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Original Research Article

Effect of Ethanol Extract of *Amaranthus Viridis* (Inine) on Potassium Bromide-Induced Haematoxicity in Wistar Rats

Innocent I. Ujah[1], Godwin S. Alor[2], Cosmas E. Achikanu[1], Onuabuchi N. Ani[1], Chukwunonso A. Nsude[1], Uchenna B. Alozieuwa[3], Jane I. Ugochukwu[4], Ebere I. Akpata[1], Justice C. Ude[1], Kenechukwu C. Onyishi[5], Innocent O. Okpako[6], Chukwuemeka O. Okechukwu[7], Obiora E. Nneji [1], Chikezie K. Ogbu[1], Chinenye A. Anieke[1] and Onyebuchi P. Ogbodo[1]

1. *Department of Applied Biochemistry, Faculty of Biological Sciences, Enugu State University of Science & Technology, Enugu State, Nigeria.*
2. *Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Nigeria.*
3. *Department of Biochemistry, Faculty of Natural & Applied Sciences, Veritas University Abuja, Nigeria.*
4. *Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria.*
5. *Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria.*
6. *Department of Molecular Biology and Biotechnology, Pan African University Institute for Basic Sciences, Technology and Innovation, Nairobi, Kenya.*
7. *Department of Food Science and Technology, Enugu State University of Science & Technology, Enugu State, Nigeria.*

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ABSTRACT

Potassium Bromate ($KBrO_3$) is an oxidative agent capable of causing hematological alterations and other toxic effects. *Amaranthus Viridis (Inine)* is a medicinal plant traditionally used for various therapeutic purposes. This study investigated the ameliorative effect of ethanol extract of *Amaranthus viridis* (Inine) on the haematological parameters in potassium bromate ($KBrO_3$) intoxicated Albino Rats. Thirty (30) rats divided into six (6) groups consisting of five rats each, group one as the normal control, group two as the negative control (50 mg/kg of $KBrO_3$ only), group three Vitamin C (100 mg/kg) + 50 mg/kg of $KBrO_3$and group four to six (200 mg/kg, 400 mg/kg, and 800 mg/kg) respectively. $KBrO_3$ exposure significantly altered some haematological indices in the rats indicating a haematotoxic effects. The results showed a dose-dependent improvement in several haematological parameters upon coadministration of *Amaranthis viridis* occurred. The red blood cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), and white blood cell count (WBC) exhibited notable recovery in comparison to the KBrO3-treated group. Platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) also demonstrated positive alterations in response to the extract. The group receiving the highest dose of the leaf extract (800 mg/kg) alongside $KBrO_3$ displayed the most significant improvement in the aforementioned parameters, indicating a potential dose-dependent protective effect against $KBrO_3$-induced haematotoxicity. These findings suggest the potential therapeutic efficacy of *Amaranthus viridis* extract in mitigating $KBrO_3$-induced hematological alterations.

Keywords: Ethanol Extract**,** *Amaranthus viridis,* Potassium Bromate ($KBrO_3$), Haematological Parameters, Albino Rats.

Introduction

Potassium bromate ($KBrO_3$) is a potent oxidizing agent used in various industries, particularly in bread-making processes, despite its well-documented adverse health effects[1]. Its exposure has been linked to renal damage, gastrointestinal disturbances, haematological alterations characterized by disruptions in red blood cell (RBC), white blood cell (WBC), and platelet counts along with changes in hemoglobin levels, haematocrit, and other crucial indices[2]. Such haematotoxic effects have prompted the exploration of potential interventions to mitigate $KBrO_3$-induced damage.

*Corresponding author. Email: j.chinecherem@yahoo.com
Tel: +2347014217837

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Amaranthus viridis, commonly known as "*Inine*" or slender amaranth, has gained attention due to its rich phytochemical composition, including flavonoids, polyphenols, and vitamins[3,4]. The medicinal properties of amaranthus species have been extensively studied, these properties are antioxidant, anti-inflammatory, and cytoprotective attributes[3]. Ethnopharmacological studies have reported the traditional use of *Amaranthus viridis* in treating various ailments, suggesting its potential therapeutic value in addressing health issues[5,6]. Haematological parameters serve as crucial indicators of an organism's physiological state, reflecting alterations in response to xenobiotic exposure, including toxic substances like $KBrO_3$[7,8]. While numerous studies have delved into the toxicological effects of $KBrO_3$, limited research has investigated potential interventions, such as herbal extracts, to reverse its haematotoxic effects. Previous research has highlighted the antioxidant and cytoprotective properties of various plant extracts in mitigating chemical-induced toxicities[9,10]. Thus, exploring the potential ameliorative effects of *Amaranthus viridis* extract on haematological parameters affected by $KBrO_3$ toxicity holds promise for developing novel therapeutic strategies. Several studies have underscored the importance of phytochemical compounds in combating oxidative stress and cellular damage induced by toxic agents[11]. The specific constituents within *Amaranthus viridis* extract, such as flavonoids and polyphenols, may exert protective effects through scavenging free radicals, modulating oxidative pathways, and

enhancing endogenous antioxidant defenses[12,13]. Through *in vivo* experiments, this research seeks to assess the protective potential of *Amaranthus viridis* extract against $KBrO_3$-induced haematotoxicity, focusing on key haematological indices including RBC count, PCV, Hb concentration, WBC count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). In this context, this study will contribute to the existing knowledge by elucidating the potential protective role of *Amaranthus Viridis* extract against $KBrO_3$-induced hematotoxicity, paving the way for future investigations into its underlying mechanisms and potential clinical applications. .

Materials and Methods

Sample Collection and Preparation of Plant Material

Fresh stems of *Amaranthus viridis* (green leaf) were collected from Umudike in Ikwuano Local Government Area of Abia State on the 8th of May 2024 and were authenticated at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike. The collected stems were chopped into smaller particles and air dried under shade on a laboratory bench over a period of 48 days before being pulverized into powder using a blender. The crude extract was prepared from the powdered material. The cold maceration technique was adopted in the preparation of plant extract. A quantity, 500 g of the powdered material was macerated in 2 litres of ethanol for 48 hours and was thereafter filtered with a Whatman filter paper. The filtrate was then concentrated to dryness in a laboratory oven at low temperature (40 ºC) to obtain a dark green pasty extract which weighed (26.89 g) and represented a 5.38% extract yield. The process was repeated until sufficient quantity of extract for the entire study was obtained.

Percentage yields in both cases were calculated using the formula:

$$\% \text{ yield} = \frac{Q}{W} \times \frac{100}{1}$$

Where Q is weight of extract obtained after extraction and W is weight of pulverized plant material macerated in methanol. The extract was preserved in the refrigerator at low temperature until use.

Acute toxicity studies

For the acute toxicity evaluation of both the crude extract and potassium bromate, the new[14] method used by[15] was adopted with modifications.

Acute toxicity evaluation of potassium bromate

Two stages were involved in the test. In the first stage, 9 Wistar rats were assigned to 3 groups (A, B and C) of 3 rats each were administered 10, 100 and 200 mg/kg of the extract respectively. The animals were thereafter monitored for the manifestations of toxicity signs and deaths within 24 hours. With zero mortality recorded, the study proceeded to the second phase which also involved the use of 9 rats assigned to 3 groups (A-C). Single oral treatment doses assigned to the groups were 400, 600 and 800 mg/kg respectively. The animals were again monitored for toxicity signs and deaths within 24 hours.

Acute toxicity value for the test agent was then calculated from the number of deaths recorded across the groups using Lorke's formula stated as:

$LD50 = \sqrt{(A \times B)}$

A= Maximum dose that produced no mortality

B= Minimum dose that killed all animals in a group

Acute toxicity evaluation of green leaf extract

In this case three stages of tests were involved. In the first stage, 9 Wistar rats assigned to 3 groups (A, B and C) of 3 rats each were administered 10, 100 and 1000 mg/kg of the extract respectively. The animals were thereafter monitored for the manifestations of toxicity signs and deaths within 24 hours. With zero mortality recorded, the study proceeded to the second phase which also involved the use of 9 rats assigned to 3 groups (A-C). Single oral treatment doses assigned to the groups were 1600, 2900 and 5000 mg/kg respectively. The animals were again monitored for toxicity signs and deaths within 24 hours. When no mortality was still observed, the highest dose (5000 mg/kg) used was repeated on another set of 3 rats during the confirmatory test stage and were monitored for mortalities within 24 hours and a further 7 days.

Acute toxicity value for the test agent was then calculated from the number of deaths recorded across the groups using Lorke's formula stated as:

$LD50 = \sqrt{(A \times B)}$

A= Maximum dose that produced no mortality

B= Minimum dose that killed all animals in a group

Experimental design for the evaluation of the effect of the crude extract of green leaf on potassium bromate induced toxicity in rats

Thirty (30) adult male Wistar rats assigned to 6 groups of 5 rats each were treated according to the order below:

Group 1: Normal control
Group 2: Negative control (50 mg/kg of KBrO3 only)
Group 3: Green leaf extract (200 mg/kg) + 50 mg/kg of KBrO3
Group 4: Green leaf extract (400 mg/kg) + 50 mg/kg of KBrO3
Group 5: Green leaf extract (800 mg/kg) + 50 mg/kg of KBrO3
Group 6: Vitamin C (100 mg/kg) + 50 mg/kg of KBrO3

All treatments were via the oral route and lasted for 14 days. Body weights were taken at the beginning and at the end of the treatment (days 1 and 14) using an electronic balance (DJ-A1000, China). At the end of the period, animals were sacrificed and blood and tissue samples were collected for the various haematological and biochemical analysis carried out.

Determination of haematological parameters

Haematological analysis of the blood samples was performed in an automated haematology analyzer (BC-2300 model, Mindray Medical Co., China) with the procedure carried as specified by the producer. The parameters which were evaluated included: red blood cells count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); platelets (PLT); (WBC) counts were obtained at once for each blood sample.

Principles of Haematological Analyzer

The haematological parameters were determined using the automated haematology analyzer according to the methods by[16], modified by[17,18] following the procedure outlined by the producer;

Procedure

To analyze a whole blood sample, the sample was presented to the diluent dispenser and the diluent key was pressed to aspirate 20µL of the sample into the dispenser. A diluted sample (about 1:300) was dispensed when the diluent key was pressed again. The sample was thoroughly mixed and presented under the suction nozzle, then the count key was pressed to aspirate into the analyzer for analysis and the result was displayed on the screen after few seconds.

Statistical analysis

Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc, Chicago, IL) was used for data analysis. Level of significance was calculated by One Way Analysis of Variance (ANOVA). Data were analyzed using Duncan Multiple Range Test and complemented with Student's t test for post-hoc test for comparisons of the means of the various doses and fractions. All data were expressed as mean ± standard deviation. $P \leq 0.05$ values or less were considered to indicate statistically significant difference between the test and control groups as well as among test groups for measured value.

Results and Discussion

Acute toxicity evaluation of the extract

No mortality was recorded in any group at all stages of the acute toxicity test, even at 5000 mg/kg. All animals instead retained their physical activities and showed no signs of toxicity (Tables 1A & 1B). The results of the phase 1 acute toxicity for potassium bromate showed there was no acute toxicity or mortality in animals at doses up to 200 mg/kg (Table 2A). For the phase 2 acute toxicity of potassium bromate, there was toxicity in

the animals at doses above 260 mg/kg, with a lethal dose (LD_{100}) of 600 mg/kg (Table 2B) Hence, the conclusion that acute toxicity value was greater than 5000 mg/kg in accordance with established international protocols for acute toxicity testing.

Table 1A: Phase 1 Acute Toxicity Evaluation of the Extract

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Animals were active and physically balanced
2	100	0/3	Animals were active and physically balanced
3	1000	0/3	Animals were active and physically balanced

Table 1B: **Phase 2 Acute Toxicity Evaluation of the Extract**

Group	Dose (mg/kg)	No. of death	Observation
1	1600	0/3	Animals were active and physically balanced
2	2900	0/3	Animals were active and physically balanced
3	5000	0/3	Animals were calm and physically inactive for a moment but soon regained physical activity.

Conclusion
LD_{50} > 5000 mg/kg extract may be safe for oral use.

Acute toxicity evaluation of potassium bromate
The acute toxicity evaluation of Potassium Bromate was conducted in two phases. In Phase 1, doses of 10, 100, and 200 mg/kg were administered, resulting in no deaths and normal physical activity in the animals. However, in Phase 2, higher doses of 260, 400, and 600 mg/kg were given, leading to increased mortality rates, with 1/3, 2/3, and 3/3 of the animals dying, respectively. As the dose increased, the animals became progressively calm, physically inactive, and eventually deceased.

Table 2a: Phase 1 Acute Toxicity Evaluation of Potassium Bromate

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Animals were active and physically balanced
2	100	0/3	Animals were active and physically balanced
3	200	0/3	Animals were active and physically balanced

Effects of the Extract On Haematological Parameters in Potassium Bromate Treated Rats
The effects of Amaranthus leaf extract on haematological parameters in potassium bromate-treated rats were investigated and shown in table 3. The results showed that potassium bromate (100 mg/kg) significantly altered haematological parameters, including reduced RBC, PCV, Hb, WBC, and PLT counts. However, co-administration of vitamin C (100 mg/kg) or Amaranthus leaf extract (200-800 mg/kg) with potassium bromate (50 mg/kg) mitigated these alterations, with the extract showing a dose-dependent protective effect. The highest dose of Amaranthus leaf extract (800 mg/kg) almost restored the haematological parameters to normal levels, indicating its potential to counteract the haematotoxic effects of potassium bromate.

Table 2b: Phase 2 Acute Toxicity Evaluation of Potassium Bromate

Group	Dose (mg/kg)	No. of death	Observation
1	260	1/3	Animals were calm and physically inactive for a moment but soon regained physical activity.
2	400	2/3	Animals were calm and physically inactive
3	600	3/3	Animals were inactive/deceased.

Acute toxicity values calculated using Lorke's formula stated as:

$LD_{50} = \sqrt{A\ x\ B}$
A= Maximum dose that produced no mortality
B= Minimum dose that killed all animals in a group
$LD_{50} = \sqrt{200\ x\ 600}$
= 346.41 mg/kg body weight

The data presented in Table 3 illustrated the effect of different treatments, particularly *Amaranthus viridis* extract in varying doses, on several crucial haematological parameters in rats subjected to potassium bromate ($KBrO_3$) toxicity. This comprehensive analysis provides insights into the potential protective role of *Amaranthus Viridis* extract against $KBrO_3$-induced hematotoxicity, shedding light reversal of $KBrO_3$-induced reductions in red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb) concentration, white blood cell (WBC) count, platelet count (PLT), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC)[19,20]. However, the administration of *Amaranthus viridis* extract showed a dose-dependent restoration of RBC counts towards normal control levels, suggesting its protective effect against $KBrO_3$-induced anaemic effect[21]. Studies have shown that antioxidant-rich plant extracts can mitigate haematotoxic effects. Previous research highlighted that flavonoids in plant extracts protected against oxidative damage to blood cells[22]. Similar protective effects of plant extracts on haematological parameters have been observed[23]. PCV is significantly reduced by $KBrO_3$ exposure, indicating anaemia or reduced erythropoiesis[24]. *Amaranthus viridis* extract restored PCV, demonstrating its potential in protecting against anemia induced by oxidative stress[25]. Similar protective effects on PCV have been reported by other researchers. For instance,[26] noted that plant-derived antioxidants help maintain haematocrit levels under oxidative stress conditions. $KBrO_3$ significantly reduces Hb concentration, indicating impaired oxygen transport capacity. *Amaranthus viridis* extract showed a dose-dependent increase in Hb levels, suggesting its role in enhancing erythropoiesis and haemoglobin synthesis. Protective effects on Hb levels have been reported by[27], who demonstrated that plant extracts with antioxidant properties can improve haemoglobin concentrations in

oxidative stress models. $KBrO_3$ exposure causes a slight decrease in WBC count, possibly indicating an impaired immune response[28]. *Amaranthus viridis* extract normalize WBC counts, indicating its potential immunomodulatory effects.

Table 3: Effects of the Extract on Haematological Parameters in Potassium Bromate Treated Rats

Treatment	RBC ($x10^6/mm^3$)	PCV (%)	Hb (g/dl)	WBC ($x10^3/mm^3$)	PLT ($x10^3/mm^3$)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	7.17±0.17^{f}	45.00±1.00^{e}	15.60±0.53^{e}	8.57±0.28^{b}	248.33±7.51^{c}	62.76±0.38^{a}	21.75±0.23^{a}	34.66±0.52^{b}
$KBrO_3$ (100 mg/kg)	4.18±0.16^{a}	29.00±1.73^{a}	9.53±0.25^{a}	7.93±0.20^{a}	228.67±2.52^{a}	69.35±2.31^{d}	22.81±0.29^{b}	32.93±1.41^{a}
Vit. C (100 mg/kg) + $KBrO_3$ (50 mg/kg)	4.91±0.04^{b}	32.33±0.58^{b}	10.93±0. 25^{a}	8.06±0.50^{ab}	239.00±4.58a^{bc}	65.85±0.76^{bc}	22.27±0.43^{ab}	33.81±0.33^{ab}
***Amaranthus* leaf extract (200 mg/kg) + $KBrO_3$ (50 mg/kg)**	5.72±0.20^{c}	38.33±1.55^{c}	12.83±0.45^{c}	8.17±0.08^{ab}	240.00±4.58^{bc}	66.99±0.33^{c}	22.43±0.41^{ab}	33.48±0.64^{ab}
***Amaranthus* leaf extract (400 mg/kg) + $KBrO_3$ (50 mg/kg)**	6.25±0.12^{d}	41.67±0.58^{d}	13.93±0.35^{d}	8.26±0.19^{ab}	234.67±8.33^{ab}	66.64±0.75^{c}	22.28±0.13^{ab}	33.44±0.53^{ab}
***Amaranthus* leaf extract (800 mg/kg) + $KBrO_3$ (50 mg/kg)**	6.76±0.28^{e}	43.33±0.58^{de}	14.57±0.32^{d}	8.51±0.29^{b}	238.67±4.51^{abc}	64.12±1.98^{a}	21.56±1.10^{a}	33.62±0.72^{ab}

Values are presented as mean ± standard deviation of replicated determination (n = 3). Means in the same column bearing different letter superscripts are statistically significantly different.

Similar findings where plant extracts with anti-inflammatory properties normalized WBC counts in inflammatory models have been reported[29]. $KBrO_3$ significantly reduces PLT count, which may impair blood coagulation. The extract significantly normalized PLT counts, indicating its possible role in maintaining haemostasis. Similar results were found by [30], who reported that plant extracts could improve platelet counts and functionality in models of induced thrombocytopenia. $KBrO_3$ increases MCV, suggesting the presence of macrocytic anaemia. The extract shows a trend towards reducing MCV, indicating its potential in normalizing red blood cell size. Similar trends were observed by [31], who found that antioxidant compounds could mitigate increases in MCV associated with oxidative stress. $KBrO_3$ slightly increased MCH, suggesting changes in haemoglobin content per cell. The extract normalized MCH values, indicating its role in maintaining haemoglobin synthesis and incorporation[32]. Dietary bioactive compounds could normalize MCH levels under stress conditions, supporting these findings. $KBrO_3$ significantly reduced MCHC, suggesting hypochromic anaemia[33]. The extract shows a trend towards normalizing MCHC, indicating its role in ensuring adequate haemoglobin content in red blood cells. Plant extracts with high antioxidant activity have been shown to normalize MCHC levels in oxidative stress models, aligning with these results[34].

Conclusion

The study demonstrates that ethanol extract of *Amaranthus viridis* significantly ameliorates haematological disturbances caused by potassium bromate intoxication in albino rats. This protective effect is evidenced by the restoration of RBC count, PCV, Hb levels, WBC count, PLT count, MCV, MCH, and MCHC towards normal values, highlighting its potential as a therapeutic agent against $KBrO_3$-induced haematotoxicity.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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