

Research Article

## Abnormal Haematological Profile caused by Potassium Bromate in Wistar Rats is corrected by *Parkia Biglobosa* Seed

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Many biological tissues and organs are affected by the toxicity of potassium bromate (KBrO<sub>3</sub>). The purpose of this study was to evaluate the *Parkia Biglobosa* (*P. Biglobosa*) seed's ability to treat KBrO<sub>3</sub>-induced haematological parameters derangement. After becoming accustomed to the lab, 24 Wistar rats were randomly assigned to groups A, B, C, and D. Group A was given distilled water to drink. Each of the groups in B, C, and D got 100 mg/kg of KBrO<sub>3</sub>. Also, for 28 days prior to sacrifice, groups C and D received 100 and 200 mg/kg of *P. biglobosa*, respectively. Blood was drawn, and the haemogram was examined using a haematology autoanalyzer. When KBrO<sub>3</sub> was added compared to the control, the results showed a substantial decrease in both haemoglobin concentration, packed cell volume (PCV), and red blood cell count from 17.26±2.84 g/dL, 39.73±2.58%, 5.12±0.83 x 10<sup>12</sup>/L to 13.25±1.25 g/dL, 27.93±1.44%, and 3.47±0.22 x 10<sup>12</sup>/L, respectively. The effect of KBrO<sub>3</sub> was dose-dependently counteracted by *P. biglobosa* treatments of 100 and 200 mg/kg body weight. However, there was no discernible difference in the MCV, MCH, and MCHC values between the control and test groups. Similar to how *P. biglobosa* reduced the effects of KBrO<sub>3</sub> in a dose-dependent manner, *P. biglobosa* also induced a substantial decrease in white blood cell count, its differentials, and platelet counts (P≤0.05). KBrO<sub>3</sub>-induced deranged haematological parameters were mitigated by *Parkia biglobosa* in a dose dependent manner. Care must be taken with the consumption of this addictive due to its numerous toxic effects. However, consumption of *P. biglobosa*, a tropical homemade food is recommended for families to benefit from the barrage of its health benefits. This will also alleviate the toxic effect of KBrO<sub>3</sub> if consumed inadvertently. Human clinical trial is needed to substantiate these findings.

**Keywords:** Haematological Parameters, *Parkia Biglobosa*, Potassium bromate

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### INTRODUCTION

When potassium bromide solution is electrolyzed or when bromine is infused into a warm potassium hydroxide solution, potassium bromate (KBrO<sub>3</sub>) is produced (Airaodion et al., 2023). It strengthens the dough and makes it bigger, and it is utilised in the United States (US) to improve flours. Due to its oxidising ability, if excess is applied to bread that is not adequately supported, residual

amounts may remain that are hazardous to the body (Kurokawa et al., 2010). When administered orally to humans or experimental animals, KBrO<sub>3</sub> is nephrotoxic and causes cancer in rats. According to studies, KBrO<sub>3</sub> causes thyroid follicular cell tumours, peritoneal mesotheliomas, and renal cell tumours (Airaodion et al. 2023; Kurokawa et al., 2010). Furthermore, research aiming at determining the route of carcinogenic action has shown that KBrO<sub>3</sub> is a full carcinogen, having both initiating and encouraging effects

on the development of rat kidney tumours (Kurokawa et al., 2010). Different organs and systems of the body are affected by potassium bromate. A recent report by Ugwu et al. (2022a) revealed that  $\text{KBrO}_3$  perturbed lipid profile of experimental rats. Another study showed that hepatic indicators were unhinged in the blood and liver cells by  $\text{KBrO}_3$  (Onyekachi et al., 2022).

Potassium bromate has been outlawed in some nations' food products: Canada, Nigeria, South Korea, Peru, the European Union, Argentina, Brazil, and a few other nations. China in 2005, India in 2016, and Sri Lanka in 2001 all enacted bans on it (Airaodion et al., 2019a). It has not, however, been outlawed in the United States. Before the Food, Drug, and Cosmetic Act's Delaney clause, which outlaws possibly carcinogenic compounds, took effect in 1958, the United States' Food and Drug Administration (FDA) approved the use of bromate. The FDA has, however, advised bakers to voluntarily discontinue using it since 1991. In California, using bromated flour necessitates the addition of a warning label (Airaodion et al., 2019b).

*Parkia biglobosa* is a dicotyledonous angiosperm in the Fabaceae family (Airaodion et al., 2019c; Thiombiano et al., 2012). It belongs to the group of vascular plants known as spermatophytes. It is a perennial deciduous plant that, under some conditions, can grow as high as 30 metres (Ntui et al., 2012). In West Africa, *P. biglobosa*, often known as African locust bean, is widely used. It is a tree of extreme importance in Burkina Faso as a source of food and money in some rural households (Termote et al., 2022). The species is indigenous to sub-Saharan Africa and is referred to as n'er'e in Francophone Africa (Hopkins, 2022). In Nigeria, the Igbos call it "Ogiri," the Yorubas call it "Iru," and the Hausas call it "Dorowa" (Onyeaghala et al., 2022). The use of the bean seeds as a soup seasoning and their monetary value have aided in their widespread acceptance.

In West Africa, notably Nigeria, the beans are commonly fermented to create "Dawadawa," a spice. The dark-colored flavour known as dawaddawa, is high in protein and lactose and widely used in local soups (Ezirim et al., 2022a; Achi, 2015; Wokoma and Aziagba, 2010). The roots of *P. biglobosa* and other parkia species, as well as paste and decoction, are used to treat a variety of skin conditions (Ajaiyeoba, 2010; Oladunmoye and Kehinde, 2011). After being soaked in water for an entire night, *P. Biglobosa* seeds are extracted by maceration. This is then filtered and used to treat diabetic mellitus (Erakhrumen et al., 2010). In folkloric medicine, it is used to treat leprosy, diarrhoea, bacterial infections, high blood pressure, and wound healing (Airaodion et al., 2020a; Ekperikpe et al., 2019). *P. Biglobosa* has significant metabolic advantages in Wistar rats. It was discovered to be hepatoprotective by reducing the rat's susceptibility to the liver-damaging effects of  $\text{KBrO}_3$  (Onyekachi et al., 2022).

Another investigation revealed that *P. biglobosa* attenuated the  $\text{KBrO}_3$ -induced coagulopathy in a dose-dependent manner (Ugwu et al., 2023). According to Ugwu et al. (2022b), *P. biglobosa* significantly raised the antioxidant levels in rats, reducing the oxidative damage brought about by potassium bromate. Ezirim et al. (2022a) showed that treatment with *P. biglobosa* prevented the testicular toxicity effect caused by  $\text{KBrO}_3$  in the rat.

It has been demonstrated that potassium bromate causes haemoglobin oxidation, oxidative stress, and impairment of the blood's antioxidant defence system (Ahmad and

Mahmood, 2016). However, this impact was lessened by taurine (Ahmad and Mahmood, 2016). Furthermore, Achukwu et al. (2009) found that  $\text{KBrO}_3$  caused thrombocytopenia in rats. However the investigation found no statistically significant differences between the test and control rats' mean cell haemoglobin concentrations, haematocrits, or total leukocyte counts. The same study suggested that consuming potassium bromate constantly and in large amounts is harmful to one's health (Achukwu et al., 2009). Despite NAFDAC's 2003 warning about the risk of employing  $\text{KBrO}_3$  as a flour enhancer, several studies in Nigeria have revealed that  $\text{KBrO}_3$  is still at the Bakeries' beck and call (Airaodion et al., 2019a,b). Researchers have recently proved that some food items in Nigeria could ameliorate the toxic effects of potassium bromate (El-Deeb and Abd-El-Hafez, 2015; Femi-Oloye et al., 2020). This study was therefore, aimed at assessing the sanative propensity of *P. Biglobosa* seed on  $\text{KBrO}_3$ -induced derangement on haematological parameters.

## MATERIALS AND METHODS

**Collection and Extraction of *Parkia Biglobosa*:** A botanist identified *P. biglobosa* seeds after purchasing them from a neighbourhood market in Ibadan, Nigeria. They were sundried and mechanically blended into powder (Moulinex). The extraction was performed using a soxhlet apparatus and ethanol as the solvent in accordance with the steps indicated by Airaodion et al. (2019b; 2020). About 10.20 percent yield extract was obtained after the ethanol was evaporated in a rotary evaporator at 35 oC. For subsequent investigation, the extract was kept in a refrigerator (4 oC).

**Animal Treatment:** Twenty-four mature male Wistar rats (*Rattus norvegicus*), weighing between 140 and 160 g, were used for the experiment. They spent seven (7) days getting used to the testing setting before the test. The rats were housed in cages made of wire mesh, and they had unrestricted access to rat food and water from the market. The animals were kept within normal ranges of temperature and humidity with 12-hour cycles of light and darkness. The Helsinki Declaration and the guidelines set by the Committee for the Purpose of Regulation and Supervision of Experiments on Animals were both adhered to during the conduct of this study, which was approved by the ethical committee on research at Abia State University, Uturu, Nigeria. The National Research Council's policy on using animals in research was also followed (NRC, 2011).

Groups A, B, C, and D of rats were created. Group A was given distilled water. For each kilogramme of body weight, animals in groups B, C, and D received 100 mg of potassium bromate. Also, groups C and D received dosages of 100 and 200 mg/kg of *P. biglobosa* seed extract respectively. Diethyl ether was used to gently sedate the animals for the final 24 hours of their 28-day therapy period before they were euthanized. The haemogram was evaluated using a haematology autoanalyzer after blood was taken through a cardiac puncture.

**Statistical Analysis:** Using one-way ANOVA and the Tukey post hoc test, the results were analyzed. P values of 0.05 were taken into consideration to be statistically

significant for all analyses, which were performed using the Graph Pad Prism software (version 8).

## RESULTS

Packed Cell Volume (PCV) and red blood cell count from  $17.26 \pm 2.84$  g/dL,  $39.73 \pm 2.58\%$ ,  $5.12 \pm 0.83 \times 10^{12}/L$  to  $13.25 \pm 1.25$ g/dL,  $27.93 \pm 1.44\%$ ,  $3.47 \pm 0.22 \times 10^{12}/L$  respectively ( $P < 0.05$ ) when  $KBrO_3$  was added compared to the control (table 1). The results showed a significant reduction in haemoglobin concentration, PCV, and red cell count from  $17.26 \pm 2.84$  g/dL,  $39.73 \pm 2.58\%$ ,  $5.12 \pm 0.83 \times 10^{12}/L$  in control animals to  $13.25 \pm 1.25$ g/dL,  $27.93 \pm 1.44\%$ ,  $3.47 \pm 0.22 \times 10^{12}/L$  respectively in animals exposed to  $KBrO_3$  only (Table 1). Treatment with 100 and 200 mg/kg body weight of *P. biglobosa* neutralized the effect of  $KBrO_3$  in a dose-dependent manner. However, MCV, MCH and MCHC values showed no significant difference ( $P > 0.05$ ) in the control and test groups (Table 1). Similarly,  $KBrO_3$  caused significant reduction in White blood cell count, its differentials and platelet count which was also mitigated in a dose-dependent manner by *P. biglobosa* as shown in Table 2.

## DISCUSSION

The main role of red blood cells is to carry carbon dioxide to the lungs for expiration and oxygen across the body for metabolism. Haemoglobin is a special protein found in the red cell and a vehicle that the red cell uses to perform these functions (Airaodion and Ogbuagu, 2020). The purpose of platelets, also known as thrombocytes, a blood component,

is to clump in response to bleeding caused by blood vessel damage and thereby producing a blood clot (Airaodion *et al.*, 2019e). Aplastic anaemia, myelodysplastic syndromes, immune thrombocytopenic purpura, chemotherapy, human immunodeficiency virus infection, and other disorders can all cause thrombocytopenia (platelet counts below  $150 \times 10^9/L$ ), which is a significant clinical issue. Platelets are necessary for haemostasis (Airaodion *et al.*, 2019f). White blood cells, sometimes referred to as leukocytes or leucocytes, are immune system cells that assist in defending the body against pathogens and foreign invaders (Airaodion *et al.*, 2019g). The importance of blood cells in the physiological activities of the body and the use of bromate as food additives in our environment makes this study critical. This study showed  $KBrO_3$  induced significant reduction ( $P < 0.05$ ) in haemoglobin concentration, platelets, total WBC count, neutrophils, lymphocytes, and monocytes compared to those in the control animals. Also, it was noted that therapy using *P. biglobosa* attenuated the effect of  $KBrO_3$ . These findings agreed with a Saudi study that used Forty five (45) Swiss Webster (SW) mice which observed significant reduction in the red cells, white blood cells (WBC) and platelets in the study groups compared to the control group (Altoom *et al.*, 2018). Another study by Mohamed and Saddek (2019) discovered a significant reduction in haemoglobin concentration, MCV and total WBC count but not for platelets. Achukwu *et al.* (2009) found only significant reduction in platelets count. Between the test and control rats, the mean Cell haemoglobin concentration, haematocrit, and total leukocyte counts did not differ statistically in their study.

**Table 1:**

Effect of *P. biglobosa* on Red Blood Cell Parameters of Potassium Bromate-induced Rats

Parameters	Control	100 mg/kg $KBrO_3$ only	100 mg/kg $KBrO_3$ + 100 mg/kg <i>P. biglobosa</i>	100 mg/kg $KBrO_3$ + 200 mg/kg <i>P. biglobosa</i>	p-value
Hb (g/dL)	$17.26 \pm 2.84$	$13.25 \pm 1.25$	$15.34 \pm 1.18$	$16.92 \pm 1.27$	0.01
PCV (%)	$39.73 \pm 2.58$	$27.93 \pm 1.44$	$34.26 \pm 1.95$	$38.27 \pm 1.22$	0.01
RBC ( $\times 10^{12}/L$ )	$5.12 \pm 0.83$	$3.47 \pm 0.22$	$4.03 \pm 0.36$	$4.98 \pm 0.72$	0.02
MCV (fL)	$75.10 \pm 7.23$	$80.49 \pm 4.53$	$85.01 \pm 6.73$	$73.60 \pm 6.15$	0.49
MCH (pg)	$28.85 \pm 3.84$	$29.54 \pm 4.24$	$30.62 \pm 3.50$	$28.69 \pm 2.31$	0.74
MCHC (g/dL)	$3.84 \pm 0.96$	$3.67 \pm 0.28$	$3.60 \pm 1.00$	$3.90 \pm 0.27$	0.82

Values are presented as Mean  $\pm$  S.D, where n = 6.

PCV = Packed Cell Volume; Hb = Haemoglobin; RBC = Red Blood Cell; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration

**Table 2:**

Effect of *P. biglobosa* on White Blood Cell and Platelets Parameters of Potassium Bromate-induced Rats

Parameters	Control	100 mg/kg $KBrO_3$ only	100 mg/kg $KBrO_3$ + 100 mg/kg <i>P. biglobosa</i>	100 mg/kg $KBrO_3$ + 200 mg/kg <i>P. biglobosa</i>	P Value
WBC ( $\times 10^9/L$ )	$9.12 \pm 1.05$	$7.03 \pm 1.37$	$7.98 \pm 2.00$	$9.08 \pm 1.15$	0.01
Platelet ( $\times 10^9/L$ )	$241.26 \pm 11.34$	$218.25 \pm 17.34$	$223.74 \pm 14.83$	$230.26 \pm 22.43$	0.00
Neutrophils ( $\times 10^9/L$ )	$5.84 \pm 1.35$	$2.46 \pm 0.11$	$3.78 \pm 0.93$	$4.79 \pm 0.38$	0.03
Lymphocytes ( $\times 10^9/L$ )	$4.17 \pm 1.12$	$2.68 \pm 0.26$	$3.10 \pm 0.75$	$3.86 \pm 1.27$	0.00
Monocytes ( $\times 10^9/L$ )	$0.80 \pm 0.07$	$0.34 \pm 0.00$	$0.40 \pm 0.01$	$0.68 \pm 0.02$	0.03

Values are presented as Mean  $\pm$  S.D, where n = 6. ; WBC = White Blood Cell

Increased lipid peroxidation and protein oxidation in both plasma and erythrocytes, which point to the development of oxidative stress, may be responsible for the reductive effect of KBrO<sub>3</sub> on blood cells, particularly red cells. The succession of oxidative lipid breakdown events is known as lipid peroxidation. During this process, free radicals "steal" electrons from the lipids in cell membranes, harming the cells. This activity is driven by a free radical chain reaction mechanism (Airaodion *et al.*, 2020b). Due to their role as oxygen carriers and the presence of lipids in their structure, red blood cells are vulnerable to lipid peroxidation. This study supports the oxidative action of KBrO<sub>3</sub>. Previous studies have reported that KBrO<sub>3</sub> possesses oxidative propensity by showing significant increases in methemoglobin and nitric oxide as well as significant decreases in plasma, hepatic, renal, and cardiac catalase, superoxide dismutase (SOD), and glutathione peroxidase activity (GPx), reduced glutathione (GSH) concentrations, and increased malondialdehyde (MDA) levels (Ugwu *et al.*, 2022c). Additionally, several toxins reduce the production of haemoglobin by preventing the activities of aminolaevulinic acid dehydratase and ferro-chelatase (Ashour *et al.*, 2007). Thus, by hindering haem-biosynthesis and reducing RBC survival, KBrO<sub>3</sub> may result in anaemia at high concentrations. In a study by Femi-Oloye *et al.* (2020), rats were administered 10 mg of KBrO<sub>3</sub> per kilogramme of body weight, and there was no discernible difference in the rats' haemoglobin levels between the test and control groups which however contradicts our study. These differences may have resulted from the doses of KBrO<sub>3</sub> used as we used 100 mg/kg against the 10mg/kg body weight used by Femi-Oloye *et al.* (2020).

The reduction of white blood cell and platelets obtained in this study corroborate with the study of Achukwu *et al.* (2009). These decreases in the leucocyte and platelet count may have been brought about by DNA strand breaks caused by the oxidative stress induced by potassium bromate in these cells. Additionally, there might have been selective megakaryocyte depression and bone marrow suppression (Hoffbrand and Petit, 2014). On the other hand, KBrO<sub>3</sub> may directly harm the platelets.

Several studies have shown attenuation or inhibition of KBrO<sub>3</sub>- induced toxicity using different nutrients. According to El-Deep *et al.* (2015), vitamin C reduced the oxidative stress caused by KBrO<sub>3</sub> on the left ventricular myocardium of adult male albino rats; the research using taurine and vanilla shown a considerable reduction in the negative effects of KBrO<sub>3</sub> toxicity on haematological parameters, renal, and testicular tissues (Mohamed and Saddek, 2019). Another investigation revealed that bilberry (*Vaccinium myrtillus* L.) extract has a protective effect against kidney damage brought about by KBrO<sub>3</sub> in mice (Bao *et al.*, 2018). Cloudy apple juice reduces rat kidney and liver damage brought on by KBrO<sub>3</sub> (Kujawska *et al.*, 2013). In a recent study, Ugwu *et al.* (2022c) demonstrated that *Corchorus olitorius* leaves ameliorated oxidative stress induced by KBrO<sub>3</sub>. While it's destructive effect on the liver was attenuated by *Parkia Biglobosa* (Onyekachi *et al.*, 2022) and *Corchorus olitorius* leaves (Aguh *et al.*, 2022).

*Parkia Biglobosa* in addition to its mitigative effect on Potassium bromate-induced deranged haematological parameters, has shown great impact on mitigating the effect of KBrO<sub>3</sub> -induced toxicity in organs and systems of the Wistar rats: *Parkia Biglobosa* seeds have been reported to

exert therapeutic effect against potassium bromate-induced testicular toxicity (Ezirim *et al.*, 2022a), sex hormones perturbations (Iwuoha *et al.*, 2022), hepatocellular injury (Onyekachi *et al.*, 2022), renal toxicity (Abali *et al.*, 2022), sperm cell abnormalities (Ezirim *et al.*, 2022b), cardiotoxicity (Ugwu *et al.*, 2022a) and coagulation abnormality (Ugwu *et al.*, 2023) induced by KBrO<sub>3</sub> on Wistar rat.

A very significant multipurpose plant, *Parkia Biglobosa* is used in the environment for food, medicine, and economic purposes (Airaodion *et al.*, 2020a). *P. biglobosa* is rich in saponins, tannins, flavonoids, resins, carbohydrates, terpenoids, phenols, sterols, and cardiac glycosides, using various aqueous and organic extracts (Musara *et al.*, 2020). Antimalarial, antihelminthic, antibacterial, antidiabetic, antihypertensive, anti-inflammatory, analgesic, anti-carcinogenic, anti-trypanosomic, and antioxidant characteristics were demonstrated by *P. biglobosa* in pharmacological studies (Airaodion *et al.*, 2020a; Musara *et al.*, 2020). It also contains high level of omega 3 which authenticates it cardioprotective and antioxidant functions (Wokoma and Aziagba, 2010). More studies should be done to unravel the latent potential of this multipurpose plant.

In conclusion, this study revealed that potassium bromate-induced deranged haematological parameters were mitigated by seed extract of *Parkia Biglobosa* in a dose dependent manner. Care must be taken with the consumption of KBrO<sub>3</sub> due to its numerous toxicological effects. However, consumption of *P. biglobosa*, a tropical homemade food is recommended for families to benefit from the barrage of its health benefit. This will also alleviate the toxicity effect of KBrO<sub>3</sub> if consumed inadvertently. Human clinical trial is needed to substantiate these findings.

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