



Research Article

Antioxidant Capacity of Methanol Extract of *Gymnema sylvestre* Leaves and its Effect on Some Physical and Biochemical Parameters of Potassium Bromate Treated Rats

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ABSTRACT

This study investigated the antioxidant capacity of methanol extract of *Gymnema sylvestre* (gurmar) leaf and its effect on some physical and biochemical parameters in potassium bromate-treated rats. Group 1: control rats, group 2: rats administered methanol extract of *Gymnema sylvestre* leaf (200 mg / kg bwt), group 3: rats administered extract of *Gymnema sylvestre* leaf (500 mg / kg bwt), group 4: (KBrO₃) - treated rats (20 mg / kg, bwt), group 5: rats treated with KBrO₃ (20 mg / kg bwt) and extract of *Gymnema sylvestre* leaf (200 mg / kg bwt) and group 6: rats treated with KBrO₃ (20 mg / kg bwt) and extract of *Gymnema sylvestre* leaf (500 mg / kg bwt). Antioxidant activity of *G. sylvestre* was carried out using Ferric Reducing Antioxidant Power (FRAP) and 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) assays. Serum electrolyte concentrations of K⁺, Na⁺ and Cl⁻ were determined using an automatic electrolyte analyzer. Methanol extracts of *G. sylvestre* leaf exhibited antioxidant activity against free radicals. In the in vivo study, there was a significant decrease (p<0.05) in the weight of liver and increase in weight of kidney of rats in KBrO₃ treated group compared with the control group. There was also significant increase in the serum electrolyte concentration of rats in KBrO₃ treated group. However, this was reversed following the administration of methanol extracts of *G. sylvestre* to the KBrO₃-treated groups. The result of this study suggests that the methanol extract of the leaves of gurmar has the potential to ameliorate KBrO₃-induced toxicity in wistar rats.

Keywords: Potassium bromate; Toxicity; *Gymnema sylvestre*; Antioxidant; Serum; Biochemical

INTRODUCTION

Food additives are used purposely in enhancing quality, taste, or quantity (Anderson and Zagorski, 2023). While some are intentionally added, others become part of food unintentionally, occurring only in trace amount due to food packaging, storage and others (WHO, 2023). Potassium bromate (KBrO₃) is a white crystalline salt soluble in water

and only slightly soluble in alcohol but bromate (KBrO₃) is a white crystalline salt, soluble in water and only slightly soluble in alcohol but insoluble in ether (Shanmugavel *et al.*, 2020). It is an oxidizing agent that helps to improve dough (Anderson and Zagorski, 2023). Though it is a flour improver, there are many reported cases of toxicity involving potassium bromate and because of this, the National Agency for Food Administration and Control (NAFDAC); the agency responsible for regulating drugs, food and chemicals in Nigeria banned the use of this chemical in 2003 on account of its deleterious effects and carcinogenicity in humans (Airadion *et al.*, 2019). Evaluation of the occurrence of potassium bromate in bread, its overall health risks to bread

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consumers and toxicity symptoms in Bamenda (Cameroon) showed that almost all bakers had experienced symptoms of potassium bromate toxicity including painful eyes, cough, diarrhea, sore throat amongst others (Nkwatoh *et al.*, 2023).

As a strong oxidizing agent, potassium bromate is reported to cause disruption of the plasma membrane of cells (Olajide *et al.*, 2016).

Gymnema sylvestre R. Br. (Gentianales: Asclepiadaceae) is commonly known as *gumar*, periploca of plant in English, “*yaryad inkura*” in Hausa, “*abere*” in Yoruba and “*oche*” in Igala. It is a large woody climbing plant that is available in dry forest of India, Asia, China, Arabian Peninsula, Australia and Africa (Jamadagni *et al.*, 2021). *Gymnema sylvestre* contains gymnemic acid which is known to selectively suppress taste responses to sweet compounds without affecting the perception of other taste elements (Turner *et al.*, 2020). It has been used in Ayurvedic medicine and the triterpene saponins (gymnemic acid), gymnema saponins and gumarin have anti-microbial, anti-obesity, anti-diabetic, anti-arthritis, anti-inflammatory and anti-tumour potentials (Jangam *et al.*, 2023).

Potassium bromates poses treat to human health when consumed in processed foods such as bread. Some special plants on the other hand, have antioxidant potentials which can counter oxidative stress. *Gymnema sylvestre* is one of such special plants. This study investigated the ameliorative effects of methanol extract of *Gymnema sylvestre* leaf on potassium bromate toxicity by evaluating body / organ weights as well as serum electrolytes.

MATERIALS AND METHODS

Collection, identification and preparation of plant material

Fresh leaves of *Gymnema sylvestre* were collected from the vicinity of Iyale (7.656933, 7.3244224) in Dekina Local Government area of Kogi state, Nigeria. The leaves were identified and authenticated on 20th October 2022 at the Herbarium Unit, Department of Biological Sciences, Federal University, Lokoja, Kogi State Nigeria with Voucher Number: 0160. *Gymnema sylvestre* leaves were washed in sink, air dried for two weeks and pulverized. Methanol extract of the leaves was achieved by extracting 100 g of the pulverized plant material in 300 mL of methanol (Sasol Chemical Industries, Limited) for 12 hours. The residue was removed by filtration and the filtrate then concentrated under reduced pressure using Buchi Rotary Evaporator (Model: R-200) at 40°C (Momoh *et al.*, 2011). The crude extract obtained was stored in capped vials awaiting use at 4°C.

Acute toxicity and phytochemical profile of *Gymnema sylvestre* methanolic extract

Toxicological analysis of *Gymnema sylvestre* by Shukla *et al.*, (2020) on the safety profile preparation of *Gymnema sylvestre* (HPGS) and estimation of its chemical constituents / acute toxicity study in rats via the administration of HPGS did not produce any toxic symptoms or show mortality at the dose level of 300 mg/kg body weight. Phytochemical analysis

indicated that HPGS contained alkaloids, saponins, and flavonoids which demonstrated the non-toxic nature of HPGS *in vivo*, suggesting a long-term usage in clinical practices when administered orally. Also, Toxicological study on albino mice treated with *Gymnema sylvestre* extracts showed LD₅₀ level at 3990 mg/kg (Khan *et al.*, 2019).

Experimental design and animal grouping

A total of 60 albino rats with average body weight of 170.0 ± 3.0 g were purchased from the Department of Biochemistry, Animal House, Prince, Abubakar Audu University, Anyigba, Kogi State, Nigeria. The animals were kept in separate aluminum metabolic cages and were subjected to 12 hours light/ darkness photoperiods. The rats were allowed free access to chicken growers mash (11.85% crude protein and 2663.3 Kcal/kg metabolizable energy) (Vital Feeds, Nigerian Limited) and good drinking water. They were allowed to acclimatize for two weeks in a well-ventilated room. The rats were handled and used in accordance with guidelines set by the Research Ethical Committee of Kogi State University, Anyigba, Nigeria. The animals were randomly distributed into six treatment groups. After the acclimatization period was completed, the animals were weighed using electronic weighing balance (RS-232C, Mettler Toledo Limited, China) and then given dosage and administration daily for 10 to 15 days as follows: Group 1: control rats, group 2: rats treated with (200 mg / kg bwt) of *Gymnema sylvestre* methanol leaf extract, group 3: rats treated with (500 mg / kg bwt) of *Gymnema sylvestre* methanol leaf extract, group 4: rats treated with (20 mg / kg bwt) of KBrO₃, Group 5: rats treated with 20 mg/kg bwt KBrO₃ and 200 mg/kg bwt of *Gymnema sylvestre* methanol leaf extract and group 6: rats treated with 20 mg / kg bwt KBrO₃ and 500mg/kg bwt of *Gymnema sylvestre* methanol leaf extract after which analyses were carried out. At the end of the experimental period (10th and 15th administration), the rats were anaesthetized in a desiccator containing cotton wool soaked in chloroform. They were then quickly brought out of the desiccators and held at the neck. The neck region was cleared of fur to expose the jugular veins. With the aid of a sharp surgical blade, the rats were sacrificed by cutting the jugular veins. Blood samples were collected into a clean plain tube and centrifuged using a Laboratory Centrifuge (SM 800B, England) at a speed of 4000 rpm for 20 minutes to get the serum (Yakubu and Akanji, 2011). The serum samples, obtained were transferred into sample containers. The liver and kidney tissues were immediately excised and weighed after which they were homogenized in ice-cold 0.25 M sucrose solution.

Antioxidant assay using 2, 2- diphenyl-1-picrylhydrazyl (DPPH) method

The free radical scavenging property of the *Gymnema sylvestre* methanol leaf extracts was determined using stable radical DPPH (2, 2-Diphenyl-1-picrylhydrazyl hydrate) according to the method of Arogba and Omede (2012). This was achieved via the reduction of the deep violet color of DPPH to light yellow when it reacts with an antioxidant. This was done by the donation of hydrogen ion from the sample to reduce the

DPPH, the change in colour was read at spectrophotometrically at 540 nm wave length.

Antioxidant assay using ferric reducing antioxidant power (FRAP) method

Ferric reducing antioxidant power (FRAP) method as described by Arogba (2015) was used. The principle of this method is based on the reduction of a colourless ferric-tripyridyltriazine complex to its blue ferrous colour formed owing to electron donation in the presence of antioxidants (Benzie and Strain, 1999).

Determination of body and organ weights

Weights of the rats were measured before the start of administration and before sacrifice on the 10th and 15th day of administration respectively. Percentage weight gain, %WG = (final average weight of rat - initial average weight of rat / initial average weight of rat) x 100. Organ weights were also measured immediately after sacrifice on the 10th and 15th day of administration respectively. The organ-weight ratio was calculated as: (final average weight of rat / average weight of organ).

Biochemical parameters

Electrolyte estimation in kidney tissues

The determination of K⁺, Na⁺ and Cl⁻ concentrations were carried out using the Semi Auto Electrolyte Analyzer (Model: AC9801, Jiangsu Audicom Medical Technology Company Limited, China).

Statistical analysis

Experimental results were expressed as mean $\pm$ standard error of mean (SEM) by one-way ANOVA using instat statistical software of Sciexperts. Significant means were separated using a post hoc at $p < 0.05$ significance level.

RESULTS

Percentage inhibition of the *Gymnema sylvestre* leaf extract increased with concentration, indicating a dose-dependent antioxidant effect. At 1000 $\mu\text{g/ml}$, the extract achieved 87.60% inhibition, close to that of quercetin (95.70%). The result of the analysis showed that despite the antioxidant tendencies of the plant extract, quercetin consistently showed higher inhibition than the extract at all shared concentrations. The columns marked with * are significantly different ($p < 0.05$), suggesting that quercetin's antioxidant capacity is significantly superior to that of the extract at those doses. At certain concentrations such as 125, 31.25, and 7.8 $\mu\text{g/ml}$, values showed moderate antioxidant activity, often between the values of the extract and quercetin.

The LC₅₀ value for quercetin was 47.84 $\mu\text{g/ml}$, while that of *Gymnema sylvestre* methanol leaf extract was 83.86 $\mu\text{g/ml}$ which means it takes a smaller amount of quercetin to reach 50% inhibition, indicating higher potency compared to the extract. Methanol extract of *Gymnema sylvestre* leaf also exhibited free radical antioxidant power against free radicals

though at a lower magnitude compared to the standard; vitamin C. Methanol extracts of *G. sylvestre* leaves exhibited antioxidant activity against DPPH radical at 83.86. This was in tandem with the report of Keerthika and Raghu (2021) who reported that ethanol extract of *G. sylvestre* leaves scavenged at 83.8. These however, were of a lower magnitude than standard antioxidant compounds; quercetin.

Body and organ weights of rats administered methanol extract of *Gymnema sylvestre* leaves and KBrO₃ for 10 and 15 days

Results of final body weight and percentage weight gain of rats administered methanol extract of *G. sylvestre* leaves and KBrO₃ after 10 and 15 days of administration were similar. The result showed significant decrease ($p < 0.05$) between the control and the KBrO₃ (20 mg/kg bwt) group. The Gs (200 mg/kg bwt) and Gs (500 mg/kg bwt) groups also showed significant difference ($p < 0.05$) with the KBrO₃ (20 mg/kg bwt) group. A comparison of the KBrO₃ (20 mg/kg bwt) + Gs (200 mg/kg bwt) and the KBrO₃ (20 mg/kg bwt) + Gs (500 mg/kg bwt) groups showed significant increase in weight gain when a higher dose Gs (500 mg/kg) of the methanol extract of the *G. sylvestre* was co-administered with KBrO₃ instead of a much lower dose Gs (200 mg/kg bwt). This present study showed a significant decrease in weight of liver ($p < 0.05$) and increase in weight of kidney ($p < 0.05$) of rats in experimental group when compared with the control group. This finding was in contrast with the report of Oyewo *et al.* (2013) but was in agreement with the report of Kawana *et al.* (1991) who reported a decrease in weight of the liver and an increase in weight of the kidney of rats administered with potassium bromate.

DISCUSSION

The results of serum electrolyte concentration for K⁺, Na⁺ and Cl⁻, after 10 and 15 days of administration showed significant increase ($p < 0.05$) between the control group and the KBrO₃ (20 mg/kg bwt) group. This was in agreement with the report of Gazuwa *et al.* (2020) who reported that elevated levels of serum electrolytes were recorded in potassium bromate treated rats and that these elevations were directly proportional to the dose of potassium bromate administered and the duration of exposure to the substance. There was no significant difference ($p > 0.05$) in serum electrolyte concentration between the control group and the Gs (200 mg/kg bwt) as well as the Gs (500 mg/kg bwt) groups after 10 and 15 days of administration. However, there was significant decrease ($p < 0.05$) in serum electrolyte concentration in comparison of the KBrO₃ (20 mg/kg bwt) group to KBrO₃ (20 mg/kg bwt) + Gs (200 mg/kg bwt) group or KBrO₃ (20 mg/kg bwt) + Gs (500 mg/kg bwt) group respectively after 10 and 15 days of administration. The serum electrolyte concentration after 10 and 15 days of administration revealed a significant increase between the normal and the KBrO₃ group at $p < 0.05$. There was also a significant decrease ($p < 0.05$) when KBrO₃-treated rats were co-administered with either Gs (200 mg/kg bwt) or Gs (500 mg/kg bwt) of methanol extract of *G. sylvestre* leaves. This report was in agreement with Adewale *et al.*

(2020) who reported that KBrO_3 causes increase in serum electrolyte concentration. However, methanol extract of *Gymnema sylvestre* leaf ameliorated the increase in concentration observed respectively in the various serum electrolytes.

CONCLUSION

The methanol leaf extract of *Gymnema sylvestre* exhibits dose-dependent antioxidant activity, though with lower potency compared to standard antioxidants; quercetin and vitamin C. The extract showed protective effects against potassium bromate (KBrO_3)-induced changes in body and organ weights and serum electrolyte disturbances in the albino rats. Specifically, Co-administration of methanol leaf extract of *Gymnema sylvestre* with KBrO_3 led to improved weight gain and mitigation of KBrO_3 -induced serum electrolyte imbalances. Methanol leaf extract of *Gymnema sylvestre* ameliorated KBrO_3 -induced alterations in kidney weights and serum electrolyte concentrations (K^+ , Na^+ , Cl^-). These findings suggest *Gymnema sylvestre* has potential antioxidant and protective properties, supporting its traditional use and possible therapeutic applications. However, further studies, including detailed mechanistic investigations, are necessary to fully establish its pharmacological applications.

AUTHORS' CONTRIBUTIONS

Conceptualization, EJO; methodology, EA.; validation, EJO. and formal analysis, EA; investigation, EA; resources, EA; data curation, AM; writing— original draft preparation, EA; writing—review and editing, A M.; supervision, EJO.; project administration, EA.; funding acquisition,EA. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this paper.

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