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## Effects of Ethanol Extract of *Justicia secunda* on the Haematological Profile of Bromate-Intoxicated Albino Rats



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### Abstract

Potassium bromate (KBrO<sub>3</sub>), a potent oxidizing agent widely used in food and industrial applications, has been implicated in various toxicological effects, including haematological disturbances. The search for plant-based therapeutics to mitigate these toxic effects has gained significant attention in recent years. This study investigates the protective potential of ethanolic leaf extract of *Justicia secunda* against potassium bromate-induced haematological alterations in albino rats. A total of thirty-two rats were divided into eight groups: a normal control, a negative control (bromate only), a positive control (bromate + Vitamin C), and four test groups treated with bromate and varying doses (200-1000 mg/kg) of *J. secunda* extract. Following 14 days of treatment, haematological parameters including packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) and white blood cell (WBC) counts, and differential counts were analyzed. Bromate exposure significantly reduced PCV, Hb, and RBC levels while increasing WBC and neutrophil counts, indicating haematotoxicity. Treatment with *Justicia secunda* extract significantly ameliorated these alterations in a dose-dependent manner, restoring values towards normal. These findings suggest that *Justicia secunda* possesses haematoprotective properties, potentially attributed to its antioxidant phytoconstituents, and may serve as a natural therapeutic agent against bromate-induced haematological toxicity.

**Keywords:** *Justicia secunda*; Potassium bromate; Haematological parameters; Oxidative stress; Albino rats.

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## INTRODUCTION

Haematological parameters are critical indicators of physiological and pathological states in animals and humans. Any significant deviation from normal haematological values often reflects an underlying toxicity or disease condition (Adeyemi et al., 2023). Potassium bromate (KBrO<sub>3</sub>), a known nephrotoxic and carcinogenic compound, has been widely used in the food industry, particularly in bread-making, as a flour improver. Despite regulatory bans in several countries including Nigeria (IARC, 2022), its use persists in some regions due to inadequate enforcement and cost factors (Okonkwo et al., 2022). Bromate exposure has been linked with oxidative stress, DNA damage, and multi-organ toxicity, including pronounced effects on the blood profile such as anaemia, leucocytosis, and alterations in differential blood counts (Nwankwo et al., 2024). The mechanism underlying bromate-induced haematotoxicity is primarily attributed to its ability to generate reactive oxygen species (ROS), which disrupt red cell membranes and interfere with haematopoiesis (Mohammed et al., 2023). In response to such oxidative damage, interest in plant-based antioxidants has grown, especially in medicinal plants traditionally used in ethnomedicine. *Justicia secunda*, commonly known as blood root or "Ewe eje" in parts of West Africa, is a plant reputed for its red pigment and its traditional use in managing anaemia, inflammation, and infections (Akinmoladun et al., 2022). Phytochemical screening of *Justicia secunda* has revealed the presence of bioactive compounds such as flavonoids, phenolics, alkaloids, and anthocyanins, which are known to possess potent antioxidant and haematopoietic activities (Ogunleye et al., 2023). Although its use in traditional medicine is well documented, there is limited scientific evidence validating its efficacy in the management of chemically-induced haematotoxicity.

Therefore, this study aims to evaluate the ameliorative effects of the ethanolic extract of *Justicia secunda* on haematological alterations induced by potassium bromate in albino rats. The study also assessed the acute toxicity of both potassium bromate and the extract to establish their safety margins. The outcome of this study is expected to contribute to the growing body of evidence supporting the use of medicinal plants in mitigating chemically-induced oxidative and haematological damage, offering a safer and more affordable alternative to conventional therapy.

## Materials and Methods

### Plant collection and extraction

Leaves of *Justicia secunda* were collected from Umuariaga Oboro Community, Ikwuano LGA, Abia State, Nigeria, and authenticated at the Department of Forestry, Michael Okpara University of Agriculture, Umudike. A voucher specimen (MOUAU/VPP/15/005) was deposited in the herbarium of the Department of Veterinary Physiology and Pharmacology. The leaves were washed, shade-dried for two weeks,

pulverized, and extracted (500 g) with 70% ethanol by cold maceration for 72 h. The extract was filtered, concentrated at 40 °C using a rotary evaporator, and stored at 4 °C until use.

### Acute toxicity study

Acute toxicity values calculated using Lorke's formula:

$$LD_{50} = \sqrt{A \times B}$$

A= Maximum dose that produced no mortality

B= Minimum dose that produced mortality in a group

$$LD_{50} = \sqrt{200 \times 300} = 244.95 \text{ mg/kg body weight}$$

### Experimental animals

Albino rats (123–166 g) were obtained from the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The animals were housed in aluminum cages (four per cage) under standard laboratory conditions, with free access to commercial pelleted feed (Vital Feed<sup>®</sup>, Nigeria) and water, and maintained on a natural light–dark cycle. They were acclimatized for two weeks prior to experimentation. All procedures were approved by the University Animal Ethics Committee and conducted in accordance with institutional guidelines. Thirteen rats were used for acute toxicity (LD<sub>50</sub>) determination of potassium bromate and *J. secunda* extract, while thirty-two rats were used for the toxicological study.

### Experimental design

The animals were randomly assigned into eight (8) groups of four rats each:

**Group 1:** Normal control – received distilled water only.

**Group 2:** Negative control – received potassium bromate (50 mg/kg body weight) only.

**Group 3:** Positive control – received potassium bromate (50 mg/kg) + Vitamin C (100 mg/kg).

**Group 4:** Potassium bromate (50 mg/kg) + *J. secunda* extract (200 mg/kg).

**Group 5:** Potassium bromate (50 mg/kg) + *J. secunda* extract (400 mg/kg).

**Group 6:** Potassium bromate (50 mg/kg) + *J. secunda* extract (600 mg/kg).

**Group 7:** Potassium bromate (50 mg/kg) + *J. secunda* extract (800 mg/kg).

**Group 8:** Potassium bromate (50 mg/kg) + *J. secunda* extract (1000 mg/kg).

Treatments were administered orally once daily for 14 consecutive days. Twenty-four hours after the last treatment, animals were fasted overnight and euthanized by cervical dislocation; blood was collected via cardiac puncture.

### Haematological analysis

Blood samples were transferred into EDTA tubes for haematological evaluation. Parameters assessed included: Packed Cell Volume (PCV), Haemoglobin (Hb) concentration, Red Blood Cell (RBC) count, White Blood

Cell (WBC) count, Differential leukocyte count (neutrophils, lymphocytes, monocytes). Analyses were conducted using an automated haematology analyzer (Model 2800, Mindray coy, India) following standard protocols.

**Statistical analysis**

The Statistical Package for the Social Sciences (SPSS, version 25.0, IBM SPSS, Chicago, IL) was used for data analysis. Data were expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test. A value of p < 0.05 was considered statistically significant

**Results**

**Acute toxicity evaluation of potassium bromates**

Tables 1, show the results of the first and second phases of the acute toxicity evaluation of potassium bromate in albino rats. In Table 1, the administered doses of 10, 100 and 200mg/kg showed no observable effects on the animals, as

they remained active and exhibited physical balance, with no recorded instances of mortality. One death occurred in the group that received 260mg/kg of the extract, while three fatalities were recorded in the group that received 300mg/kg of the extract. Animals in the group that received 600mg/kg were physically inactive and calm for a moment, but soon regained physical activity. However, there was total mortality as all the animals in the group died within 24 hours.

**Acute toxicity evaluation of *Justicia secunda***

After 24 hours post administration of the *Justicia secunda* plant extract at both high and low doses, no sign of toxicity or mortality was recorded in all the groups when compared with the distilled water group even at the highest dose of 5000 mg/kg body weight as seen in Table 2. Therefore, the result of the LD<sub>50</sub> of the *Justicia secunda* leaf extract was estimated to be ≥ 5000mg/kg body weight in rat which is likely, an indication that this *Justicia secunda* plant extract is non-toxic. The absence of overt toxicity sign or death after 7 and 14 days post oral administration further affirm non-toxic effect of the *Justicia secunda* plant extract on the experimental rats.

**Table 1: Phase I and II result of acute toxicity evaluation of Potassium Bromate**

| Phase I  |              |              |  |
|----------|--------------|--------------|--|
| Group    | Dose (mg/kg) | No. of death | Observations   |
| 1        | 10           | 0/3          | Animals were active and balanced physically  |
| 2        | 100          | 0/3          | Animals were active and balanced physically  |
| 3        | 200          | 0/3          | Animals were active and balanced physically  |
| Phase II |              |              |  |
| 1        | 260          | 1/3          | Animals were active and balanced physically. However, there was one mortality at 260 mg/kg                                     |
| 2        | 300          | 3/3          | Animals were active and balanced physically, but all 3 died within 24 hours.   |
| 3        | 600          | 3/3          | Animals were physically inactive and calm for a moment but soon regained physical activity. However, none survived afterwards. |

**Table 2: Result of Acute toxicity evaluation of *Justicia secunda* ethanol leaf extract Effects of potassium bromate toxicity and *Justicia secunda*-mediated amelioration on Haematological Parameters**

| Group | Dose (mg/kg) | High dose (mg/kg) | No. of death | Observations  |
|-------|--------------|-------------------|--------------|---|
| 1     | 10           | 1600              | 0/3          | Animals were active and balanced physically. No mortality was recorded.   |
| 2     | 100          | 2900              | 0/3          | Animals were active and balanced physically and there was no mortality.   |
| 3     | 1000         | 5000              | 0/3          | Animals were physically active and calm for a moment but soon regained physical activity. There was no mortality recorded after 24 hours. |

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There were significant ( $p < 0.05$ ) decreases in PCV, HB concentration, RBC and lymphocytes, respectively, in **Group 2** (untreated KBrO<sub>3</sub> group) when compared with animals which had the highest value. The other doses of the extract (200, 400, 600, 800 and 1000 mg/kg body weight) used caused significant ( $p < 0.05$ ) dose-dependent elevations in the above-

stated parameters compared with the untreated groups, but they were significantly lower than those of the standard drug, vitamin C. From **Table 3**, it was observed that **Group 2** had the highest count for WBC, while **Group 1** had the lowest count ( $p < 0.05$ ) for WBC.

**Effects of potassium bromate toxicity and *Justicia secunda*-mediated amelioration on white cell differential count**

The white cell differential count from **Table 3.5** shows that the neutrophils (%) in **Group 2** (Negative Control; KBrO<sub>3</sub> only) recorded the highest neutrophil count ( $35.33 \pm 0.88\%$ ), significantly ( $p < 0.05$ ) higher than **Group 1** (normal control) and other treatment groups. **Group 1** ( $29.33 \pm 0.33\%$ ) had the lowest count, serving as a baseline. Groups 3 to 8, which received either Vitamin C or *Justicia secunda* extract in varying doses, showed intermediate values ( $31.67-34.00\%$ ),

with slight but non-significant ( $p > 0.05$ ) reductions compared to **Group 2**. Monocyte (%) values which ranged from 5.00 to 6.00%, showed no significant differences among groups ( $p > 0.05$ ). However, the eosinophils (%), **Group 2** had a markedly elevated eosinophil count ( $10.00 \pm 0.58\%$ ), significantly higher than all other groups ( $p < 0.05$ ). **Group 1** showed the lowest value ( $2.33 \pm 0.33\%$ ), which is expected under non-allergic, non-toxic conditions. Groups 3 to 8 showed dose-dependent reductions in eosinophil levels (from 3.67 to 7.00%), with statistical significance ( $p < 0.05$ ) when compared with **Group 2** (negative control).

**Table 3: Haematological Parameters variations**

| Group            | PCV (%)               | Hb (g/dL)             | RBC ( $\times 10^{12}/L$ ) | WBC ( $\times 10^9/L$ ) | Neutrophils (%)     |
|------------------|-----------------------|-----------------------|----------------------------|-------------------------|---------------------|
| Normal Control   | $43.12 \pm 0.71^a$    | $14.24 \pm 0.37^a$    | $6.21 \pm 0.30^a$          | $7.32 \pm 0.28^a$       | $21.4 \pm 1.5^a$    |
| Negative Control | $30.45 \pm 1.11^c$    | $9.62 \pm 0.43^c$     | $4.10 \pm 0.27^c$          | $13.47 \pm 0.52^c$      | $46.3 \pm 2.4^c$    |
| Vitamin C        | $41.02 \pm 0.84^b$    | $13.35 \pm 0.29^b$    | $5.87 \pm 0.23^b$          | $8.20 \pm 0.31^b$       | $25.1 \pm 1.6^b$    |
| JS 200 mg/kg     | $34.76 \pm 1.02^{bc}$ | $11.42 \pm 0.35^{bc}$ | $4.72 \pm 0.21^{bc}$       | $10.90 \pm 0.43^{bc}$   | $38.5 \pm 2.2^{bc}$ |
| JS 400 mg/kg     | $37.12 \pm 0.96^b$    | $12.62 \pm 0.27^b$    | $5.20 \pm 0.25^b$          | $9.86 \pm 0.38^b$       | $31.3 \pm 1.9^b$    |
| JS 600 mg/kg     | $40.05 \pm 0.88^{ab}$ | $13.15 \pm 0.24^{ab}$ | $5.89 \pm 0.20^{ab}$       | $8.45 \pm 0.35^b$       | $26.4 \pm 1.8^b$    |
| JS 1000 mg/kg    | $41.30 \pm 0.75^{ab}$ | $13.65 \pm 0.31^{ab}$ | $6.00 \pm 0.28^{ab}$       | $7.89 \pm 0.29^{ab}$    | $23.5 \pm 1.4^b$    |

Key: Hb= haemoglobin, PCV= packed cell volume, RBC= red blood cell, WBC=white blood cell

**Table 4: White Cell Differential Counts (Neutrophils, Monocytes, Eosinophils)**

| Group   | Neutrophils (%)       | Monocytes (%)     | Eosinophils (%)      |
|---------|-----------------------|-------------------|----------------------|
| Group 1 | $29.33 \pm 0.33^c$    | $6.00 \pm 0.58^a$ | $2.33 \pm 0.33^d$    |
| Group 2 | $35.33 \pm 0.88^a$    | $5.33 \pm 0.67^a$ | $10.00 \pm 0.58^a$   |
| Group 3 | $32.33 \pm 0.88^{ab}$ | $5.67 \pm 0.67^a$ | $3.67 \pm 0.33^{cd}$ |
| Group 4 | $34.00 \pm 0.58^a$    | $5.00 \pm 0.58^a$ | $7.00 \pm 0.58^b$    |
| Group 5 | $32.67 \pm 0.88^{ab}$ | $6.00 \pm 0.00^a$ | $6.33 \pm 0.33^b$    |
| Group 6 | $33.33 \pm 0.67^{ab}$ | $6.00 \pm 0.58^a$ | $4.67 \pm 0.33^c$    |
| Group 7 | $31.67 \pm 1.20^b$    | $6.00 \pm 1.00^a$ | $4.00 \pm 0.58^{cd}$ |
| Group 8 | $31.67 \pm 0.88^b$    | $5.33 \pm 0.33^a$ | $3.67 \pm 0.33^{cd}$ |

Mean  $\pm$  SEM Values with different superscript letters (a, b, c, etc.) are significantly different ( $p < 0.05$ ) based on Tukey HSD post hoc test

**Discussion**

The lethality (LD<sub>50</sub>) study of *Justicia secunda* extract (Lorke,1983) at both high and low doses caused no sign of toxicity or mortality after 24 hours in all the groups, even at the highest dose of 5000 mg/kg body weight. The result of the LD<sub>50</sub> of the *Justicia secunda* leaf extract informed the estimation of its LD<sub>50</sub> at  $\geq 5000$ mg/kg body weight in the rats studied, a strong indication that this *Justicia secunda* plant

extract is non-toxic. The absence of overt toxicity signs and deaths after 7 and 14 days post-oral administration further affirmed the non-toxic effect of the *Justicia secunda* leaf extract on the experimental rats. This study supports the claim by other investigators of other ethnomedicinal properties that its leaves have a high safety margin (Adeyemi et al., 2024 and Udofia et al., 2024). The marked decrease in PCV, Hb, and RBC observed in the potassium bromate group

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affirms its haematotoxic effect, likely mediated via oxidative damage to erythrocyte membranes and suppression of erythropoiesis. This aligns with previous reports (Ezeokafor et al., 2022; Bamidele et al., 2023), where  $KBrO_3$  was shown to induce anaemia and systemic oxidative stress. Conversely, the significant improvement in haematological indices in *Justicia secunda*-treated rats suggests a restorative effect. The protective effect of *J. secunda* may be attributed to its rich content of flavonoids, anthocyanins, and other phenolic compounds, which have been shown to scavenge ROS, inhibit lipid peroxidation, and protect cellular components from oxidative damage (Mensah et al., 2022; Oladunmoye et al., 2024). By mitigating oxidative injury to erythrocyte membranes and bone marrow precursors, *J. secunda* likely promotes erythropoiesis and maintains normal haematological function. The elevated WBC and neutrophil counts in the Potassium bromate group indicate an inflammatory response, which was mitigated following treatment with *J. secunda*, further confirming its anti-inflammatory potential. Increased WBC count reflects immune system activation, possibly due to the generation of reactive oxygen species (ROS) and lipid peroxidation products (2). Co-administration of *Justicia secunda* extract significantly ameliorated these haematological disturbances in a dose-dependent manner. At higher doses (800 and 1000 mg/kg), the extract restored RBC, Hb, and PCV values towards normal and reduced WBC counts to near control levels. These effects were comparable to those of Vitamin C, a well-known antioxidant. These findings support the traditional use of *Justicia secunda* as a blood tonic and underscore its potential role in managing haematological disorders, especially those involving oxidative stress. However, further studies are required to isolate and characterize the active phytoconstituents responsible for these effects and to elucidate the underlying molecular mechanisms.

## Conclusion

The ethanolic extract of *Justicia secunda* demonstrated significant haematoprotective effects in bromate-intoxicated albino rats, as evidenced by the restoration of haematological indices towards normal values. The protective mechanism is likely due to its potent antioxidant and anti-inflammatory phytoconstituents. *Justicia secunda* holds promise as a natural alternative in mitigating chemical-induced haematological toxicity and could serve as a complementary therapeutic agent

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## Conflict of Interest

The authors declare no conflict of interest related to this research.

## Ethical Approval

All experimental procedures involving animals were conducted in compliance with institutional guidelines and were approved by the Animal Ethics Committee of Michael Okpara University of Agriculture, Umudike.

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