolics particularly among other bioactive compounds present in the leaf of *Theobroma cacao* leaf in the feed can easily participate in the scavenging of the free radicals caused by potassium bromate or modulate some enzymatic pathways [41]. In a similar vein, the drop in total protein concentration in the potassium bromate-treated group in this study may have resulted from a disruption in the metabolic pathways required for the manufacture of crucial proteins essential to the operation of these organs. The group fed fortified feed had a return to normal metabolic function, which clarified the leaf's anti-oxidant defense against potassium bromate-induced oxidative damage. To counteract the damage caused by oxygen-free radicals, tissues have evolved an antioxidant defense system that comprises non-enzymatic antioxidants as well as enzymatic activities like superoxide dismutase (SOD) and catalase (CAT [42–44]. Superoxide dismutase is a ubiquitous antioxidant enzyme with cofactor Cu^{2+} and Zn^{2+} in the form found in the eukaryote and Fe^{2+} and Mn^{2+} in the form found in prokaryotes [45]. The increase in SOD activity in this investigation supported the hypothesis that potassium bromate causes oxidative damage. However, the increased antioxidant enzyme activity served to remove free radicals and stop lipid peroxidation. However, the endogenous SOD activity was not stimulated because the free radicals produced by potassium bromate were neutralized by the *Theobroma cacao* leaf's antioxidant activity. Glutathione is a tripeptide scavenging molecule against the free radical [46,47]. GSH is a vital intracellular and extracellular protective antioxidant against oxidative stress. The increase in the GSH level in the liver and serum is different from what is in the kidney also explains the degree to which the tissue is affected. The increased levels in the liver and serum were due to low concentrations of free radicals hence the rate of usage and production is balanced. Therefore there is no induction for the production of GSH due to the antioxidant function of the fortified feed. However, in the kidney, the reduction in GSH was due to an imbalance as a result of increased demand caused by a high level of free radicals. Malondialdehyde is a by-product of lipid peroxidation in the cell membrane [48]. As a result, an increase in MDA tissue levels may be proportionate to the degree of oxidative damage to the tissue. In addition, the levels of malondialdehyde, a lipid peroxidation end product that is stable, may be used to determine how free radicals are produced and how their activities are regulated [49]. The literature indicating that those tissues are one of the targets of potassium bromate oxidative damage was validated by the current study's reported rise in MDA levels in the liver, kidney, and serum. Additionally, the decrease in MDA levels in the tissues of the groups fed *Theobroma cacao*-fortified feed supported the existence of an antioxidant bioactive molecule in the leaf. AST and ALT are present in the hepatic cells of the liver as well as other tissues such as the pancreas, heart, red blood cell muscle, and kidney and can thus act as biomarkers for the liver if there is no leakage due to injury to the other tissues [50–52]. The enzyme levels are useful in identifying acute hepatocellular diseases as well as assessing subtle and early changes in biliary obstruction and active cirrhosis [53]. The reduction in ALT activity might be related to the increased activity of the drug-metabolizing enzyme to detoxify potassium bromate in the liver, shielding the liver cell from direct injury [54–56]. However, the normal ALT activity in the animal-fed fortified feed could be due to the antioxidant potential of the

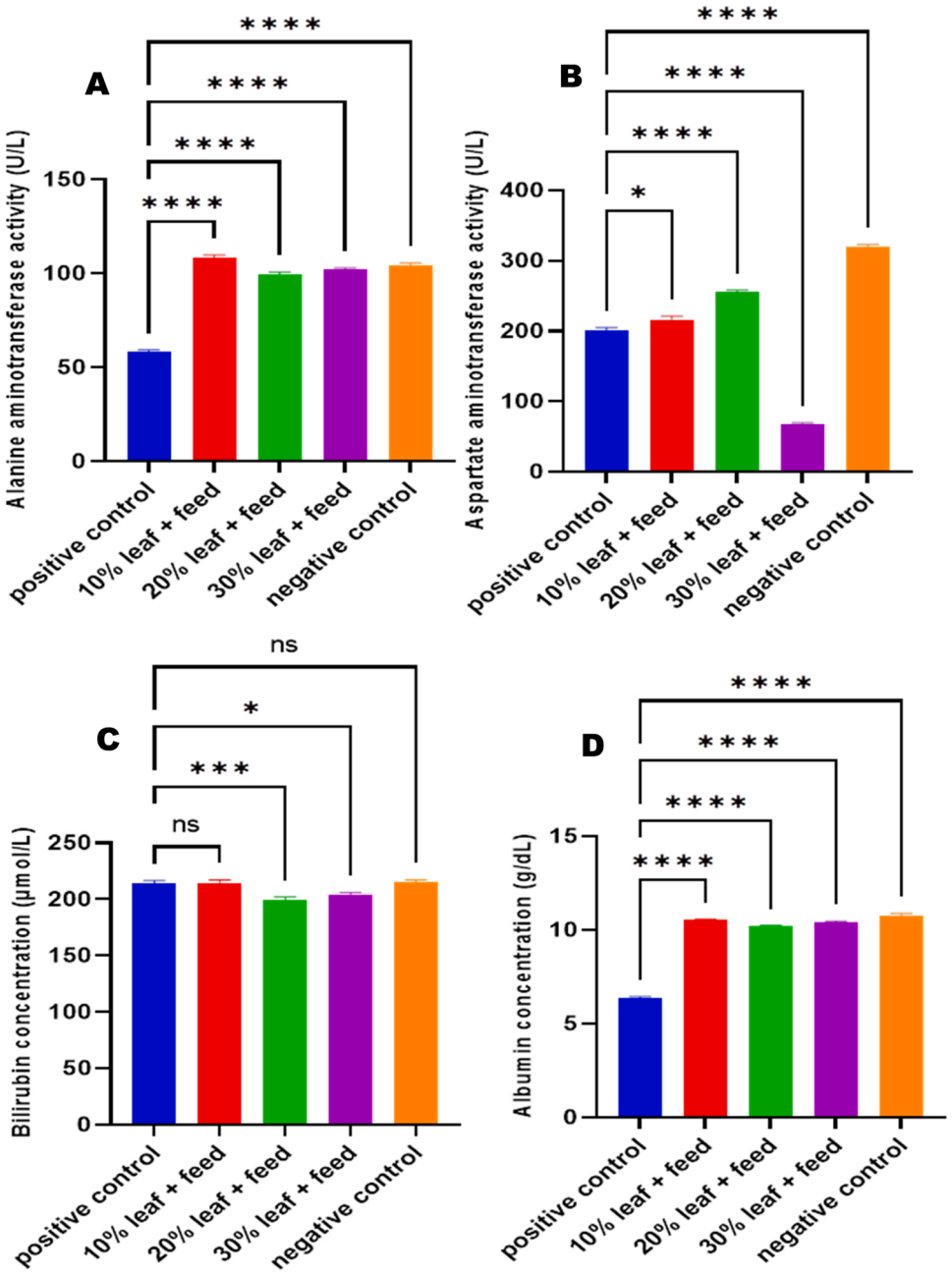

Fig. 8. Effect of *Theobroma cacao* fortified feed on serum alanine aminotransferase activity (A), aspartate aminotransferase activity (B), bilirubin concentration (C), and albumin concentration (D) in rats administered potassium bromate for a 14-day treatment. Values are presented as mean ± SEM (n = 4). Bars with different superscripts are significantly different ($p < 0.05$).

bioactive components in the leaf. Similarly, AST activity was decreased in PB-exposed rats, but it was restored by a *Theobroma cacao* leaf fortified diet. Bilirubin is the anionic by-product of the breakdown of RBC haemoglobin. An increase in serum bilirubin levels may be related to impaired liver function or haemolytic anaemia [57]. Therefore an assault on the liver by potassium bromate which has been reported by this study could be linked to an increase in serum bilirubin observed, however the abnormality was ameliorated by *Theobroma cacao* fortified feed. Albumin is a crucial plasma protein generated predominantly by liver parenchymal cells [58,59]. Albumin is an essential biomarker of hepatic function that can be influenced by dietary status and liver disease [60]. The lower albumin concentration revealed in this investigation indicated that the liver's synthesis of albumin was interrupted. However, the disruption caused by PB toxicity to liver function was mitigated in the group administered *Theobroma cacao*-fortified feed, resulting in a normalization of albumin levels. The increased alp activity by potassium bromate could be due to de novo synthesis; however, a decrease in the kidney in the potassium bromate group was due to a damaged membrane resulting in the leakage of the enzyme. In either liver or kidney tissue, the groups fed fortified feed led to a restored activity which was a result of *Theobroma cacao* protection. Urea is a waste product from the amino acid catabolism [61] while creatinine is the by-product of creatine catabolism [62]. The concentration of these products in the serum is regulated by the kidney; hence compromise in its functional status will lead to an increase in the serum. The rise in serum urea concentration in the positive control group might be due to the effect of potassium bromate on renal function. However, there was a restoration in the rats given fortified feed, indicating the effectiveness of *Theobroma cacao* in

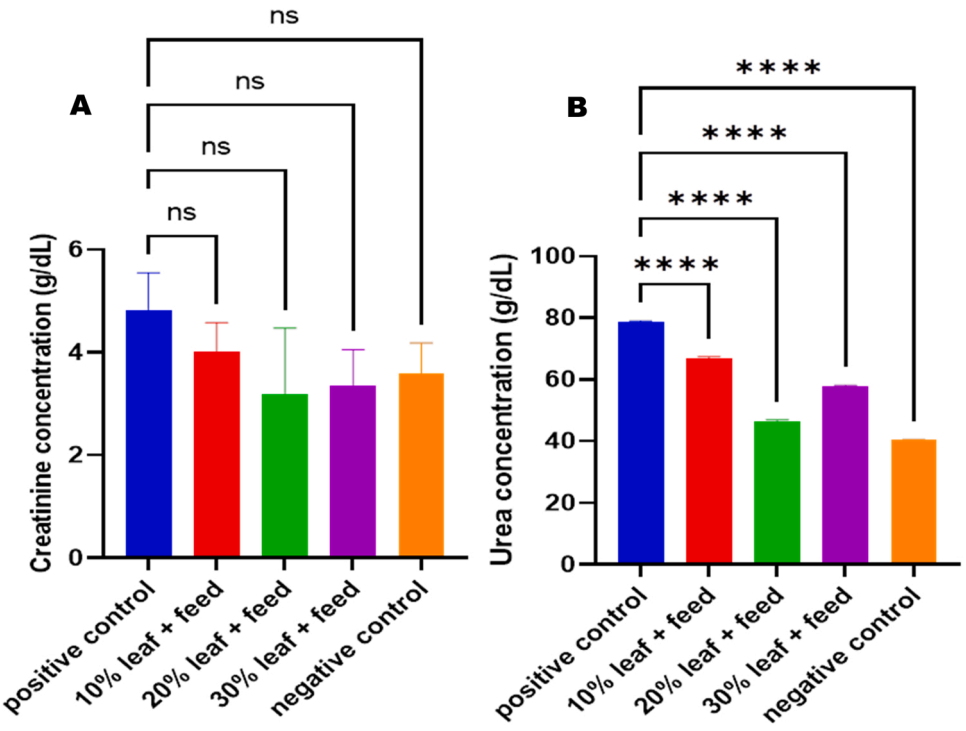

Fig. 9. Effect of *Theobroma cacao* fortified feed on serum creatinine concentration (A) and urea concentration (B) in rats administered potassium bromate for a 14-day treatment. Values are presented as mean ± SEM (n = 4). Bars with different superscripts are significantly different ($p < 0.05$).

Table 3
Effect of *Theobroma cacao* fortified feed on the hematological parameters of rats administered potassium bromate for a 14-day treatment.

Group	Positive control	10 % leaf+ feed	20 % leaf + feed	30 % leaf + feed	Negative control
WBC (10^3 μL)	7.57 ± 3.79^a	5.37 ± 0.35^a	8.80 ± 6.42^a	8.50 ± 4.42^a	9.93 ± 2.72^a
RBC (10^6 μL)	6.38 ± 0.52^a	7.16 ± 0.50^a	3.57 ± 1.71^a	6.68 ± 1.11^a	5.64 ± 2.64^a
HGB (g/dL)	12.87 ± 1.07^a	13.27 ± 0.71^a	11.90 ± 0.90^a	12.23 ± 0.23^a	14.40 ± 0.15^a
HCT (%)	40.06 ± 1.64^a	41.93 ± 3.02^a	21.93 ± 9.35^a	39.73 ± 1.59^a	32.70 ± 15.35^a
MCV (fL)	62.97 ± 3.13^a	58.53 ± 0.12^a	61.47 ± 2.38^a	59.43 ± 1.56^a	38.63 ± 19.31^a
MCH (pg)	20.10 ± 0.86^a	18.56 ± 0.78^a	47.30 ± 15.39^a	18.56 ± 0.20^a	11.57 ± 5.18^a
MCHC (%)	32.10 ± 2.05^a	31.76 ± 1.37^a	37.86 ± 2.00^a	30.83 ± 0.63^a	20.13 ± 10.06^a
PLT (10^3 μL)	836.67 ± 87.76^a	556.67 ± 85.16^a	850.00 ± 313.65^a	656.33 ± 74.48^a	715.00 ± 195.16^a

Values are presented as mean ± SEM (n = 4). Bars with different superscripts are significantly different ($p < 0.05$).

combating potassium bromate toxicity. Furthermore, the effect of potassium bromate was not pronounced in the creatine metabolism based on the data provided in this present study. The hematopoietic system is an essential system to assess toxicity in humans and animals [63,64]. This present study has demonstrated the haematological cells or haemopoietin apparatus were not prone to oxidative damage by potassium bromate. This might possibly mean that the manufacturing process of blood cells was not disrupted or that the blood cells were not damaged by the oxidative stress caused by potassium bromate. Similarly, the photomicrographs of the liver and kidney cells were normal because the potassium bromate impact was mitigated by the *Theobroma cacao* fortified feed. The degradation of liver and kidney cells associated with oxidative damage by potassium bromate was minimized or avoided in groups fed fortified feed, which might be attributed to the antioxidant activity of *Theobroma cacao* leaf in the fortified feed.

5. Conclusion

Rats fed a diet enriched with *Theobroma cacao* leaf did not exhibit the biochemical and histological changes brought on by the oxidative damage caused by potassium bromate. This might be due to the free radical scavenging activity of several bioactive compounds of the leaf, particularly the flavonoids, which have antioxidant qualities, or by the fiber in the leaves of the fortifying leaf, which has metal chelating activities.

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CRediT authorship contribution statement

Nwonuma Charles Obiora: Conceptualization, Methodology, Formal analysis, **Abdurrahman Jadesola Edna:** Formal analysis, Writing – original draft, **Rotimi Damilare Emmanuel:** validation and editing, **Evbuomwan, Ikponmwosa Owen:** formal analysis and validation, **Lele, Kelechi Charity:** Project administration and methodology, **Alejolowo, Omokolade Oluwaseyi:** Visualization and Supervision, **Ezea, Samson Chukwuemeka:** Formal analysis, Validation, and editing, Asogwa, Nnaemeka Tobechukwu: Funding acquisition and methodology, and **Oludipe Emmanuel Olorunleke:** Methodology, Formal analysis and validation.

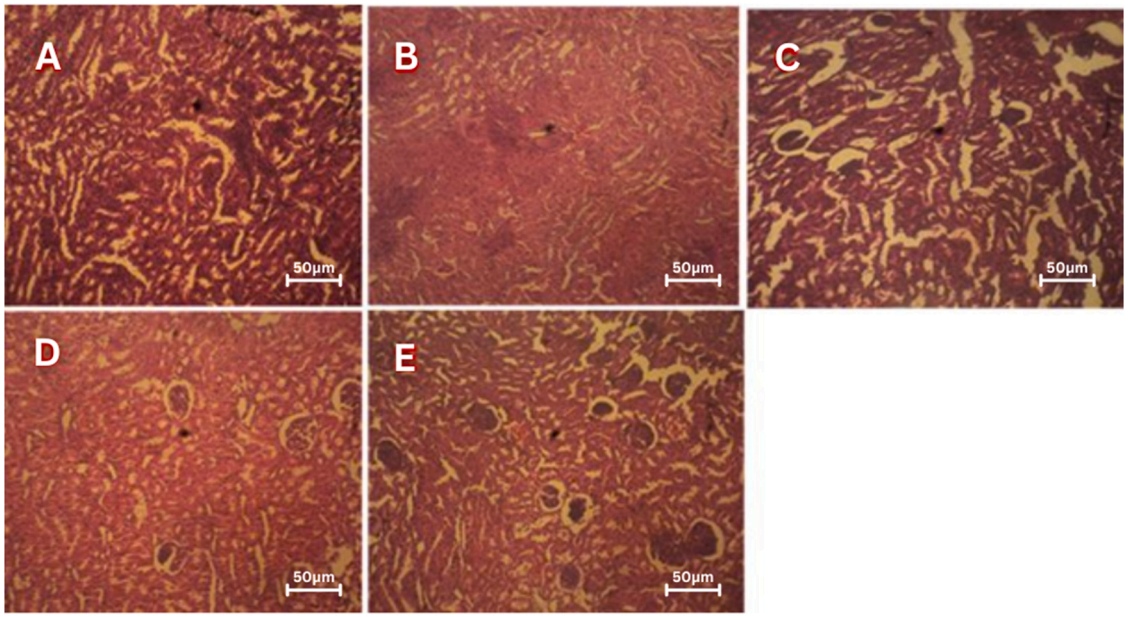

Fig. 10. Photomicrograph of the kidney (H&E X100): Group **A**, Potassium bromate solution, micrograph showed a highly proliferated renal tissue with slightly distorted glomeruli. Group **B**, Potassium bromate solution +10 % *Theobroma cacao* leaf fortified feed, micrograph showed an obliterated renal tissue with a highly branching tubule. Group **C**, Potassium bromate solution +20 % *Theobroma cacao* leaf fortified feed, Micrograph showed a mildly infiltrated glomerulus. Group **D**, Potassium bromate solution +30 % *Theobroma cacao* leaf fortified feed, micrograph showed grossly normal Bowman's capsule with mild infiltration. Group **E**, negative control, micrograph showed an infiltrated and highly proliferated tissue.

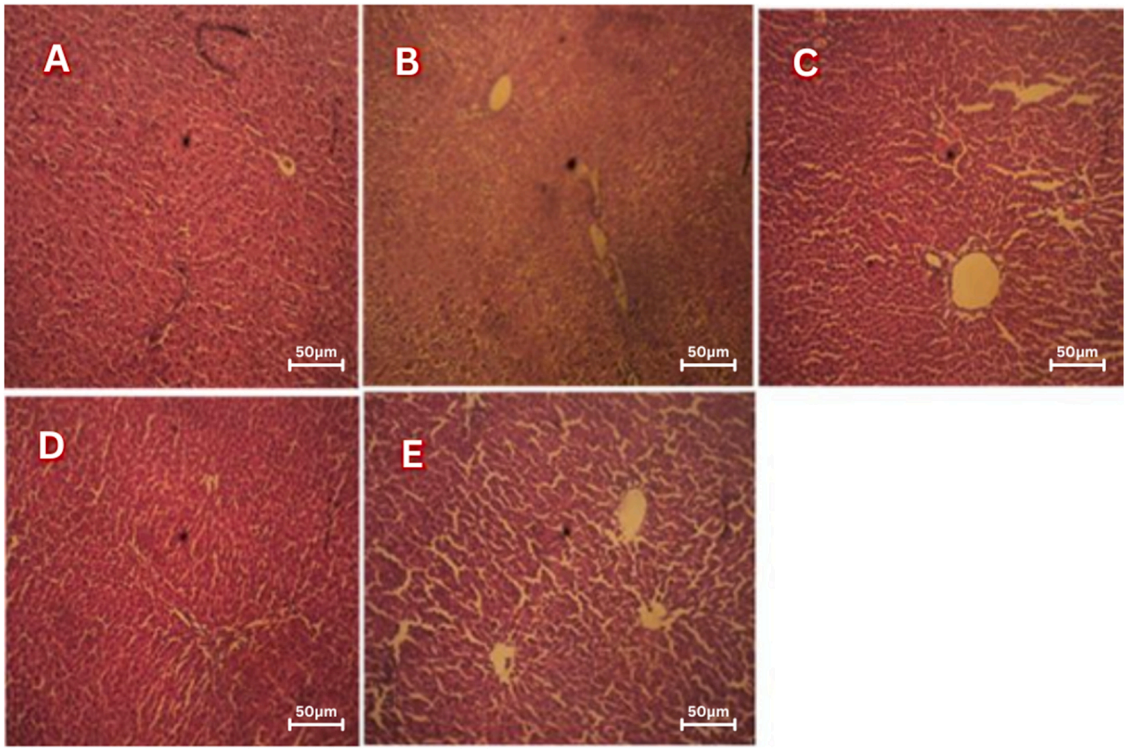

Fig. 11. Photomicrograph of the liver (H&E X100): Group **A**, Potassium bromate solution, micrograph showed a grossly normal hepatic nodule. Group **B** Potassium bromate solution +10 % *Theobroma cacao* leaf fortified feed, micrograph showed a mild proliferation around the central vein. Group **C**, Potassium bromate solution +20 % *Theobroma cacao* leaf fortified feed, micrograph showed a normal hepatic nodule with proliferated cells around the hepatic artery as a result of mild infiltration. Group **D**, Potassium bromate solution +30 % *Theobroma cacao* leaf fortified feed, micrograph showed a grossly normal hepatic tissue Group **E**, negative control, micrograph showed a normal hepatic nodule.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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