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Research Article

Effect of Ethanol Extract of *Aframomum angustifolium* Seed on the Induction of Nephrotoxicity by Bromate in Wistar Rats

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Abstract

Background: The continued use of bromate due to its oxidizing property poses health hazards since it is an established nephrotoxic agent.

Objectives: This study evaluated the capacity of the ethanol extract of *Aframomum angustifolium* seeds to ameliorate the nephrotoxicity of potassium bromate in Wistar rats.

Methods: In stage I of this study, the main phytochemical groups in the seeds were quantified using spectrophotometric procedures. The acute and sub-chronic toxicities of the extract were studied by monitoring physical and biochemical parameters in stage II. In stage III, the reno-protective effect of the extract were determined by administering 350 and 750 mg/kg bw of the extract with 30 mg/kg bw potassium bromate orally. The reno-protective study lasted for 56 days and the effect of treatment on biomarkers was determined on days 28 and 56.

Results: The phytochemical groups (i.e., alkaloids, flavonoids, saponins, tannins, ascorbic acid, and alpha-tocopherol) were detected in the seeds. The acute and sub-chronic oral administration of the extract did not induce any significant toxic reactions across the studied concentrations. The sub-chronic administration of the extract reduced average weight gain in the treated groups. The obtained results in the reno-protective and histological studies indicated that the seed extract offers protection against the induced oxidative assault by bromate.

Conclusion: In general, the co-administration of the ethanol extract of *A. angustifolium* seeds with bromate can reduce its nephrotoxicity in a dose-dependent manner.

Keywords: *Aframomum angustifolium*, Bromate, Nephrotoxicity, Reno-protection, Phytochemicals

Background

Potassium bromate ($KBrO_3$) is a strong oxidizing agent that is extensively used as a maturing agent for flour and a dough conditioner (1). It is also a component of permanent hair weaving solutions and can be generated during the ozonation of bromine-contaminated water (2). Based on the established nephrotoxicity of bromate in rats, the Centre for Science in the Public Interest petitioned the Food and Drug Administration on the use of bromate as a food additive. The use of bromate by bakers has been banned in several countries including Nigeria (3). Despite the ban, the routine analyses of bread and other confectionery products in Nigeria indicate non-compliance (4).

The mechanism of bromate toxicity in the kidney is the induction of oxidative stress by bromine radicals released from the enzymatic reduction of bromate. Oxidative stress characterized by an increased concentration of free radicals has been implicated in the onset and progression of several diseases. The oxidative process is attenuated by phytochemicals due to their antioxidant property (1,5). Several natural compounds can reduce the nephrotoxicity of bromate due to their antioxidant property (6-10). *Aframomum angustifolium* was selected for this study based on availability, cost, and conventional use as a spice in Nigerian delicacies. In our previous research, it was observed that the antioxidant property of the extract is not significantly affected by heat processing (10).

The aim of this study was to determine if the co-administration of the *A. angustifolium* seed extract offers any protection against bromate-induced nephrotoxicity in rats.

Materials and Methods

Sample Preparation and Extraction

The dry fruit pods of *A. angustifolium* were purchased from a local vendor at Ebelle Market, the Igueben Local Government Area of Edo State. They were authenticated in the Department of Biological Sciences, Samuel

Adegboyega University, Ogwa, Edo State, Nigeria.

The seeds were homogenized to a fine powder using a mechanical blender, and the homogenate was macerated in ethanol for seventy-two hours (11). Then, the extract was filtered using a clean muslin cloth and concentrated using a rotary evaporator. The concentrate was dried to powder in a desiccator and then stored in air-tight bottles at room temperature.

Methods

The research was conducted in three stages, viz. of phytochemical screening, acute and sub-chronic toxicity study, and assessment of the reno-protective effect of the extract.

Stage I: Phytochemical Screening

The concentrations of alkaloids, tannins, saponins, flavonoids, and vitamins C and E were assayed in homogenate using spectrophotometric procedures described by Harborne (12), Van Buren and Robinson (13), Obadoni and Ochuko (14), Boham and Kocipai-Abyazan (15), Roe and Kuether (16) and Agoreyo et al (17), respectively.

Stage II: Acute and Sub-chronic Toxicity Tests

The acute toxicity of the extract was estimated using the method of Lorke (18). Six experimental groups (groups 1-6) each comprising 3 Wistar rats were used for this study. Group 1 served as control while groups 2-6 received via the oral route 10, 100, 1000, 3000, and 5000 mg/kg bw of the extract, respectively. Another four groups (groups I-IV) each containing 3 Wistar rats were applied for the sub-chronic toxicity study. The control group (group I) received distilled water while groups II, III, and IV received a daily dose of 1000, 2000, and 3000 mg/kg bw of the extract for 28 days orally with the aid of a gavage. The blood samples were collected by cardiac puncture under anaesthesia and the serum activities of several parameters were analyzed as well. Such parameters were alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (*AST*), gamma-glutamyl transferase (GGT) as well as serum concentrations of total protein (TP), bilirubin (BIL), bicarbonate ion (HCO_3^-), sodium ion (Na^+), potassium ion (K^+), and creatinine (Cr). The kidney tissue activity of superoxide dismutase (SOD) and catalase (CAT) were also determined using a standard spectrophotometric procedure.

Stage III: Assessment of the Reno-protective Effect of the Extract

The reno-protective effect of the extract against bromate-induced nephrotoxicity was determined using four experimental groups (groups A-D) comprising six rats each. Groups A and B served as normal and positive controls, respectively, while groups C and D served as the test groups. Group B received 30 mg bromate/kgbw/ only while groups C and D received 30 mgBr/kg bw co-administered with 350 and 750 mg/kg bw of the ethanol extract. The administration of extracts and toxin to the respective groups lasted for 56 days. In each group, three rats were sacrificed on the 28th day and the other half was sacrificed on the 56th day. The blood samples were collected via cardiac puncture into plain sample bottles under anesthesia and serum concentrations of TP, BIL, HCO_3^-, Na^+, K^+, and Cr were analyzed in addition to determining the kidney tissue activity of SOD and CAT using the standard spectrophotometric procedure.

Histological studies on kidney tissues were performed in the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin, Benin.

Statistical Analysis

The results were expressed as mean ± standard error of the mean. The analysis of variance was conducted using Microsoft Excel 2013, and $P < 0.05$ was considered statistically significant.

Results

The obtained results regarding the quantitative screening for some major phytochemical groups are presented in Table 1. Tannins were observed to be the highest at 120 mg/g of seed homogenate. The ascorbic acid concentration in the homogenate was higher than alpha-tocopherol. Tables 2 and 3 provide a summary of acute and sub-chronic toxicity studies, respectively. In the acute toxicity study, the experimental groups were physically observed for toxic responses. Only group 6, which received the highest dose of the extract (5000 mg/kg bw), displayed drowsiness within the first 24 hours after receiving the dose. The sub-chronic oral administration of the extract at 1000, 2000, and 3000 mg/kg bw of the extract significantly inhibited average weight gain in the study groups. Within the study period, average weight gain was significantly higher in the control group compared to the treated groups. There were no significant differences in the ALP, GGT, and SOD activity, as well as the serum concentrations of TP, HCO_3^-, and K^+. AST activity was significantly higher in

Table 1. Concentration of Phytochemical Groups

Parameter	Result
Alkaloids (mg/g)	3.67 ± 0.33
Flavonoids (µg/g quercetin equivalents)	373.32 ± 3.94
Saponins (µg/g)	80.00 ± 3.33
Tannins (mg/g)	120 ± 0.23
Ascorbic acid (µg/g)	608.07 ± 0.07
Alpha-tocopherol (µg/g)	204.45 ± 0.00

Table 2. Summary of Acute Toxicity Study

	Group 1 (Control)	Group 2 (10 mg/kg bw)	Group 3 (100 mg/kg bw)	Group 4 (1000 mg/kg bw)	Group 5 (3000 mg/kg bw)	Group 6 (5000 mg/kg bw)
Number of animals	3	3	3	3	3	3
Mortality	Nil	Nil	Nil	Nil	Nil	Nil
Physical signs of toxicity	Nil	Nil	Nil	Nil	Nil	Nil
Physical observation	Normal	Normal	Normal	Normal	Normal	Drowsiness
Time of onset	Nil	Nil	Nil	Nil	Nil	Within 24 hours
Time of recovery	Nil	Nil	Nil	Nil	Nil	Two days after the onset

Note. Group 1 = Control group; Group 2 = Received a single dose of 10 mg/kg bw of the extract; Group 3 = Received a single dose of 100 mg/kg bw of the extract; Group 4 = Received a single dose of 1000 mg/kg bw of the extract; Group 5 = Received a single dose of 3000 mg/kg bw of the extract; Group 6 = Received a single dose of 5000 mg/kg bw of the extract.

Table 3. Effect of Extract on Biochemical Parameters After Sub-chronic Exposure

Parameters	Group 1 (Mean ± SEM)	Group II (Mean ± SEM)	Group III (Mean ± SEM)	Group IV (Mean ± SEM)
ALP (U/L)	27.67 ± 0.52 [b]	22.43 ± 1.64 [a]	28.77 ± 2.27 [b]	30.23 ± 0.95 [b]
ALT (U/L)	20.67 ± 1.67 [a]	21.12 ± 3.75 [a]	21.00 ± 0.58 [a]	21.33 ± 0.53 [a]
AST (U/L)	54.28 ± 0.92 [b]	46.00 ± 2.43 [b]	48.76 ± 3.32 [b]	60.72 ± 1.59 [a]
GGT (U/L)	6.18 ± 1.02	5.40 ± 1.02	5.02 ± 2.04	5.79 ± 1.34
Total protein (g/L)	6.04 ± 0.21	5.94 ± 0.08	6.91 ± 0.36	7.04 ± 0.20
BIL (mg/L)	2.81 ± 0.16 [b]	1.33 ± 0.09 [a]	1.09 ± 0.05 [a]	1.54 ± 0.03 [a]
HCO_3^- (mmol/L)	20.71 ± 0.22	16.41 ± 0.38	16.75 ± 0.27	21.59 ± 0.95
Na^+ (mmol/L)	109.09 ± 2.62 [b]	93.94 ± 1.52 [a]	110.61 ± 1.52 [b]	113.64 ± 2.62 [b]
K^+ (mmol/L)	4.57 ± 0.14	4.96 ± 0.17	5.29 ± 0.11	5.97 ± 0.34
Cr (mg/dL)	0.61 ± 0.22	0.54 ± 0.38	0.68 ± 2.27	0.62 ± 0.95
CAT (U/mg protein)	17.56 ± 0.97 [a]	21.47 ± 2.59 [b]	16.28 ± 0.63 [a]	15.03 ± 1.70 [a]
SOD (U/mg protein)	10.30 ± 0.34	9.98 ± 0.12	11.50 ± 0.42	11.08 ± 0.86

Note. SEM: Standard error of the mean; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; BIL: Bilirubin; HCO_3^-: Bicarbonate ion; Na^+: Sodium ion; K^+: Potassium ion: CAT: Catalase; Cr, Creatinine; SOD: Superoxide dismutase; Group I: Control group, received distilled water daily; Group II: Received 1000 mg/kg bw of the extract daily; Group III: Received 2000 mg/kg bw of the extract daily; Group IV: Received 3000 mg/kg bw of the extract daily

[a,b] Different letters represents a significant difference at $P < 0.05$.

group IV that received the highest dose. The result is presented in Table 3.

The results of the reno-protective potential of the extracts against bromate-induced toxicity are reported in Tables 5 and 6. After 28 days, the serum concentrations of HCO_3^-, K^+, and TP, as well as SOD activity were not significantly different ($P > 0.05$) across the groups. Na^+ and Cr concentrations were significantly lower ($P < 0.05$) in groups A and D, respectively (Table 5). The serum concentrations of K^+ and TP were not significantly different across the groups after 56 days. Based on the results, the concentrations of HCO_3^-, Na^+, and Cr, in addition to SOD activity elevated in group B ($P < 0.05$) compared to other groups.

Table 4. Effect of extract on average weight gain during sub-chronic exposure

Group	Mean (g) ± SEM
I	23.74 ± 5.28 [b]
II	8.69 ± 5.08 [a]
III	7.93 ± 5.73 [a]
IV	10.15 ± 3.38 [a]

Note. SEM: Standard error of the mean. Group I: Control group, received distilled water daily; Group II: Received 1000 mg/kg bw of the extract daily; Group III: Received 2000 mg/kg bw of the extract daily; Group IV: Received 3000 mg/kg bw of the extract daily.

[a,b] Different letters indicate a significant difference at $P < 0.05$.

Plates 1-8 present changes in the microstructure of kidney tissues revealed by histological evaluations. The microstructure of the kidney tissue from group A (normal control) was not affected after 28 days of treatment. Group B (positive control) showed patchy tubular necrosis while groups C and D indicated normal tubules and glomerulus (Figures 1 and 2).

After 56 days, the microstructure of kidney tissues from groups A and D was normal while the tubular necrosis in group B became more obvious. The kidney tissue from group C represented normal tubules and dense infiltration of lymphocytes.

Discussion and Conclusion

The impact of phytochemicals on redox reactions *in*

Table 5. Effect of the Co-administration of Extract and Bromate of Some Kidney Function Indicators After Treatment for 28 Days

Parameter (Serum)	Group A (Mean ± SEM)	Group B (Mean ± SEM)	Group C (Mean ± SEM)	Group D (Mean ± SEM)
HCO_3^- (mmol/L)	27.56 ± 0.57	28.53 ± 0.71	25.02 ± 0.52	27.43 ± 0.66
Na^+ (mmol/L)	84.29 ± 2.81 [a]	124.29 ± 1.93	119.62 ± 2.4 [b]	116.25 ± 0.82 [b]
K^+ (mmol/L)	4.57 ± 0.1	4.96 ± 0.17	5.29 ± 0.11	5.97 ± 0.24
Cr (mg/dl)	2.94 ± 0.55 [b]	3.23 ± 0.32 [b]	2.35 ± 0.19 [b]	1.33 ± 0.07 [a]
TP (g/L)	6.3 ± 0.03	6.85 ± 0.12	6.82 ± 0.31	6.44 ± 0.14
SOD (U/mg protein)	10.02 ± 0.36	12.59 ± 0.51	11.60 ± 0.14	11.11 ± 0.72
CAT (U/mg protein)	18.36 ± 1.0 [a]	22.06 ± 1.87 [b]	16.54 ± 0.92 [a]	16.79 ± 2.25 [a]

Note. SEM: Standard error of the mean; HCO_3^-: Bicarbonate ion; Na^+: Sodium ion; K^+: Potassium ion; TP: Total protein; CAT: Catalase; Cr, Creatinine; SOD: Superoxide dismutase; Group A: Normal control group, received distilled water daily; Group B: Positive control group, received a daily dose of 30 mg/kg bw bromate; Group C: Test group 1, received a daily dose of 30 mg/kg bw bromate co-administered with 350 mg/kg bw extract; Group D: Test group 2, received a daily dose of 30 mg/kg bw bromate co-administered with 750 mg/kg bw extract.
[a,b] Different letters indicate a significant difference at $P<0.05$.

Table 6. Effect of the Co-administration of the Extract and Bromate of Some Kidney Function Indicators After Treatment for 56 Days

Parameter (Serum)	Group A (Mean ± SEM)	Group B (Mean ± SEM)	Group C (Mean ± SEM)	Group D (Mean ± SEM)
HCO_3^- (mmol/L)	24.1 ± 0.25 [b]	25.35 ± 2.66 [b]	18.51 ± 2.84 [a]	21.79 ± 0.23
Na^+ (mmol/L)	122.69 ± 4.70 [a]	149.68 ± 1.67 [b]	132.20 ± 2.54 [c]	135.40 ± 4.36 [c]
K^+ (mmol/L)	5.49 ± 0.4	7.08 ± 0.94	5.92 ± 0.47	5.14 ± 0.11
Cr (mg/dL)	1.88 ± 0.22 [a]	4.21 ± 0.17 [b]	2.58 ± 0.25 [a]	2.12 ± 0.42 [a]
TP	4.99 ± 0.48	5.75 ± 0.46	4.71 ± 0.55	5.05 ± 0.12
SOD (U/mg protein)	8.44 ± 0.33 [b]	11.82 ± 0.74 [b]	6.60 ± 0.59 [a]	7.41 ± 0.69 [a]
CAT (U/mg protein)	22.54 ± 3.44 [a]	22.34 ± 6.04 [a]	28.85 ± 4.66 [b]	21.59 ± 3.26 [a]

Note. SEM: Standard error of the mean; HCO_3^-: Bicarbonate ion; Na^+: Sodium ion; K^+: Potassium ion; Cr: Creatinine; TP: Total protein; CAT: Catalase; SOD: Superoxide dismutase; Group A: Normal control group received distilled water daily; Group B: Positive control group received a daily dose of 30 mg/kg bw bromate; Group C: Test group 1 received a daily dose of 30 mg/kg bw bromate co-administered with 350 mg/kg bw extract; Group D: Test group 2 received a daily dose of 30 mg/kg bw bromate co-administered with 750 mg/kg bw extract
[a,b,c] Different letters represent a significant difference at $P<0.05$.

vivo are well documented and available data indicate that they are safer alternatives compared to synthetic antioxidants (19-22). This research focused on the reno-protective effect of the ethanol extract of *A. angustifolium* by studying subtle changes in biochemical indicators and histology of the kidney tissue upon co-administration with bromate.

The botanical genus *Aframomum* consists of a variety of closely related species defined by both their proximate and phytochemical composition. The species a*ngustifolium* and *danielli* are closely related and only identified by chemotaxonomy due to similarities in their biochemical composition (23,24). The reported proximate composition for *A. danielli* indicates the moisture content of 10.4 ± 0.1%, ash of 9.3 ± 0.1%, protein of 8.5 ± 1.0%, fats of 23.1 ± 0.6, and available carbohydrates of 11.9 ± 0.1% (25,26). Flavonoids and diterpenoids are major phytochemical groups that are used as chemo-markers for this genus (27,28). The main phytochemical groups in the genus *Aframomum* includes flavonoids, terpenes, alkaloids, tannins, saponins, terpenes, steroids, and cardiac glycosides (29,30). The result of the phytochemical analysis in this research corresponds with the reports on the phytochemical composition of the members of the genus (Table 1).

The current emphasis on research and development of drugs from plant materials worldwide is associated with obtaining adequate information on the toxicity and efficacy of the utilized plants. The acute toxicity

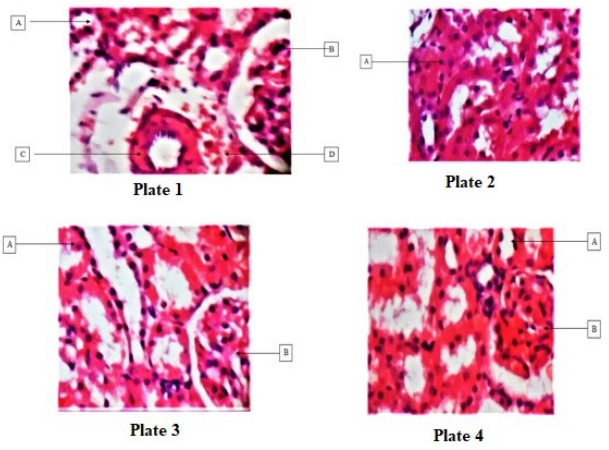

Figure 1. Plate 1: Showing (A) tubules, (B) glomerulus, (C) arcuate artery, and (D) interstitial space (H&E ×100). Plate 2: Illustrating (A) patchy tubular necrosis (H&E; ×100). Plate 3: Displaying (A) normal tubules and (B) glomerulus (H&E; ×100). Plate 4: Depicting (A) normal tubules and (B) glomerulus (H&E; ×100).

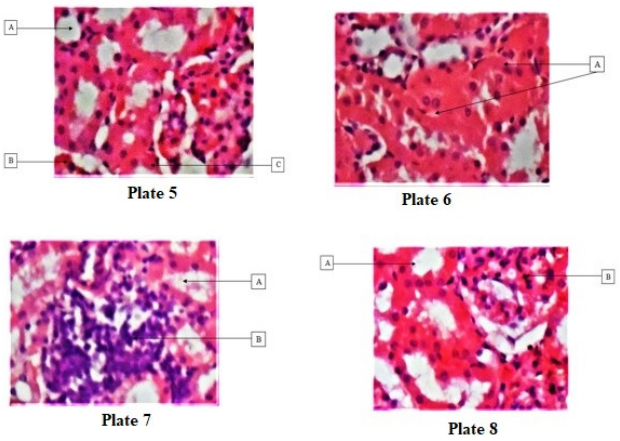

Figure 2. Plate 5: Displaying (A) normal tubules and (B) glomerulus (H&E; ×100). Plate 6: Showing (A) patchy tubular necrosis (H&E; ×100). Plate 7: Depicting (A) normal tubules and (B) dense infiltrates of lymphocytes (H&E; ×100). Plate 8: Illustrating (A) normal tubules and (B) normal glomerulus (H&E; ×100).

of the seed extract was studied to ascertain the dose range. There was no mortality during toxicity studies. In the acute toxicity study, single doses of the extract at 10, 100, and 1000 mg/kg bw were initially administered to three groups and then, two higher doses (3000 and 5000 mg/kg bw) were administered to two new groups, respectively. No observable physical manifestations were found following the administration of the first three doses. However, drowsiness was observed only in the group receiving 5000 mg/kg bw which cleared off within the first forty-eight hours of the fourteen days of monitoring. The report of this study based on the Hodge and Sterner toxicity scale indicated that this extract is non-toxic (31). The very frequent use of *A. angustifolium* seeds as a spice and a component of herbal mixtures with no immediate adverse effects support this observation. Fresh tomatoes and biscuits were preserved with the extracts of *A. danielli* at concentrations of ≥5% w/w with no reports on toxicity (32,33).

The sub-chronic administration of 1000, 2000, and 3000 mg/kg bw of the extract to Wistar rats inhibited average weight gain compared to the control group (Table 4). All animals in the experimental set-up were allowed free access to food and water during the study. A similar observation was previously reported, confirming that the average weight gain of all the rats receiving 300 mg/kg bw of the *A. melegueta* extract was significantly lower compared to the control group (34).

The assayed serum biomarkers following chronic exposure represented no significant alteration. Serum AST and ALP activities, as well as the TP elevated by the highest dose while demonstrating a reduction in the group that received 1000 mg/kg bw compared to the control. The concentrations of serum BIL, HCO_3^-, Na^+, K^+, and Cr, as well as SOD and CAT activities were not generally significantly different from the obtained results for the control group. The observed increase in AST and ALT in this study may indicate an alteration in hepatic cellular integrity. The *A. melegueta* extract has also been reported to be useful in the treatment of diabetes, the only concern being the elevation in the activities of liver enzymes (34,35). The reports on the hepatotoxicity of *Aframomum* species represent a difference. The administration of the *A. melegueta* extract at 200 and 400 mg/kg bw, respectively, showed a significant ($P<0.05$) reversal effect that palliated the deleterious effect of cadmium on the liver (36). The researchers suggested that the aqueous extracts of *A. melegueta,* when orally administered, could ameliorate cadmium-induced oxidative stress in male Wistar rats in a dose-dependent manner. There is a dearth of information on the nephrotoxicity of *A. angustifolium.* However, the sub-chronic administration of the aqueous extract of *A. melegueta* caused no apparent histological change in the kidney (37).

The nephrotoxicity of bromate is mediated by oxidative stress (1,38). The capacity of the extract from *A. angustifolium* seeds for inhibiting the oxidative process was the focal point of the research. The extracts from *Aframomum* species have antioxidant capacity (37,38). In the final phase of this study, bromate administered as $KBrO_3$ (30 mg/kg bw) was orally co-administered with two doses of the extract, namely, 350 (group C) and 750 mg/kg bw (group D). After twenty-eight days, the histological evaluation of kidney tissues from various groups revealed patchy tubular necrosis in group B. Groups C and D showed normal kidney microstructure comparable to group A (normal control). Serum Na^+ concentration and Cr significantly increased in group B compared to the control and the treated groups. The antioxidant activities of enzymes SOD and CAT also elevated in this group possibly due to the observed necrosis whereas the activity in the treated groups was not significantly different from the control group (Tables 5 and 6).

The histological analyses of the kidney tissue revealed a progression of tubular necrosis in group B after 56 days. There was no tubular necrosis in the kidney tissues from groups A, C, and D. The tubules in group C were densely infiltrated by lymphocytes suggesting the dose-dependent effect of the extract against bromate-induced oxidative stress in the kidney.

Chronic exposure to a mild dose or acute exposure to a lethal dose of bromate has been reported to inhibit the activities of antioxidant enzymes. The activities of SOD and CAT were not significantly inhibited in this study. The sustained activities support available data that 30 mg/kg bw is in the range classified as the least observable adverse effect level of the toxin. However, HCO_3^-, Na^+, K^+, and Cr significantly increased in group B (the positive control group) compared to the treated groups.

The result of this study indicated that the consumption of phytochemicals with antioxidant properties can protect rat kidneys from oxidative damage that can be induced by bromate. It is suggested that future studies evaluate the biochemical mechanism of the observed effects.

Authors' Contributions

NPO designed the experiment and supervised the work. IEE performed the experiment and wrote the manuscript.

Conflict of Interest Disclosures

None.

Ethical Issues

The treatment of the animals conformed to the guidelines of the Principles of Laboratory Animal Care (NIH Publication 85- 23, revised 1985).

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