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TABLE OF CONTENTS

WEST AFRICAN JOURNAL OF MEDICINE

ORIGINAL ARTICLE

Blood Coagulation Normalization Effect of *Parkia Biglobosa* Seed on Potassium Bromate-induced Coagulopathy

Effet de Normalisation de la Coagulation Sanguine de la Graine de Parkia Biglobosa sur la Coagulopathie Induite par le Bromate de Potassium

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ABSTRACT

BACKGROUND: Potassium bromate ($KBrO_3$) has been reported to be toxic, adversely affecting many body tissues and organs. The aim of this study was to determine the blood coagulation effect of *Parkia biglobosa* (P. biglobosa) seed on potassium bromate induced coagulopathy.

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 for 28 days. At the end of the treatment period, blood samples were collected in sodium citrate bottles and were used for analysis of Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT), fibrinogen and vitamin K levels using standard methods.

RESULTS: Administration of potassium bromate increased Prothrombin Time (PT) from 11.67±2.15 seconds (in control animals) to 19.53±2.83 seconds. Treatment with 100 and 200 mg/kg body weight of *P. biglobosa* seed extract neutralized this effect in a dose-dependent manner. Likewise, $KBrO_3$ was observed to have significantly elevated Activated Partial Thromboplastin Time (APTT) from 29.67±3.93 to 41.10±4.79 seconds and Thrombin Time (TT) from 15.36±2.06 to 25.43±2.83 seconds when compared with those in the control group. The result further showed that exposure of animals to $KBrO_3$ significantly declined the levels of fibrinogen (from 4.05±0.72 to 2.59±0.30 g/dL) and vitamin K (from 3.18±0.73 to 1.84±0.18 ng/mL) when compared with the untreated animals. The effect of $KBrO_3$ on PT, APTT, TT, Fibrinogen and vitamin k were attenuated by P. biglobosa in a dose-dependent manner.

CONCLUSION: The results of this investigation demonstrated that potassium bromate caused prolongation of PT, aPTT and TT and decreased levels of fibrinogen and vitamin K, but *P. biglobosa* treatment counteracted these effects. Thus, it is recommended that these results be investigated in clinical trials in human volunteers. **WAJM 2023; 40(2): 148–154.**

Keywords: Blood coagulation, Coagulopathy, Parkia biglobosa, Potassium bromate.

RÉSUMÉ

CONTEXTE: On a signalé que le bromate de potassium (KBrO3) est toxique et qu'il a des effets néfastes sur de nombreux tissus et organes du corps. Le but de cette étude était de déterminer l'effet de la graine de *Parkia biglobosa (P. biglobosa)* sur la coagulopathie induite par le bromate de potassium.

MÉTHODOLOGIE: *P. biglobosa* a été extrait à l'aide d'un extracteur soxhlet avec de l'éthanol comme solvant. Vingt-quatre rats Wistar mâles adultes ont été acclimatés dans des conditions de laboratoire et ont été répartis au hasard en groupes A, B, C et D. Le groupe A a reçu de l'eau distillée par voie orale. Les animaux des groupes B, C et D ont reçu 100 mg/kg de poids corporel de bromate de potassium, mais les groupes C et D ont également été traités avec 100 et 200 mg/kg de poids corporel de *P. biglobosa* respectivement. Le bromate de potassium et *P. biglobosa* ont été fraîchement préparés quotidiennement et administrés aux rats par gavage oral pendant 28 jours. A la fin de la période de traitement, des échantillons de sang ont été collectés dans des bouteilles de citrate de sodium et ont été utilisés pour l'analyse du temps de prothrombine (PT), du temps de thromboplastine partielle activée (APTT), du temps de thrombine (TT), du fibrinogène et des niveaux de vitamine K en utilisant des méthodes standard.

RÉSULTATS: L'administration de bromate de potassium a augmenté le temps de prothrombine (PT) de 11,67±2,15 secondes (chez les animaux témoins) à 19,53±2,83 secondes. Un traitement avec 100 et 200 mg/kg de poids corporel a neutralisé cet effet de manière dose-dépendante. De même, on a observé que le KBrO3 augmentait significativement le temps de thromboplastine partielle activée (TCA) de 29,67±3,93 à 41,10±4,79 secondes et le temps de thrombine (TT) de 15,36±2,06 à 25,43±2,83 secondes par rapport aux animaux du groupe témoin. Le résultat a également montré que l'exposition des animaux au KBrO3 a réduit de manière significative les niveaux de fibrinogène (de 4,05±0,72 à 2,59±0,30 g/dL) et de vitamine K (de 3,18±0,73 à 1,84±0,18 ng/mL) par rapport aux animaux non traités. L'effet du KBrO3 sur le PT, l'aPTT, le TT, le Fibrinogène et la vitamine K a été atténué par *P. biglobosa* de manière dose-dépendante.

CONCLUSION: Les résultats de cette étude ont démontré que le bromate de potassium a provoqué une prolongation du PT, de l'aPTT et du TT et a diminué les niveaux de fibrinogène et de vitamine K, mais le traitement par *P. biglobosa* a contrecarré cet effet. Il est donc recommandé que ces résultats soient étudiés dans des essais cliniques sur des volontaires humains. **WAJM 2023; 40(2): 148–154.**

Mots-clés: Coagulation sanguine, Coagulopathie, *Parkia biglobosa,* Bromate de potassium.

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INTRODUCTION

Haemostasis, a typical physiological occurrence in the body's defense mechanism, turns blood into a thick, jelly-like mass at the site of injury or damage and inhibits blood loss following bleeding.[1] One of the processes in the hemostasis mechanism, blood coagulation results in a blood clot that stops the flow of blood to the injured area. The mechanism of coagulation is a multi-step procedure that involves the activation of several enzymes, each of which uses a unique substrate to become an active enzyme.[2] This proteolytic cascade continues until thrombin is produced. The subsequent breakdown of soluble fibrinogen into insoluble fibrin by newly produced thrombin causes the development of a blood clot. Pathological disorders, such as venous thrombosis, may result from any improperness in the coagulation cascade.[3] Reducing thrombus formation and inhibiting the coagulation cascade can also prevent atherosclerosis and a number of cardiovascular illnesses.[4]

A genetic bleeding illness known as hemophilia is brought on by a lack, or very rarely, malfunction, of a specific coagulation factor. This is the most prevalent severe inherited hemorrhagic condition in people.[5] A flaw or mutation in the gene responsible for the production of the clotting factor almost always causes hemophilia.[6] In critically ill patients, coagulopathies are frequently present, and they raise mortality.[7,8]

Potassium bromate ($KBrO_3$), a typical food ingredient used in the manufacturing of bread, is also found in drinking water samples as a result of ozone disinfection. When rats were given $KBrO_3$, it was shown that this led to oxidative stress and passively decreased the blood's antioxidant capacity.[9] Dietary antioxidants can halt or delay the oxidation of susceptible cellular substrates, reducing oxidative stress. Phenolic substances such flavonoids, phenolic acids, diterpenes, saponins, and tannins have attracted a lot of interest because of their significant antioxidative activity.[10] Antioxidants are therefore essential to include in our diets in order to protect against a number of chronic illnesses associated to oxidative damage. Antioxidants are also essential for preserving food quality since they can prevent lipids from deteriorating as a result of oxidation.[11]

African locust bean is *Parkia biglobosa*'s common name. It belongs to the Leguminosae genus of legumes and is a perennial tree.[12] The plant's seeds are enclosed in a yellowish, mealy, and sweet-tasting edible pulp.[13] It is a well-known fact that this plant has a large amount of phenolic compounds.[14] The bark of the plant included epigallocatechin, epicatechin 3-O-gallate, and epigallocatechin 3-O-gallate.[15] Leaf extract contains heart and saponin glycosides.[16] The fruit's pulp and seeds are rich in protein and lactose.[15] Examples of antinutritional elements included in the seeds are phytate, tannin, oxalate, and hydrogen cyanide.[12] Along with having various positive effects, the *P. biglobosa* extract has antibacterial,[14] antidiabetic,[17] antifungal,[18] anti-inflammatory,[19] anti-diarrheal,[20] anti-hypertensive,[21] hypoglycemic,[22] and hypolipidemic[22] properties. According to a recent study by Ezirim *et al.*[23] *P. biglobosa* seed has the ability to treat testicular toxicity caused by potassium bromate. The goal of this study was to find out if it could shield Wistar rats from the coagulopathy brought on by potassium bromate.

MATERIALS AND METHODS

Procurement of Chemical and Kits

Potassium bromate ($KBrO_3$) and the biochemical kits for the determination of coagulation factors were purchased from Cephas Global Resources Limited (A division of Deliving Stone Int'l), E Line 444 (along Fin Bank/Eco Bank), Head Bridge Market, Onitsha, Anambra State, Nigeria.

Collection and Extraction of *Parkia biglobosa*

A botanist identified the *P. biglobosa* (African locust bean) seeds after they were purchased from a local market in Ibadan, Nigeria. After being sun-dried, they were ground into powder using a mechanical blender (Moulinex). Using a soxhlet apparatus and ethanol as the solvent, the extraction was completed in accordance with the steps described by Airaodion *et al.*[24,25] About 25 g of the sample powder and a round bottom flask with a capacity of 250 mL of ethanol were added to the soxhlet extractor and condenser on a heating mantle. The solvent was heated by the heating mantle and began to evaporate as it 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.

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 lab 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 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 in conducting the animal experiments.[26] At random, they were put into groups A, B, C, and D. Group A received oral distilled water as the usual control. Although groups C and D likewise received 100 and 200 mg/kg body weight of *P. biglobosa*, respectively, the animals in groups B, C, and D received 100 mg/kg body weight of potassium bromate. Fresh potassium bromate and *P. biglobosa* were administered to the rats every day by oral gavage for 28 days. 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.

Determination of Prothrombin Time, Activated Partial Thromboplastin Time, Thrombin Time and Fibrinogen Level

Analysis of PT, aPPT, TT and fibrinogen was done using the methods described by Clarisse *et al.*[27]

Statistical Analysis

The outcomes are shown as the mean ± standard deviation. The level of group homogeneity was assessed using Tukey's test and one-way Analysis of Variance (ANOVA). All analyses were carried out using the Graph Pad Prism software (version 8), and P values ≤0.05 were considered statistically significant.

RESULTS

Administration of potassium bromate increased prothrombin time (PT) from 11.67±2.15 seconds (in control animals) to 19.53±2.83 seconds (Figure 1). Treatment with 100 and 200 mg/kg body weight neutralized this effect in a dose-dependent manner. Likewise, $KBrO_3$ was observed to have significantly elevated activated partial thromboplastin time (aPTT) from 29.67±3.93 to 41.10±4.79 seconds and thrombin time (TT) from 15.36±2.06 to 25.43±2.83 seconds when compared with those in the control group respectively (Figures 2 and 3). Figures 4 and 5 show that exposure of animals to $KBrO_3$ significantly declined the levels of fibrinogen (from 4.05±0.72 to 2.59±0.30 g/dL) and vitamin K (from 3.18±0.73 to 1.84±0.18 ng/mL) when compared with the untreated animals. The effect of $KBrO_3$ on PT, aPTT, TT, Fibrinogen and vitamin k were attenuated by *P. biglobosa* in a dose-dependent manner.

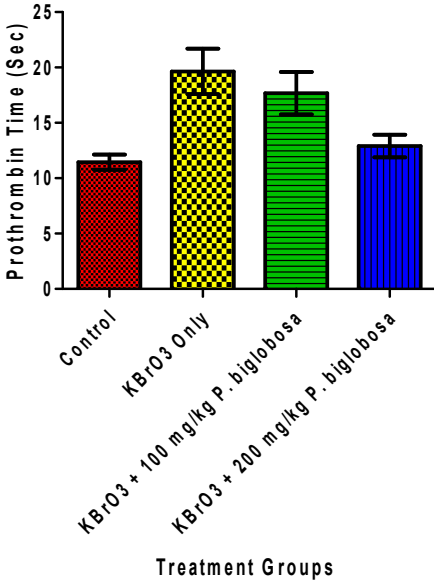

Fig. 1: Effect of *Parkia Biglobosa* Seed on the Prothrombin Time of Potassium Bromate-induced Coagulopathy.
Values are presented as Mean ± SD, where n = 6. P = 0.03

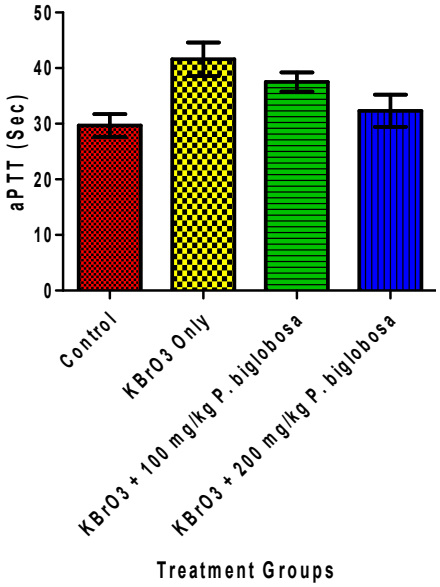

Fig. 2: Effect of *Parkia Biglobosa* Seed on the Activated Partial Thromboplastin *Time (aPTT)* of Potassium Bromate-induced Coagulopathy
Values are presented as Mean ± SD, where n = 6. P = 0.02

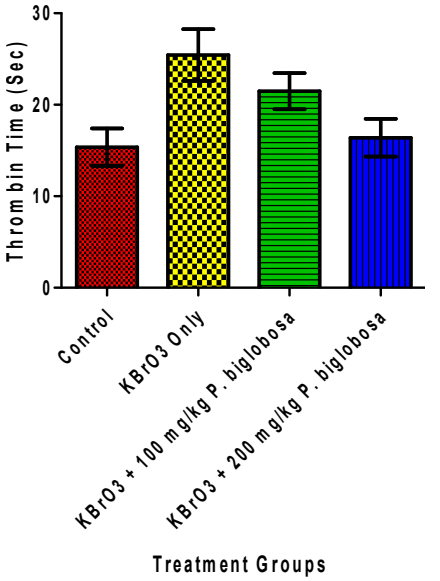

Fig. 3: Effect of *Parkia Biglobosa* Seed on the Thrombin Time of Potassium Bromate-induced Coagulopathy
Values are presented as *Mean ± SD*, where n = 6. P = 0.03

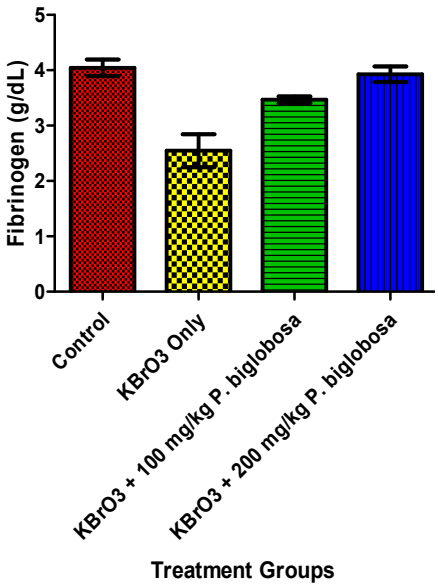

Fig. 4: Effect of *Parkia Biglobosa* Seed on Fibrinogen Level of Potassium Bromate-induced Coagulopathy.
Values are presented as Mean ± SD, where n = 6. P = 0.03

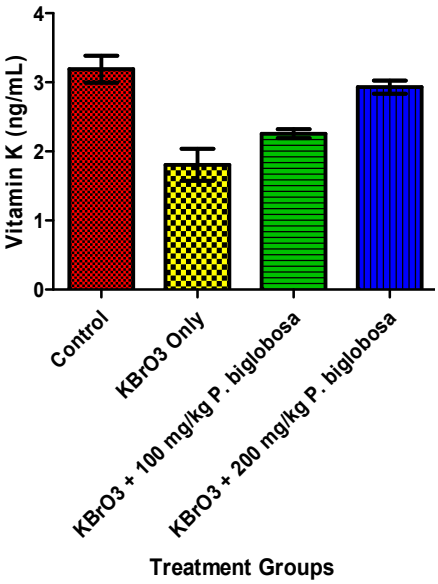

Fig. 5: Effect of *Parkia Biglobosa* Seed on Vitamin K level of Potassium Bromate-induced Coagulopathy.
Values are presented as Mean ± SD, where n = 6. P = 0.02

DISCUSSION

A vital physiological mechanism known as hemostasis is the action that causes a blood vessel to stop bleeding.[28] Local vasoconstriction, platelet plug formation, and the coagulation cascade are the main components of hemostasis. Primary hemostasis is the first, fleeting reflexive vasoconstriction with platelet adhesion, aggregation, and activation, while secondary hemostasis involves the creation and stabilization of fibrin clots.[29] One of the most important processes in mediating hemostasis is blood coagulation. Flowing liquid blood plasma is transformed into a soft, viscous gel that traps the biological components of red blood cells and platelets, preventing blood from extravasating.[30]

Numerous coagulation tests, including prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen, and vitamin K levels, can be used to determine the risk of bleeding.[31] Therefore, the impact of potassium bromate on coagulation and subsequent administration of *P. biglobosa* extract were assessed using these tests.

An *in vitro* test called Prothrombin Time (PT)/International Normalized Ratio (INR) is used to assess how well the common and intrinsic coagulation pathways are working. It precisely monitors the time, which under physiological conditions ranges between 10 and 13 seconds,[32] that a clot must form following thromboplastin addition to a specific blood sample. The dynamics of the extrinsic and common coagulation pathways working are evaluated using the *in vitro* test known as the activated partial thromboplastin time (aPTT). Since the intrinsic route depends on contact activation, this requirement can be met *in vitro* by including the phospholipid cephalin, kaolin, silica, or other similarly negatively charged compounds. This is one of the initial tests performed to assess hemophilia.[33] Though it may differ between different laboratories, the normal range is 25 to 35 seconds.[34] A prolonged aPTT may be indicative of diseases such as disseminated intravascular coagulation (DIC), von Willebrand disease, and hemophilia.[34]

Important evaluation tests used to identify the intrinsic and extrinsic coagulation pathways are the aPTT and PT.[35] This study found that when compared to the control group, $KBrO_3$ considerably raised aPTT and PT. According to reports, the cause of an extended aPTT is suppression of intrinsic coagulation factors.[2,36] This reaction may be related to potassium bromate's hyperlipidemic impact.[37] High cholesterol diet in rabbits was observed to significantly increase coagulation factors II, VII, and X.[38] In response to a higher prothrombin catabolic rate, an increase in plasma concentration of clotting factors is stimulated.[39]

When animals exposed to $KBrO_3$ alone were compared to those in the control group, this study revealed a significant reduction in fibrinogen concentration (Figure 4), and these reduced levels cause an increase in bleeding duration. Therefore, it can be deduced that potassium bromate's effect on inhibiting platelet aggregation may be related to the fact that platelets require fibrinogen to trigger platelet aggregation.[40] According to a study reported by Achukwu *et al.*,[41] $KBrO_3$ decreased animal platelet count and prevented platelet aggregation. The bleeding time has been reported to increase when platelet aggregation is inhibited.[1] Therefore, the inhibition of platelet aggregation may be the mechanism through which $KBrO_3$ affects coagulation factors in our study. Chemicals that act on the phospholipase C pathway to decrease platelet activity may have been present in $KBrO_3$. This impact is comparable to that of a well-known anticoagulant medication Heparin, which may be the most likely cause of the prolonged bleeding period. Heparin is known to enhance the production of anti-thrombin III (AT-III), which in turn stimulates the formation of complexes with thrombin and inactivates numerous coagulation factors, hence prolonging the aPTT.[36] Factor Xa and thrombin are two active clotting factors that AT-III delays. The thrombin-antithrombin (TAT) complex can be used to evaluate the increase in thrombin inhibition caused by integration with AT-III.[42] By interacting with protein S, AT-III also contributes to the reduction of thrombin and the deactivation of factors VIIIa and Va.[43]

In this study, thrombin time (TT) was considerably increased by $KBrO_3$ in comparison to the control group ($p = 0.03$) (Figure 3). A laboratory test called the thrombin time measures how much fibrin is produced from fibrinogen as a result of thrombin's action. The average thrombin time is normally between 14 and 16 seconds or less than 20 seconds. A prolonged thrombin time may be a sign of thrombin inhibitors, dysfibrinogenemia, hypofibrinogenemia, or both.[44] When *P. biglobosa* extract was administered, the impact of $KBrO_3$ on thrombin time which is prolongation of TT was reduced.

This study also found that compared to the control group, animals exposed to $KBrO_3$ had considerably lower levels of vitamin K. (Figure 5). Treatment with *P. biglobosa*, however, reversed this impact. Given that vitamin K is known to promote blood clotting, $KBrO_3$'s reduction of it may have prevented the treated animals' blood from clotting, prolonging the bleeding time. Depending on the aetiology and severity, increased bleeding diathesis may be treated in a variety of ways, and vitamin K may be taken alone or in conjunction with other pro-coagulant medicines.[45,46] Before liver and kidney biopsies, central venous catheterization, and tracheostomies, PT is not a reliable indicator of bleeding risk in non-anticoagulated patients.[47–50] However, if the PT-INR is higher than 1.6, the death rate for individuals with a haemothorax and chest tube drainage increases.[51] According to a prior study, the prevalence of vitamin K deficiency is over 25%, peaking around the time of intensive care unit (ICU) admission.[52] Although it is still unknown whether vitamin K insufficiency is harmful or not in non-bleeding circumstances, it is well recognized that vitamin K has other numerous benefits outside its undeniable function in coagulation. For instance, vitamin K functions as a cofactor for vitamin K-dependent proteins, which are important for cancer management, bone metabolism, and cardiovascular health.[53]

In human patients with hepatotoxicity, hemostatic system disturbances are well documented.[54] The balance

between the coagulant, anticoagulant, and fibrinolytic pathways controls how quickly clots form and dissolve during hemostasis.[55,56] Tissue factor (TF) causes the coagulation system to become active, which leads to the production of thrombin and the development of insoluble fibrin clots. Plasmin mediates the dissolution of fibrin clots and plasminogen activator inhibitor-1 (PAI-1) inhibits it. Prothrombin time increases in those who experience liver injury, and this change is correlated with the level of toxicity.[57–59] A damaged liver's decreased ability to produce coagulation factors has been documented.[60] Sinusoidal endothelial cells are damaged in liver injuries in both human and animal models,[61,62] which would encourage activation of the coagulation system. Additionally, proinflammatory cytokines like tumor necrosis factor are produced after human poisoning[60] as well as in animal models.[63] A number of serine proteases, including thrombin, are produced when the coagulation cascade is activated, and this intracellular signaling is brought on by the cleavage of the protease-activated receptor-1 (PAR-1).[64] A variety of liver cells express this receptor.[65,66] Additionally, $KBrO_3$ is hepatotoxic according to a study reported by Bayomy *et al.*[67] Therefore, the hepatotoxicity of $KBrO_3$ may be blamed for the negative changes in the coagulation factors of treated animals in our investigation. In a comparable investigation, Ugwu *et al.*[68] found that *P. biglobosa* treatment had the ability to reduce the oxidative stress that $KBrO_3$ treatment caused in the plasma, liver, kidney, and heart of treated rats. Its medicinal components may possibly be responsible for the reduction in $KBrO_3$-induced coagulopathy.

CONCLUSION

The results of this investigation demonstrated that potassium bromate caused prolongation of PT, aPTT, TT and decreased plasma levels of fibrinogen and vitamin K. *P. biglobosa* treatment counteracted these effects and brought about reduction of PT, aPTT and TT as well as elevation of fibrinogen and vitamin K levels. Thus, it is recommended that these results be investigated in clinical trials in human volunteers.

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