

## Original Research Article

# Hepatocellular Injury Ameliorated by a Common African Food, *Parkia Biglobosa*

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### ABSTRACT

**Background:** *Parkia biglobosa* seed has been reported to possess hepatoprotective potential. Therefore, this study sought to investigate its ability in ameliorating KBrO<sub>3</sub>-induced hepatotoxicity.

**Methodology:** *P. biglobosa* was extracted with soxhlet extractor with ethanol as the solvent. Twenty-four adult male Wistar rats were acclimatized under laboratory conditions and were randomly grouped into A, B, C and D. Group A was given distilled water orally. Animals in groups B, C and D were administered 100 mg/kg body weight of potassium bromate, but groups C and D were also treated with 100 and 200 mg/kg body weight of *P. biglobosa* respectively. Both potassium bromate and *P. biglobosa* were freshly prepared on daily basis and administered to rats by oral gavage. After 28 days of treatment, the animals were sacrificed under mild diethyl ether anaesthetization 24 hours after cessation of last treatment. Blood and liver tissue were collected.

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**Results:** The findings demonstrated that, when compared to the control group, KBrO<sub>3</sub> caused a significant increase ( $p < 0.05$ ) in ALT, AST, LDH, ALP, total bilirubin (TB), conjugated bilirubin (CB), and unconjugated bilirubin (UB) levels, but decreased total protein, albumin and globulin in the serum of animals. In the liver cells, KBrO<sub>3</sub> reduced hepatic biomarkers. These perturbations were neutralized in the groups treated with 100 and 200 mg/kg body weight respectively.

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**Conclusion:** The result of the present study revealed that KBrO<sub>3</sub> is hepatotoxic at a dose of 100 mg/kg body weight. The result further suggests that *P. biglobosa* possesses hepatoprotective properties in rats *in vivo*. This study can be replicated in human trial.

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**Keywords:** Hepatoprotective potential; *Parkia biglobosa* seed; potassium bromate

### 1. INTRODUCTION

Liver disease is a primary cause of death worldwide and a major global issue [1]. In the UK, the standardized mortality rate grew by more than 400% between 1970 and 2010 [2]. Infectious, carcinogenic, immune-mediated, and toxic substances can all cause harm to the liver because of its specific structure and functions [3]. Alterations in the concentrations of liver enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) are early indicators of hepatic damage [4,5]. ALT is found in the

cytoplasm of liver cells and in extrahepatic sources like the skeletal and cardiac muscles, although its quantity in the liver is significantly larger [3]. Although it is not unique to hepatocyte membrane damage, an increase in the serum level of ALT is highly predictive of extrahepatic injury [5]. In contrast, AST is present in the cytoplasm of all tissues other than the bones, albeit when compared to other tissues, it is most abundant in the liver and skeletal muscle [6]. Thus, hepatocellular injury can be distinguished from skeletal muscle injury because, in the former, the rise in ALT is greater than that in AST, resulting in a lower AST/ALT ratio than that

which is possible in skeletal muscle injury [7]. On the other hand, high levels of bilirubin and low levels of glutathione indicate the death of hepatocytes [9,10], while alkaline phosphatase (ALP) is a sign of cholestasis [8].

The creation of bread, cheese, and beer all employ potassium bromate (KBrO<sub>3</sub>), a common food ingredient. Due to its capacity to enrich dough and increase its volume and flexibility, it is frequently used in the production of bread [11]. The International Agency for Research on Cancer (IARC) categorized this molecule as a potential carcinogen in 1999, according to Mahmud et al. [12], yet despite this classification, the compound is still used industrially in some nations (including Nigeria). The well-known toxin and cancer-causing substance is still utilized in the production of bread in different cities of Nigeria [13,14]. The harmful effect of KBrO<sub>3</sub> was shown in a study by Bayomy et al. [15] which was supported by elevated levels of malonaldehyde in the liver tissue, AST and ALT serum enzymes, and glutathione (GSH) and superoxide dismutase (SOD) in the liver tissues.

Parkia peas, which belong to the Fabaceae family (subfamily Mimosoideae), are highly prevalent in tropical regions like Africa. The *Parkia biglobosa*, one of the several species in this genus, is well recognized for its usage in cooking. The different tribes in Nigeria have distinct names for the plant known as African locust bean. It is known as "Ogili" among the Ibo, "Dorawa" among the Hausa, and "Iru" among the Yoruba [16,17]. The African locust bean seeds are cleaned, cooked till soft, washed and dehulled, and then allowed to ferment for a few days to produce this culinary product. After being shaped into balls, the fermented product is allowed to dry [18]. Analgesic, antihypertensive, antifungal, anti-inflammatory, cardioprotective, and hepatoprotective applications of African locust bean seed have all been described [17,19-25]. Therefore, the purpose of this study is to further assess how African locust bean protects against potassium bromate-induced hepatic enzyme distortion.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Extraction of *Parkia biglobosa*

Having purchased *P. biglobosa* (African locust bean) seeds from a local market in Ibadan, western Nigeria, the seeds were identified by a botanist. These were sun-dried and ground into powder using a mechanical blender (Moulinex). The extraction of the food product was done using a soxhlet apparatus, with ethanol as the solvent; the steps outlined by Airaodion et al. [26,27] were followed. About 25 g of the sample powder in 250ml of ethanol in a round bottom flask was added to the soxhlet extractor and condenser on a heating mantle. Heat generated by the heating mantle was channeled to the solvent until it began to evaporate; the vapour was passed through the apparatus to the condenser. The condensate dropped into a reservoir that housed the sample-containing thimble. When the solvent level reached the siphon and was poured back into the flask with a flat bottom, the cycle was resumed. The operation was given a total of 18 hours. With a yield of 2.55 g and a percentage yield of 10.20 percent, the ethanol was evaporated in a rotary evaporator at 35 °C at the end of the process. The extract was kept in the refrigerator for further analysis.

### 2.2 Animal Treatment

Twenty-four (24) mature male Wistar rats (*Rattus norvegicus*) weighing between 140 and 160 g were used in the experiment. They were acclimated in a laboratory setting for seven (7) days prior to the trial. The rats were housed in wire-mesh cages with free access to commercial rat food and water. The animals were kept in standard temperature and humidity conditions with 12-hour alternating cycles of light and dark. This inquiry was carried out in accordance with the Declaration of Helsinki and the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals. Additionally, NIH policy was followed when doing animal experiments [28]. The rats were picked at random and grouped into groups

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A, B, C, and D. Group A (control group) received oral distilled water; groups C and D received 100 and 200 mg/kg body weight of *P. biglobosa*, respectively, while the animals in group B received 100 mg/kg body weight of potassium bromate. The rats in groups B and C received 100mg/kg body weight of *P. biglobosa* in addition. Fresh potassium bromate and *P. biglobosa* were administered to rats every day by oral gavage. The animals had 28 days of successive treatments before being killed. The animals were sacrificed while being gently sedated with diethyl ether twenty-four hours following the last treatment. Through a heart puncture, blood was taken.

### 2.3 Preparation of Liver homogenate

The animals were dissected, and the liver was taken out. After that, the liver of each rat were weighed. The liver tissue was homogenized in 50 mMol/ L Tris-HCl buffer using a Teflon homogenizer (pH 7.4). The combination was centrifuged at 5000 rpm for 15 minutes, and the supernatants were stored in the freezer until they were required for biochemical analysis.

### 2.4 Determination of Hepatic biomarkers

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activities were analysed from the liver and serum homogenates following the method of Reitman and Frankel; Randox commercial Enzyme kits were used [28]. Other biomarkers, namely Total Protein (TP), Albumin (ALB), Globulin (GLO), Total Bilirubin (TB), Conjugated Bilirubin (CB), and Unconjugated Bilirubin (UB), were also assayed from liver and serum homogenates following procedures previously described by Airaodion *et al* [25].

### 2.5 Statistical Analysis

The results are presented as the mean minus the standard deviation. Turkey's test was used after one-way analysis of variance (ANOVA) to

determine how homogeneous the groups were. Graph Pad Prism Software Version 8.00 was used for all analyses, and *p* values below 0.05 were regarded as statistically significant.

## 3. RESULTS

As shown in Table 1, the findings demonstrated that, when compared to the control group, potassium bromate caused a significant increase ( $p < 0.05$ ) in the ALT, AST, LDH, ALP, total bilirubin (TB), conjugated bilirubin (CB), and unconjugated bilirubin (UB) levels in the serum of animals treated with only 100 mg/kg body weight of potassium bromate. The serum levels of these biomarkers in those rats treated with potassium bromate, as well as 100 and 200 mg/kg body weight doses of *P. biglobosa* extract, however, decreased significantly in a dose-dependent manner. The serum levels of total protein (TP), albumin (ALB), and globulin (GLO), on the other hand, decreased significantly in the group of rats treated with only 100 mg/kg body weight of potassium bromate, when compared with those in the control group (Table 1). An additional treatment with 100mg and 200mg/kg body weight of locust bean seed extract resulted in a significant increase in the serum levels of TP, ALB, and GLO, in a dose-dependent manner.

Table 2 shows that the activities of AST, ALT, and LDH as well as the levels of TP and ALB in the liver cells of those rats given 100 mg/kg body weight dose of potassium bromate was significantly decreased ( $p < 0.05$ ), when compared to the rats in the control group. The additional administration of 100 mg/kg and 200 mg/kg body weight doses of *P. biglobosa* extract attenuated this decrease in a dose-dependent manner. The changes observed in the levels of the other biomarkers in the liver cells were of no statistical significance (Table 2).

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**Table 1: Effect of *P. biglobosa* on Hepatic Biomarkers in the Serum of Potassium Bromate-induced Rats**

Hepatic Biomarkers	Control	KBrO <sub>3</sub> Only	KBrO <sub>3</sub> + 100 mg/kg <i>P. biglobosa</i>	KBrO <sub>3</sub> + 200 mg/kg <i>P. biglobosa</i>	p-value
AST (U/L)	54.27±3.34	86.25±4.37	79.56±4.52	60.27±3.85	0.02
ALT (U/L)	151.53±9.53	211.28±8.93	198.47±6.36	173.96±4.83	0.04
LDH (U/L)	7.43±0.82	13.79±2.83	10.42±1.42	7.96±2.04	0.03
ALP (U/L)	73.45±6.47	102.00±5.93	94.02±2.27	79.22±4.83	0.02
TP (g/dL)	7.89±1.03	4.89±1.23	5.67±0.84	7.09±0.59	0.01
ALB (g/dL)	5.05±0.92	2.91±0.18	3.35±0.27	4.34±0.44	0.04
GLO (g/dL)	2.84±0.22	1.98±0.25	2.32±0.17	2.75±0.21	0.03
TB (g/dL)	0.09±0.00	0.22±0.01	0.19±0.01	0.11±0.00	0.02
CB (g/dL)	0.04±0.00	0.09±0.00	0.09±0.00	0.05±0.00	0.02
UB (g/dL)	0.05±0.00	0.13±0.01	0.10±0.00	0.06±0.00	0.02

Values are presented as Mean±SD, where n = 6.

**Legend:** AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, LDH = Lactate Dehydrogenase, ALP = Alkaline Phosphatase, TP = Total Protein, ALB = Albumin, GLO = Globulin, TB = Total Bilirubin, CB = Conjugated Bilirubin, UB = Unconjugated Bilirubin

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**Table 2: Effect of *P. biglobosa* on Hepatic Biomarkers in the Liver of Potassium Bromate-induced Rats**

Hepatic Biomarkers	Control	KBrO <sub>3</sub> Only	KBrO <sub>3</sub> + 100 mg/kg <i>P. biglobosa</i>	KBrO <sub>3</sub> + 200 mg/kg <i>P. biglobosa</i>	p-value
AST (U/L)	48.46±2.27	29.75 ±1.88	36.67 ±1.89	45.44±3.01	0.04
ALT (U/L)	128.19±4.29	97.45±5.55	108.33±3.10	122.84±5.25	0.02
LDH (U/L)	9.35±2.11	7.84±2.22	8.17±1.18	9.00±0.96	0.01
ALP (U/L)	78.85±3.29	81.27±3.55	81.84±4.18	79.79±3.21	2.96
TP (g/dL)	9.35±1.85	7.27±1.44	7.93±1.26	8.88±1.19	0.05
ALB (g/dL)	6.99±1.21	5.04±0.66	5.37±1.06	6.29±1.37	0.05
GLO (g/dL)	2.36±0.28	2.23±0.11	2.56±0.44	2.59±0.28	1.96
TB (g/dL)	0.34±0.01	0.29±0.03	0.29±0.02	0.32±0.02	1.74

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CB (g/dL)	0.21±0.02	0.18±0.00	0.19±0.04	0.19±0.03	3.02
UB (g/dL)	0.13±0.00	0.11±0.00	0.10±0.00	0.13±0.00	5.67

Values are presented as Mean±SD, where n = 6.

**Legend:** AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, LDH = Lactate Dehydrogenase, ALP = Alkaline Phosphatase, TP = Total Protein, ALB = Albumin, GLO = Globulin, TB = Total Bilirubin, CB = Conjugated Bilirubin, UB = Unconjugated Bilirubin

#### 4. DISCUSSION

One of the main characteristics of liver injury is changes in particular liver enzymes released into the blood when the liver cells deteriorate [29]. The serum levels of the aminotransferases rapidly rise when liver cells suffer ischemic injury, either from a lack of blood or oxygen [30]. With the exposure of one group of rats to KBrO<sub>3</sub> alone, the levels of AST, ALT, and ALP sharply increased, suggesting liver cell necrosis brought about by oxygen deprivation (Table 1). Similar results were obtained in a study by Hassan et al. [31] where rats given potassium bromate displayed elevated ALT, AST, and ALP activity. Numerous risk factors, such as potassium bromate, alcohol, environmental contaminants, irradiation, and medications cause oxidative stress on liver cells [25,32]. The use of natural antioxidants found in medicinal plants has the power to scavenge free radicals created by oxidative stress, hence lessening liver damage caused by reactive oxygen species (ROS) [32]. One of these therapeutic plants with antioxidant properties is *Parkia biglobosa*, which is an everyday diet in Africa [16,33]. When the African locust bean seed extract was administered to the rats in the present research, the increase in serum aminotransferase activity brought about by the administration of potassium bromate was attenuated; the extent of attenuation depended on the dose of *Parkia biglobosa* extract administered. This supports the view that *Parkia biglobosa* seed extract has hepatoprotective characteristics as reported by Saleh et al. [16].

Although it is mostly a liver enzyme, alkaline phosphatase (ALP) can also be present in bone. Its activity may be increased in physiological

circumstances, such as during pregnancy, but it is increased in pathological circumstances, such as hepatic congestion brought about by right-sided heart failure [34]. Additionally, certain medications including glucocorticoids and anti-convulsants, as well as high-fat diets, may cause it to rise [35]. The finding of Hassan et al. [31] is in agreement with the rise in ALP activity in the serum of Wistar rats fed with 100mg/Kg body weight of KBrO<sub>3</sub> in the index study.

The increased activity of indicators like ALT, AST, and LDH in the liver tissue of animals without pretreatment with *P. biglobosa* may be the result of hepatocyte cellular necrosis, which increases cell permeability. Alteration in the level of Lactate dehydrogenase is a measure of cell damage, including liver toxicity and artery endothelial instability [36]. The considerable rise in LDH activity seen in rats treated with KBrO<sub>3</sub> alone may be indicative of the onset of cytolysis, which is a potential marker of membrane damage, including damage to blood vessel endothelial membranes. Reactive oxygen species (ROS) are produced in endothelial cells as a result of this endothelial membrane rupture, whether directly or indirectly, as was previously observed [37]. Free radicals attack unsaturated fatty acids in the membranes, causing membrane lipid peroxidation that reduces membrane fluidity, enzyme leakage, loss of receptor activity, and damage to membrane proteins that result in cell inactivation [38]. Oxidative stress results from a reduction in the antioxidant defense system when lipid peroxidation increases [39]. This shows that the treatment of KBrO<sub>3</sub> may have compromised the liver membrane of the rats, allowing for increased levels of AST, ALT, and LDH in the

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liver as well as subsequent penetration. However, when mice were given 100 and 200 mg/kg body weight of *P. biglobosa* seed extract, this impact was lessened. According to a recent report, *P. biglobosa* seed lowers the production of ROS in Wistar rats' hearts, livers, and kidneys [34]. So, the antioxidant activity of *P. biglobosa* seed may be the cause of its protective effects against KBrO<sub>3</sub>-induced hepatotoxicity.

In this investigation, KBrO<sub>3</sub> dramatically decreased the serum concentrations of total protein, albumin, and globulin when compared to the control group. According to Ekam and Udosen's report [40], a drop in serum protein during hepatotoxicity simply denotes the presence of para-proteins or a drop in antibody synthesis. This decrease is consistent with their findings. The level of total protein, albumin, and globulin may fall as a result of liver dysfunction, malnutrition and malabsorption, diarrhea, nephrosis, alpha-1-antitripsin deficiency, acute hemolytic anemia, hypogammaglobulinemia/agammaglobulinemia, loss through the urine in severe kidney disease, pregnancy, and other conditions. Long-term hepatic cell death lowers serum levels of total protein, albumin and globulin, and generates additional hepatic releases to worsen hepatic dysfunction [40]. Other hemeoproteins include cytochromes, catalase, peroxidase, tryptophan pyrrolase, and a small pool of free heme.

Bilirubin is the breakdown result of the heme moiety of hemeoglobin [41]. Hyperbilirubinaemia is caused by an increase in the amount of directly reacted bilirubin in the blood. This syndrome is toxic and can lead to jaundice, auditory impairment, and neurotoxicity with subsequent brain damage [41]. On the other hand, modest unconjugated hyperbilirubinaemia functions as a mild antioxidant and may provide defense against the development of tumors and cardiovascular diseases [42]. Low concentrations of directly reacting bilirubin can sometimes lead to cardiac issues as well as strokes in humans [42]. Under a variety of clinical circumstances, serum bilirubin levels are frequently elevated. The fact that bilirubin is

linked to serum albumin throughout blood circulation lessens the possibility of toxicity considered to be brought on by free bilirubin [43]. Bilirubin binds to albumin with a high affinity, but is quickly and selectively taken up by the liver, conjugated with glucuronate, and secreted into bile [42]. As a result, bilirubin is transformed in the liver into bilirubin glucuronic acid and eliminated along with bile. Comparing the KBrO<sub>3</sub>-treated rats to the control animals revealed a statistically significant increase in the serum levels of total, conjugated, and unconjugated (indirect) bilirubin, which may indicate tissue injuries. However, in animals that received both KBrO<sub>3</sub> and *P. biglobosa* seed extract at the same time, this effect was reduced.

Folk medicine has long recognized the wide range of therapeutic uses of *P. biglobosa* seed, and scientific development has produced ample proof for the majority of these claims. The hepatoprotective potential of this plant has been further proven by the current *in vivo* study. Based on a significant increase (P<0.05) in hepatic biomarkers in the KBrO<sub>3</sub> group when compared to the control group, it was determined in this study that KBrO<sub>3</sub> intoxication in rats caused hepatocellular damage. Concurrent administration of *P. biglobosa* seed extract to rats alleviated KBrO<sub>3</sub>-induced changes in rat serum hepatic biomarkers in a dose-dependent manner, indicating that this plant may have hepatoprotective properties. Our findings seem to support an earlier finding by Airaodion et al. [25] that *Parkia biglobosa* protects the liver from acute ethanol-induced oxidative stress in Wistar rats.

In comparison to the control group, Table 2 demonstrates that the administration of KBrO<sub>3</sub> decreased the activities of AST, ALT, LDH, and ALP as well as the concentrations of total protein, albumin, globulin, total bilirubin, conjugated, and unconjugated bilirubin in the liver. Since the liver is where these biomarkers are primarily produced, the findings of this study may suggest that KBrO<sub>3</sub> decreased either the synthesis of these parameters or increased their

efflux from the liver thereby increasing their level in the blood. As a result of the *P. biglobosa* seed extract treatment, these perturbations were attenuated.

## 5. CONCLUSION

The result of the present study revealed that KBrO<sub>3</sub> is hepatotoxic at a dose of 100 mg/kg body weight. The result further suggests that *P. biglobosa* possesses hepatoprotective properties in rats *in vivo*. This study can be replicated in human trial.

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