EFFECTS OF *GYMNEMA SYLVESTRE* LEAVES EXTRACT ON LIVER AND KIDNEY OF POTASSIUM BROMATE-TREATED RATS

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ABSTRACT

This study investigated the effect of methanol extract of Gymnema sylvestre (Gs) leaves on body/organ weight and selected biochemical parameters of serum, liver, and kidney of potassium bromate-treated rats. The animals were grouped into six groups with the control as group 1 while others were administered $KBrO_3$ (20 mg/kg body weight (bwt)) only, Gs extract (200 mg/kg bwt) only, Gs extract (500 mg/kg bwt) only or with $KBrO_3$ (20 mg/kg bwt) and Gs extract (200 or 500 mg/kg bwt). Liver and kidney function indices were determined by spectrophotometric assay of the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and blood urea nitrogen (BUN) and creatinine concentrations respectively, while serum electrolyte: Na^+, K^+, Cl^-, Ca^{2+} and HCO_3^- concentrations were determined using an automatic electrolyte analyzer. $KBrO_3$ induced a significant increase ($p<0.05$) in the liver and kidney function indices when compared to the control after 10 and 15 days of administration. The increase in activity of the serum enzymes confirmed the property of $KBrO_3$ as a membrane labilisers. The administration of methanol extract of Gs leaves to $KBrO_3$-treated rats significantly reversed ($p<0.05$) the increase in activity of the liver enzymes as well as the electrolyte concentrations for both days. This is suggestive of the hepatoprotective potential and ability to protect the urinary tract by the plant extract. The results of this study suggest the possible application of the methanol extract of Gs leaves as a remedy for $KBrO_3$ toxicity.

Keywords: Potassium bromate, *Gymnema sylvestre*, Blood glucose, Liver function, Kidney function

INTRODUCTION

In the food industry, food additives are used purposely to enhance quality, taste, or quantity (Anderson and Zagorski, 2023). While some are intentionally added, others become part of food unintentionally, occurring only in trace amounts due to food packaging, storage, and other handling (WHO, 2023). Potassium bromate ($KBrO_3$) 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). Desirable as it may as 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 (Airaodion

et al., 2019). Evaluation of the occurrence of potassium bromate in bread, its overall health risks to bread consumers, and toxicity symptoms in Bamenda (Cameroon) showed that almost all bakers had experienced symptoms of potassium bromate toxicity including painful eyes, cough, diarrhea, and sore throat (Nkwatoh *et al*., 2023).

Being a strong oxidizing agent, potassium bromate is reported to disrupt the plasma membrane of cells (Olajide *et al*., 2016). Also, Oshikoya (2009) reported the toxicity of acute oral administration of potassium bromate thereby supporting the findings of Akanji *et al*. (2008) who reported the toxicity of potassium bromate at 10 mg/kg body weight to kidney, liver, and small intestine cells of the exposed rat. Another study has also indicated that exposure to bromate causes renal toxicity in men and experimental animals through peroxidation of membrane lipids and DNA damage (Uchida *et al*., 2006). Furthermore, potassium bromate induces renal oxidative stress which is known to cause renal failure, methaemoglobinaemia, and kidney cancer (Parsons and Chipman, 2000)

Gymnema sylvestre R. Br. (Gentianales: Asclepiadaceae) is commonly known as periploca of plant in English, "yaryad inkura" in Hausa, 'abere" in Yoruba, "aviukusugar" in Ebira and "oche" in Igala. It is a large woody climbing plant that is available in dry forests of India, Asia, China, the Arabian peninsula, Australia, and Africa (Jamadagni *et al*., 2021). *G. 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-arthritic, anti-inflammatory, and anti-tumor properties (Satdive *et al*., 2003; Kanetkar *et al*., 2007; Jangam *et al*., 2023; Ghosh *et al*., 2023).

Due to the common use of potassium bromate, a relatively large number of people are exposed to the compound. A survey on potassium bromate in bread samples revealed the presence of bromate in breads selected for such analysis (Turner *et al*., 2020). This study therefore investigated the effect of potassium bromate administered with methanol extract of *G. sylvestre* leaf extract on body/organ weight, some liver enzymes, and kidney function indices.

MATERIALS AND METHODS

Collection, Identification, and Preparation of Plant Material: Fresh leaves of *G. sylvestre* were collected from the vicinity of Ajaokuta Steel Complex (GPS 7.584627930029824, 6.714444912183313), Ajaokuta Local Government Area, Kogi State, Nigeria. The leaves were identified (Utteridge and Bramley, 2016) and authenticated on 16th January 2019 at the Herbarium Unit, Department of Biological Sciences, Federal University, Lokoja, Kogi State Nigeria with Voucher Number: 0150. *G. sylvestre* leaves were washed in a sink, air-dried for two weeks, and pulverized. To obtain the methanol extract, 100 g of the pulverized plant material was extracted in 300 ml of methanol (Sasol Chemical Industries, Limited) for 12 hours. The residue was removed by filtration and the filtrate was then concentrated under reduced pressure using a Büchi Rotary Evaporator (Model: R-200) at 40°C (Momoh *et al*., 2011). The crude extract obtained was stored in capped vials pending use at 4°C.

Acute Toxicity and Phytochemical Profile of *Gymnema sylvestre* Methanolic Extract: Toxicological analysis of *G. sylvestre* by Shukla *et al*. (2020) on the safety profile of homeopathic preparation of *G. 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 long-term usage in clinical practices when administered orally. Also, a toxicology study on albino mice treated with *G. sylvestre* extracts showed an LD_{50} level of 3990 mg/kg (Khan *et al*., 2019).

Experimental Design and Animal Grouping: A total of 54 rats with an average body weight of 150.0 ± 3.0 g were purchased from the

Department of Biochemistry, Animal House, University of Nigeria Nsukka, Enugu State. The animals were kept in separate aluminum metabolic cages and were subjected to 12-hour 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 per guidelines set by the Research Ethical Committee of Kogi State University, Anyigba, Nigeria. The animals were randomly distributed into six treatment groups, replicated three times with each replicate having three rats (Table 1).

Table 1: Animal grouping and administration of methanol extract of *G. sylvestre* leaves and potassium bromate

Group	Dose of methanol extract of *G. sylvestre* leaves or potassium bromate administered in mg/kg body weight	Number of rats
1	Control rats were treated with drinking water and chicken growers mash (diet).	9
2	200 mg/kg body weight of methanol extract of *G. sylvestre* leaves.	9
3	500 mg/kg body weight of methanol extract of *G. sylvestre* leaves.	9
4	20 mg/kg body weight of potassium bromate ($KBrO_3$)	9
5	20 mg/kg body weight of potassium bromate + 200 mg/kg body weight of methanol extract of *G. sylvestre* leaf.	9
6	20 mg/kg body weight of potassium bromate + 500 mg/kg body weight of methanol extract of *G. sylvestre* leaf	9

After the acclimatization period was completed, the animals were weighed using an electronic weighing balance (RS-232C, Mettler Toledo Limited, China) and then administered several treatments daily for up to 10 and 15 days as follows: Group 1: control rats, Group 2: rats administered methanol extract of *G. sylvestre* leaves (200 mg/kg bwt), Group 3: rats administered methanol extract of *G. sylvestre* leaves (500 mg/kg bwt), Group 4: ($KBrO_3$) - treated rats (20 mg/kg, bwt), group 5: rats treated with $KBrO_3$ (20 mg/kg bwt) and methanol extract of *G. sylvestre* leaves (200 mg/kg bwt) and group 6: rats treated with $KBrO_3$ (20 mg/kg bwt) and methanol extract of *G sylvestre* leaves (500 mg /kg bwt) after which the several analyses discussed shortly were carried out.

Collection of Blood Sample: 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 razor blade, the rats were sacrificed by cutting the jugular veins, and blood samples were collected by allowing them to drip down into clean plain bottles. The liver and kidney tissues were immediately excised and weighed after which they were homogenized in ice-cold 0.25 M sucrose solution. The blood was then 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).

Antioxidant Assay Using 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) Method: The free radical scavenging property of the *G. 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 colour of DPPH to light yellow when it reacts with an antioxidant. This was done by the donation of a hydrogen ion from the sample to reduce the DPPH, The change in colour was read spectrophotometrically at 540 nm wavelength (Blois, 1958)

Antioxidant Assay Using Ferric Reducing Antioxidant Power (FRAP) Method: The 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 Organs Weights: The weights of the rats were measured before the start of administration and before sacrifice on the 10^{th} and 15^{th} 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 10^{th} and 15^{th} day of administration respectively. The organ-weight ratio was calculated as: (the final average weight of the rat / average weight of the organ).

Biochemical Parameters

Determination of Blood Glucose: Blood glucose concentration was determined using a glucometer which uses the electrochemical technology in the electrochemical test strips containing an enzyme called glucose oxidase. Glucose oxidase reacts with glucose in the blood sample and creates an acid called gluconic acid. The gluconic acid formed then reacts with another chemical in the testing strip called ferricyanide. The ferricyanide and the gluconic acid again react with each other to form ferrocyanide. Immediately after the ferrocyanide was formed, the glucometer runs an electronic current through the blood sample on the strip, and the current thus produced is capable of reading the ferrocyanide and identifying the amount of glucose present in the blood sample on the testing strip per Beer-Lambert's law of absorbance spectrophotometry.

Estimation of liver function parameters: Determination of serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activity were carried out using Randox kit (Randox Laboratories Limited, 55 Diamond Road, Crumlin) instructions, while the activity of alkaline phosphatase was determined using Teco Diagnostics Kit instructions.

Estimation of kidney function parameters: Serum creatinine concentration and blood urea nitrogen (BUN) were determined using Agape Diagnostics Kits (Agape, Switzerland) instructions.

Electrolyte estimation in kidney tissues: The determination of Na^+, K^+, Ca^{2+}, Na^+, Cl^- and HCO_3^- concentrations was carried out using the Semi-Auto Electrolyte Analyzer (Model: AC9801, Jiangsu Audicom Medical Technology Company Limited, China).

Statistical Analysis: The data was analyzed by one-way ANOVA using the instant statistical software of Sciexperts. Significant means were separated using a post hoc at $p<0.05$ significance level. Experimental results were expressed as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Free Radical Scavenging Activity of Methanol Extract of *Gymnema sylvestre* Leaves: 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 as shown in Table 2. Methanol extract of *G. sylvestre* leaves exhibited free radical antioxidant power against free radicals though at a lower magnitude compared to the standard; vitamin C as shown in Table 3.

Body and Organ Weights of Rats Administered Methanol Extract of *Gymnema sylvestre* Leaves and $KBrO_3$ for 10 and 15 Days: Results of final body weight and percentage weight gain of rats administered methanol extract of *G. sylvestre* leaves and $KBrO_3$ after 10 and 15 days of administration were similar as shown in Table 4. The result showed a significant decrease ($p<0.05$) between the control and the $KBrO_3$ (20 mg/kg bwt) group. The Gs (200 mg/kg bwt) and Gs (500 mg/kg bwt) groups also showed significant differences ($p<0.05$) with the $KBrO_3$ (20 mg/kg bwt) group. A comparison of

the $KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt) and the $KBrO_3$ (20 mg/kg bwt) + Gs (500 mg/kg bwt) groups showed a 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 $KBrO_3$ instead of a much lower dose Gs (200 mg /kg bwt).

Table 2: Free radical scavenging activity of methanol extract of *Gymnema sylvestre* leaf using DPPH

SN	Concentration (µg/ml)	Percentage inhibition	
		Extract	Quercetin
1	1000	87.70 ± 4.50	95.80 ±0.90*
2	500	82.72 ±0.781	87.63 ±1.85*
3	250	69.54 ±0.862	79.93 ±4.31*
4	125	55.39 ±0.710	67.97 ±6.50*
5	62.5	43.47±0.651	52.90 ±2.52*
6	31.25	30.59 ±0.603	45.67 ±1.26*
7	15.6	24.60 ±1.106	32.20 ±1.78*
8	7.8	12.76 ±1.656	17.50 ±1.76*
LC_{50}		83.86*	47.84

**Significant mean at p<0.05 using student pairwise comparison (t-test)*

Table 3: Free radical scavenging activity of methanol extract of *Gymnema sylvestre* leaves using ferric reducing antioxidant power (FRAP) method

Sample Concentration	Ration (mg/ml)	FRAP value
Extract	50	1.50 ± 0.28
Vitamin C	50	2.00 ± 0.00*

**Significant mean at p<0.05 using student pairwise comparison (t-test)*

This present study showed a significant decrease in the weight of the liver ($p<0.05$) and an increase in the weight of the kidney ($p<0.05$) of rats in the experimental group when compared with the control group. This finding was in contrast with the report of Oyewo *et al*. (2013) but agreed with the report of Kawana *et al*. (1991) who reported a decrease in the weight of the liver and an increase in the weight of the kidneys of rats administered with potassium bromate.

Blood Glucose Level: The result of blood glucose level revealed a significant decrease ($p<0.05$) in blood glucose level between the control and the $KBrO_3$ (20 mg/kg bwt) group after 10 days of administration and no significant difference ($p>0.05$) between the two groups after 15 days of administration. There was no significant difference ($p>0.05$) in blood glucose level between the Gs (200 mg/kg bwt) and the Gs (500 mg/kg bwt) after 10 days of administration but there was a significant decrease ($p<0.05$) in the blood glucose values between these two groups after 15 days of administration. Also, there was no significant difference ($p>0.05$) in blood glucose level between the $KBrO_3$ (20 mg/kg bwt) group and the $KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt) group after 10 days of administration, but there was a significant increase ($p<0.05$) in the values of these two groups after 15 days of administration. Similarly, there was no significant difference ($p>0.05$) in blood glucose concentration between $KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt) and $KBrO_3$ (20 mg/kg bwt) + Gs (500 mg/kg bwt) group after 10 days of administration but a significant decrease ($p<0.05$) in blood glucose level was observed after 15 days of administration. Figures 1 and 2 showed that there was no significant difference ($p>0.05$) in the blood glucose level of the control group for both 10 and 15 days of administration. There was a significant increase ($p<0.05$) in blood glucose levels between the 10 and 15 days of administration in the $KBrO_3$ group. Furthermore, Figures 1 and 2 showed a significant increase ($p<0.05$) in blood glucose levels for 10 and 15 days of administration in the Gs (200 mg/kg bwt) group. There was no significant difference ($p>0.05$) between the results of the 10^{th} and 15^{th} day of administration of the Gs (500 mg/kg bwt) group. However, there was a significant decrease ($p<0.05$) in blood glucose level between the 10th and 15th day administration result of the $KBrO_3$ (20 mg/kg bwt) + Gs (500 mg/kg bwt) group.

As earlier reported by Paliwal *et al*. (2009), the first confirmation of *G. sylvestre* used in human diabetics came almost a century back when it was demonstrated that the leaves of *G. sylvestre* reduced urine glucose in diabetics.

Paliwal *et al*. (2009) demonstrated that *G. sylvestre* leaf powder has positive and encouraging effects on blood glucose levels.

Table 4: Body and organ weight of rats administered methanol extract of *Gymnema sylvestre* leaves and $KBrO_3$ for 10 and 15 days

Groups	The initial weight of the rat (g)	Final body weight of rats (g)	(%) Weight gain	Weight of liver at sacrifice (g)	Weight of kidney at sacrifice (g)	Liver body weight ratio	Kidney body weight Ratio
	10 days						
Control	106.87 ± 0.94	151.00 ± 4.25	38.85^{e}	5.60 ± 0.27^{b}	0.93 ± 0.03^{a}	27.31 ± 1.30^{a}	163.43 ± 3.71^{d}
GS (200 mg/kg bwt)	134.6 ± 2.16	147.50 ± 0.76	9.58^{c}	3.96 ± 0.1^{a}	0.88 ± 0.04^{a}	37.25 ± 0.88^{b}	167.61 ± 7.74^{d}
GS (500 mg/kg bwt)	134.6 ± 0.9	159.67 ± 0.69	18.62^{d}	4.61 ± 0.21^{a}	1.08 ± 0.03^{a}	34.71 ± 1.31^{b}	147.84 ± 3.86^{b}
$KBrO_3$ (20 mg/kg bwt)	192.75 ± 2.63	177.67 ± 0.84	-7.82^{a}	4.89 ± 0.10^{a}	1.22 ± 0.03^{b}	36.33 ± 0.66^{b}	145.63 ± 3.42^{b}
$KBrO_3$ (2 $KBrO_3$ (20 mg/kg bwt) + GS (200 mg/kg bwt)	176.6 ± 1.12	160.67 ± 0.51	-9.02^{a}	5.45 ± 0.28^{b}	1.17 ± 0.04^{a}	29.48 ± 1.31^{a}	137.32 ± 3.76^{a}
$KBrO_3$ (20 mg/kg bwt) + GS (500 mg/kg bwt)	154.40 ± 1.68	159.50 ± 0.17	3.30^{b}	4.03 ± 0.003^{a}	1.01 ± 0.01^{a}	39.57 ± 0.04^{d}	157.92 ± 0.88^{c}
	15 days						
Control	106.87 ± 0.94	151.00 ± 4.25	38.85^{d}	5.60 ± 0.27^{b}	0.93 ± 0.03^{a}	27.31 ± 1.30^{a}	163.43 ± 3.71^{c}
GS (200 mg/kg bwt)	134.60 ± 2.16	168.67 ± 1.02	25.31^{c}	4.62 ± 0.26^{a}	1.04 ± 0.01^{a}	36.51 ± 2.20^{b}	162.18 ± 2.17^{c}
GS (500 mg/kg bwt)	134.60 ± 0.90	167.33 ± 2.22	24.32^{c}	4.91 ± 0.18^{a}	1.01 ± 0.00^{a}	34.08 ± 0.91^{b}	165.67 ± 1.67^{c}
$KBrO_3$ (20 mg/kg bwt)	192.75 ± 2.63	181.67 ± 0.51	-5.75^{a}	5.50 ± 0.12^{b}	1.30 ± 0.02^{b}	33.03 ± 0.73^{b}	139.75 ± 2.20^{a}
$KBrO_3$ (20 mg/kg bwt) + GS (200 mg/kg bwt)	176.60 ± 1.12	170.01 ± 0.30	-3.73^{a}	5.33 ± 0.16^{b}	1.10 ± 0.03^{a}	31.90 ± 0.90^{ab}	154.55 ± 3.24^{b}
$KBrO_3$ (20 mg/kg bwt) + GS (500 mg/kg bwt)	154. 40 ± 1.70	177.33 ± 0.84	14.85^{b}	5.02 ± 0.14^{a}	1.19 ± 0.02^{a}	35.32 ± 0.9^{b}	149.02 ± 2.49^{b}

a-f means on the same column with different letter superscripts are significantly different ($p<0.05$), GS = Gymnema sylvestre

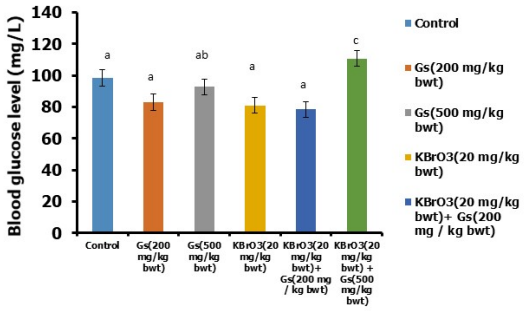

Figure 1: Graph showing the effect of $KBrO_3$ and methanol extract of *Gymnema sylvestre* leaves on the blood glucose level of $KBrO_3$-treated rats after 10 days of administration. Key: *a-f bars with different letter superscripts are significantly different (p<0.05), GS = Gymnema sylvestre*

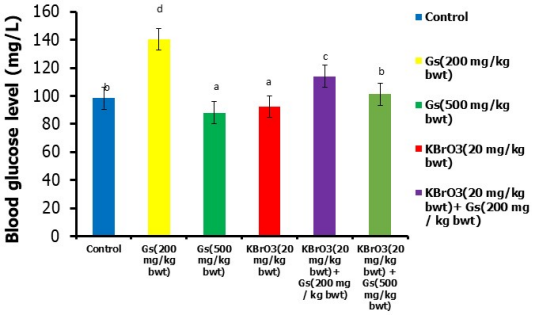

Figure 2: Graph showing the effect of $KBrO_3$ and methanol extract of *Gymnema sylvestre* leaves on the blood glucose level of $KBrO_3$-treated rats after 15 days of administration. Key: *a-f bars with different letter superscripts are significantly different (p<0.05), GS = Gymnema sylvestre*

No harmful effect was observed on the health status of the subjects and thus, which indicates that *G. sylvestre* powder is effective in lowering fasting as well as postprandial blood glucose levels. The day 10 and 15 results of blood glucose levels in this present research indicated that both potassium bromate and *G. sylvestre* did not show any significant perturbation of serum glucose concentration. The reason for this is not clear but it may be because the dose of bromate administered may not have caused damage severe enough to affect the glomerular glucose retention in the blood and also as the rats were not induced with any form of glucose.

Liver Function Parameters: The result of this present study via the assessment of liver function by spectrophotometric measurement of serum levels of AST, ALT, and ALP revealed that bromate caused an increase in the levels of the enzymes in the potassium bromate treated group which was reversed by co-administration of methanol extract of the *G. sylvestre* leaves (Table 5). This was in tandem with the report of Bayomy *et al.* (2016) who reported a significant rise in levels of ALT, AST, and ALP in liver tissues of rats treated with potassium bromate, and the effects were subsequently ameliorated by administration of Vitamin C. Elevated levels of these enzymes occur as a result of damage of cellular tissues within the liver which leads to leakage of the enzymes from the hepatocyte into the blood following hepatocellular injury (Kedderis, 1996). An increase in AST level was noticed in the potassium bromate-treated rats leading to weakness and redness of the eye was seen during the early stage of this study. This was in agreement with the report of Turner *et al.* (2020) who reported redness of the eye and weakness of rats on exposure to potassium bromate. However, these effects subsided after subsequent co-administration of methanol extract of *G. sylvestre* leaves. This indicates the hepatoprotective potential of the plant extract against bromate-induced damage. The trend of the results for this study was similar for rats treated for either 10 or 15 days of administration.

Blood Urea Nitrogen: The result of BUN activity showed a significant increase ($p<0.05$) after both 10 and 15 days of administration when compared with the control and $KBrO_3$ (20 mg/kg bwt) group (Figures 3 and 4). This was in tandem with the report of Gazuwa *et al.* (2020). There was no significant difference ($p>0.05$) between the Gs (200 mg/kg bwt) and the Gs (500 mg/kg bwt) groups after 10 and 15 days of administration. After both days, there was a significant decrease ($p<0.05$) in BUN concentration when the $KBrO_3$ (20 mg/kg bwt) group was compared to the $KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt) group. In addition, there was a significant decrease ($p<0.05$) when the values of BUN concentration for $KBrO_3$ (20 mg/kg bwt) group were compared with those of the $KBrO_3$ (20 mg/kg bwt) + Gs (500 mg/kg bwt) group.

Table 5: Liver function parameters of $KBrO_3$-treated rats administered methanol extract of *Gymnema sylvestre* leaves

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
	Day 10		
Control	8.75 ± 0.40^a	28.25 ±1.52^c	38.25 ± 1.34^c
GS (200 mg/kg bwt)	16.38 ± 0.25^b	22.33 ± 0.5^b	25.83 ± 0.63^c
GS (500 mg/kg bwt)	11.17 ± 0.25^a	29.57 ±0.42^c	15.67 ± 1.02^b
$KBrO_3$ (20 mg/kg bwt)	21.87 ± 0.54^c	44.17 ±1.27^d	30.27 ± 0.54^d
$KBrO_3$(20 mg/kg bwt) + GS (200 mg/kg bwt)	15.83 ± 0.01^b	26.13 ± 0.27^{bc}	27.00 ± 0.90^c
$KBrO_3$(20 mg/kg bwt) + GS (500 mg/kg bwt)	14.27 ± 0.37^b	14.00 ±0.67^a	10.03 ± 0.37^a
	Day 15		
Control	8.75 ± 0.40^b	28.25 ± 1.52^c	38.25 ± 1.34^d
GS (200 mg/kg bwt)	7.27 ± 0.21^b	16.93 ± 0.42^b	30.33 ± 0.51^c
GS (500 mg/kg bwt)	2.33 ± 0.19^a	18.33 ± 0.51^b	12.00 ± 0.70^a
$KBrO_3$ (20 mg/kg bwt)	16.93 ±0.17^c	15.83 ± 0.48^b	50.83 ± 0.43^e
$KBrO_3$(20 mg/kg bwt) + GS (200 mg/kg bwt)	3.33 ± 0.19^a	8.53 ± 0.43^a	17.66 ± 0.51^b
$KBrO_3$(20 mg/kg bwt) + GS (500 mg/kg bwt)	2.67 ± 0.19^a	13.67 ±0.38^b	24.33 ± 0.51^c

a-f means on the same column with different letter superscripts are significantly different (p<0.05), GS = Gymnema sylvestre

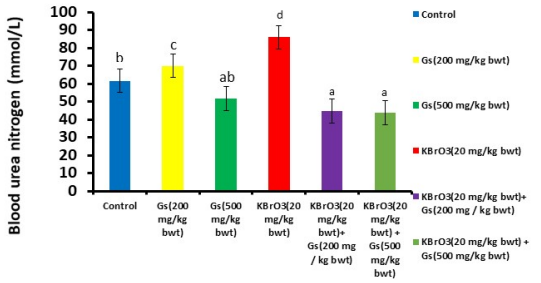

Figure 3: Graph showing the effect of $KBrO_3$ and methanol extract of *Gymnema sylvestre* leaves on the blood urea nitrogen of $KBrO_3$-treated rats after 10 days of administration. Key: *a-f bars with different letter superscripts are significantly different (p<0.05), GS = Gymnema sylvestre*

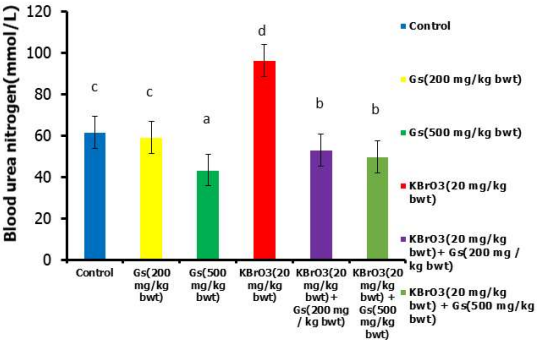

Figure 4: Graph showing the effect of $KBrO_3$ and methanol extract of *Gymnema sylvestre* leaves on the blood urea nitrogen of $KBrO_3$-treated rats after 15 days of administration. Key: *a-f bars with different letter superscripts are significantly different (p<0.05), GS = Gymnema sylvestre*

The graph of BUN concentration (mmol/L) against the days of administration revealed that there was no significant difference (p>0.05) in the BUN concentration of the control after 10 and 15 days of administration. There was a significant increase (p<0.05) in BUN concentration between the 10th and 15th days of administration for the $KBrO_3$ (20 mg/kg bwt) group. The graph also indicated a significant increase (p<0.05) in BUN concentration after 10 and 15 days of administration in the Gs (200 mg/kg bwt) group. In the $KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt) group there was a significant increase (p<0.05) in BUN concentration between 10 and 15 days of administration. A Significant increase (p<0.05) was also observed in the Gs (500 mg/kg bwt) group. However, there was no significant difference (p>0.05) between the two days for the $KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt) group.

These findings were similar to the report of Oshikoya (2009) who reported that potassium bromate caused an increase in plasma BUN concentration. The BUN concentration after 10 and 15 days showed a significant increase between the normal and the $KBrO_3$ group at p<0.05. There was a significant decrease (p<0.05) in the BUN and serum creatinine concentration when the $KBrO_3$-treated rats were co-administered with either Gs (200 mg/kg bwt) or Gs (500 mg/kg bwt) of the methanol extract of *G. sylvestre* leaves. Hence, the methanol extract of *G. Sylvestre* leaves ameliorated the effect of $KBrO_3$ on kidney function indices.

Serum Creatinine Concentration: The result of the serum creatinine assay revealed a significant increase (p<0.05) in serum creatinine

concentration when the values of the control group were compared to those of the $KBrO_3$ (20 mg/kg bwt) group after 10 and 15 days of administration. This was in tandem with the report of Altoom *et al*. (2018) who reported that potassium bromate caused an increase in plasma BUN and creatinine concentration.

A comparison between the Gs (200 mg/kg bwt) and the Gs (500 mg/kg bwt) group after both 10 and 15 days showed a significant decrease ($p<0.05$) in serum creatinine concentration. A comparison of $KBrO_3$ (20 mg/kg bwt) group to $KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt) group, after 10 and 15 days of the administration, revealed a significant decrease ($p<0.05$) in serum creatinine concentration. A comparison between the $KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt) group and the $KBrO_3$ (20 mg/kg bwt) + Gs(500 mg/kg bwt) group after both days indicated that there was no significant difference ($p>0.05$) in serum creatinine concentration. A significant decrease ($p<0.05$) in serum creatinine concentration occurred between $KBrO_3$ (20 mg/kg bwt) group and the $KBrO_3$ (20 mg/kg bwt) + Gs (500 mg/kg bwt) group after both 10 and 15 days of administration. This was a result of the antioxidant capacity of the co-administered methanol extract of *G. sylvestre.* This is in tandem with the report of Gumbar *et al*. (2023) who stated that the antioxidant properties of *G. sylvestre* restored renal oxidative stress markers, reducing kidney inflammation and injury but in disagreement with their report that gymnemic acid demonstrated greater antioxidant efficacy than the standard, vitamin E in restoring antioxidant potentials and mitochondria enzymes.

The graph of serum creatinine concentration against the days of administration (Figures 5 and 6) indicated that there was no significant difference ($p>0.05$) in the values of the control after 10 and 15 days of administration. However, there was a significant increase ($p<0.05$) between the two days of administration concerning the $KBrO_3$ (20 mg/kg bwt) group.

The graph indicated that there was no significant difference ($p>0.05$) between the two days for the Gs (200 mg/kg bwt) and Gs (500 mg/kg bwt) groups, but there was a significant increase ($p<0.05$) in-between the 10 and 15 days of administration of the $KBrO_3$ (20 mg/kg bwt) + Gs (500 mg/kg bwt) group.

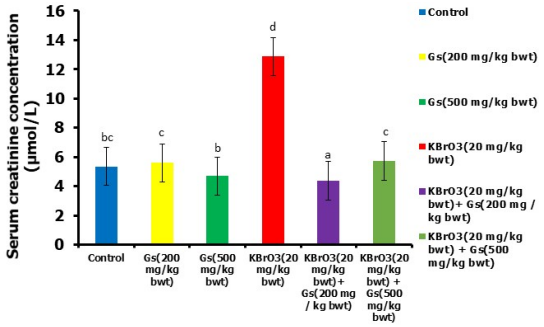

Figure 5: Graph showing the effects of $KBrO_3$ and methanol extract of *Gymnema sylvestre* leaves on the serum creatinine concentration of $KBrO_3$-treated rats after 10 days of administration. Key: *a-f bars with different letter superscripts are significantly different (p<0.05), GS = Gymnema sylvestre*

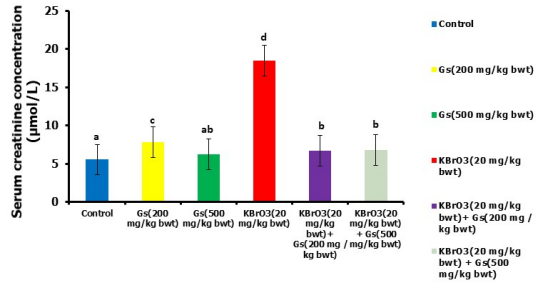

Figure 6: Graph showing the effects of $KBrO_3$ and methanol extract of *Gymnema sylvestre* leaves on the serum creatinine concentration of $KBrO_3$-treated rats after 15 days of administration. Key: *a-f bars with different letter superscripts are significantly different (p<0.05), GS = Gymnema sylvestre*

This was in agreement with Adewale *et al*. (2020) who reported nephrotoxicity and oxidative stress in rats administered with $KBrO_3$.

Serum Electrolyte: The results of serum electrolyte concentration for Na^+, K^+, Cl^-, Ca^{2+}, and HCO_3^- after 10 and 15 days of administration showed a significant increase ($p<0.05$) between the control group and the $KBrO_3$ (20 mg/kg bwt) group (Table 6). 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.

Table 6: Serum electrolyte concentration of potassium bromate treated rats administered methanol extract of *Gymnema sylvestre* leaves

Group	Na^+ (mMol/l) Day10	Na^+ (mMol/l) Day 15	K^+ (mMol/l) Day 10	K^+ (mMol/l) Day15	Cl^- (mmol/l) Day 10
Normal	142.25 ± 1.77^a	142.25 ± 1.77^a	7.18 ± 0.33^a	7.18 ± 0.33^a	109.50 ± 1.64^b
Gs (200 mg/kg bwt)	140.33 ± 0.69^a	142.33 ± 3.01^a	6.50 ± 0.29^a	7.70 ± 0.58^a	105.67 ± 1.64^b
Gs (500 mg/kg bwt)	137.00 ± 1.76^a	141.33 ± 1.54^a	6.76 ± 0.25^a	6.87 ± 0.18^a	105.33 ±0.84^b
$KBrO_3$ (20 mg/kg bwt)	355.60 ± 0.28^b	362.83 ± 2.08^b	16.17 ± 0.24^b	14.27 ± 0.13^b	260.00 ± 1.67^c
$KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt)	138.33 ± 0.51^a	145.67 ± 0.69^a	6.73 ± 0.20^a	5.80 ± 0.03^a	100.33 ± 0.69^a
$KBrO_3$ (20 mg/kg bwt + Gs (500 mg/kg bwt)	140.66 ± 1.39^a	145.67 ± 1.02^a	6.83 ± 0.12a	6.16 ± 0.18 ^{a}	108.34 ± 0.38^b
	Cl^- (mmol/l) Day 15	**Ca^{2+} (mmol/l) Day 10**	**Ca^{2+} (mmol/l) Day 15**	**HCO_3^- (mmol/l) Day 10**	**HCO_3^- (mmol/l) Day 15**
Normal	109.50 ± 1.64^a	1.00 ± 0.04^a	1.00 ± 0.04^a	26.00 ± 0.98^a	26.00 ± 0.98^a
Gs (200 mg/kg bwt)	108.67 ± 1.02^a	0.70 ± 0.00^a	1.04 ± 0.02^a	24.67 ± 0.19^a	26.67 ± 1.50^a
Gs (500 mg/kg bwt)	107.67 ± 0.69^a	1.09 ± 0.01^a	0.73 ± 0.03^a	24.00 ± 0.58^a	25.00 ± 0.67^a
$KBrO_3$ (20 mg/kg bwt)	267.50 ±2.50^b	2.70 ± 0.01^b	2.09 ± 0.08^b	71.00 ± 1.88^b	63.33 ± 1.75^b
$KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt)	111.66 ± 0.84^a	1.0 ± 0.05^a	0.68 ± 0.03^a	26.33 ± 0.84^a	25.00 ± 0.67^a
$KBrO_3$ (20 mg/kg bwt + Gs (500 mg/kg bwt)	110.67 ± 0.69^a	1.08 ± 0.02^a	0.73 ± 0.05^a	27.23 ± 0.89^a	24.33 ± 1.26^a

a-f means on the same column with different letter superscripts are significantly different ($p<0.05$), GS = Gymnema sylvestre

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 $KBrO_3$ (20 mg/kg bwt) group to $KBrO_3$ (20 mg/kg bwt) + Gs (200 mg/kg bwt) group or $KBrO_3$ (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 $KBrO_3$ group at $p<0.05$. There was also a significant decrease ($p<0.05$) when $KBrO_3$-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 an increase in serum electrolyte concentration. However, the methanol extract of *G. sylvestre* leaves ameliorated the increase in concentration observed respectively in the various serum electrolytes. Serum electrolytes of animals in the combination therapy followed a similar trend to that of BUN and serum creatinine levels as reported.

Conclusion: In conclusion, the methanol extract of *G. sylvestre* leaves through its antioxidant potential was responsible for the observed restoration of biochemical indices of renal and liver functions of potassium bromate-treated rats administered with the methanol extracts of *G. sylvestre leaves.* These evidences support the use of this plant in native medicine.

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REFERENCES

ADEWALE, O. O., AREMU, K. H. and ADEYEMO, A. T. (2020). Assessment of combined toxic effects of potassium bromate and sodium nitrite in some key renal markers in male Wistar rats. *Research Journal of Health Sciences*, 8(1): 6 – 17.

AIRAODION, A. I., EWA, O., OGBUAGU, E. O., OGBUAGU, U., AGUNBIADE, A. P. and OLORUNTOBA, A. P. (2019). Evaluation of potassium bromate in bread in Ibadan metropolis: fifteen years after ban in Nigeria. *Asian Food Science Journal*, 7(4): 1 – 7.

AKANJI, M., NAFIU, M. and YAKUBU, M. (2008). Enzyme activities and histopathology of selected tissues in rats treated with potassium bromate. *African Journal of Biomedical Research*, 11(1): 87 – 95.

ALTOOM, N. G., AJAREM, J., ALLAM, A. A., MAODAA, S. N. and ABDEL-MAKSOUD, M. A. (2018). Deleterious effects of potassium bromate administration on renal and hepatic tissues of Swiss mice. *Saudi Journal of Biological Sciences*, 25(2): 278 – 284.

ANDERSON, E. and ZAGORSKI, J. (2023). *Trending – Potassium Bromate.* Centre for Research on Ingredient Safety, Michigan State University, East Lansing, Michigan, United States. https://www.canr.msu.edu/news/potassium-bromate# Accessed October 17, 2023.

AROGBA, S. S. (2015). Effect of processing on antioxidant activity of conventional mango (*Mangifera indica*) seed. *Journal of Environmental Science, Toxicology and Food Technology*, 9(4): 50 - 55.

AROGBA, S. S. and OMEDE, A. (2012). Comparative antioxidant activity of processed mango *(Mangifera indica)* and bush mango *(Irvingia gabonensis)* kernels. *Nigerian Food Journal*, 30(2): 17 – 21.

BAYOMY, N. A., SOLIMAN, G. M. and ABDELAZIZ, E. Z. (2016). Effect of potassium bromate on the liver of adult male albino rat and a possible protective role of vitamin C: histological, immunohistochemical, and

biochemical study. *The Anatomical Record*, 299(9): 1256 – 1269.

BENZIE, I. F. and STRAIN, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology,* 299: 15 – 27.

BLOIS, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181: 1199 – 1200.

GAZUWA, S. Y., DABAK, J. D,. JARYUM, K. H. and OLUWA, I. (2020). Relationship between dose and duration of administration of potassium bromate on selected electrolytes and hepatorenal parameters in male albino Wistar rats. *European Journal of Nutrition and Food Safety*, 11(4): 214 – 220.

GHOSH, A. R., ALSAYARI, A., HABIB, A. H., WAHAB, S., NADIG, A. P., RAFEEQ, M. M., BINOTHMAN, N., ALJADANI, M., AL-DHUAYAN, I. S., ALAQEEL, N. K. and KHALID, M. (2023). Anti-tumor potential of *Gymnema sylvestre* saponin rich fraction on *in vitro* breast cancer cell lines and *in vivo* tumor-bearing mouse models. *Antioxidants*, 12: 134. https://doi.org/10.3390/antiox12010134

GUMBAR, S., BHARDWAJ, S., MEHAN, S., KHAN, Z., NARULA, A.S., KALFIN, R., TABREZ, S., ZUGHAIBI, T. and WASI, S. (2023). Renal mitochondrial restroration by gymnemic acid in gentamicin-mediated experimental nephrotoxicity: evidences from serum, kidney and histopathological alterations. *Frontiers in Pharmacology*, 14: 1218506. https://doi.org/10.3389/fphar.2023.1218506

JAMADAGNI, P. S., PAWAR, S. D., JAMADAGNI, S. B., GAUTAM, M., GAIDHANI, S. N., PRASAD, G. P. and GURAV, A. M. (2021). Recent updates in research on *Gymnema sylvestre*. *Pharmacognosy Reviews*, 15(30): 128 – 133.

JANGAM, A., TIRUNAVALLI, S. K., ADIMOOLAM, B. M., KASIREDDY, B., PATNAIK, S. S., ERUKKAMBATTU, J., THOTA, J. R., ANDUGULAPATI, S. B. and ADDLAGATTA, A. (2023). Anti-inflammatory and antioxidant activities of *Gymnema sylvestre* extract rescue acute respiratory distress syndrome in rats via modulating the NF-κB/MAPK pathway. *Inflammopharmacology*, 31(2): 823 – 844.

KANETKAR, P., SINGHAL, R. and KAMAT, M. (2007). *Gymnema sylvestre:* a memoir. *Journal of Clinical Biochemistry and Nutrition*, 41(2): 77 – 81.

KAWANA, K., NAKAOKA, T., HORIGUCHI, Y., WATANABE, S., WATANABE, S. and KAWAUCHI, S. (1991). Toxicological study of potassium bromate. II. Hepatotoxic effects of the potassium bromate and benzo (a) pyrene simultaneous administration in mice. *Japanese Journal of Toxicological and Environmental Health,* 37(4): 266 – 275.

KEDDERIS, G. L. (1996). Biochemical basis of hepatocellular injury. *Toxicologic Pathology*, 24(1): 77 – 83.

KEERTHIKA, R. and RAGHU, S. (2021). Efficacy of Gymnema sylvestre as a potent antioxidant: an in vitro study. *Annals of Medical and Health Sciences Research,* 11(2): 6 – 9.

KHAN, F., SARKER, M. M. R., MING, L. C., MOHAMED, I. N., ZHAO, C., SHEIKH, B. Y., TSONG, H. F. and RASHID, M. A. (2019). Comprehensive review on phytochemicals, pharmacological and clinical potentials of *Gymnema sylvestre*. *Frontiers in Pharmacology*, 10: 1223. https://doi.org/10.33891fphar2019.01223

MOMOH, S., YUSUF, O., ADAMU, M., AGWU, C. and ATANU, F. O. (2011). Evaluation of the phytochemical composition and hypoglycemic activity of methanol leaf extracts of *Costus afer* in albino rats. *British Journal of Pharmaceutical Research,* 1(1): 1 – 8.

NKWATOH, T. N., FON, T. P. and NAVTI, L. K. (2023). Potassium bromate in bread, health risks to bread consumers and toxicity symptoms amongst bakers in Bamenda, North West Region of

Cameroon. *Heliyon*, 9(2): e13146. https://doi.org/10.1016/j.heliyon.2023.e13146

OLAJIDE, J. E., AKANJI, M. A. and DAIKWO, M. A. (2016). Modulation of enzyme activities following the coadministration of potassium bromate and chloroquine in selected tissues and serum of albino rats. *Animal Research International*, 13(1): 2359 – 2367.

OSHIKOYA, K. A. (2009). Response: accidental potassium bromate poisoning causing acute renal failure. *Online Journal of Health and Allied Sciences*, 8(3): 10 – 20.

OYEWO, O. O., ONYIJE, F. M. and AWOMIRAN, P. O. (2013). Hepatotoxic effect of potassium bromate on the liver of Wistar rats. *Journal of Morphological Sciences*, 30(2): 107 – 114.

PALIWAL, R., KATHORI, S. and UPADHYAY, B. (2009). Effect of Gurmar (*Gymnema sylvestre*) powder intervention on the blood glucose levels among diabetics. *Studies on Ethno-Medicine*, 3(2): 133 – 135.

PARSONS, J. L. and CHIPMAN, J. K. (2000). The role of glutathione in DNA damage by potassium bromate *in vitro*. *Mutagenesis*, 15(4): 311 – 316.

SATDIVE, R. K., ABHILASH, P. and FULZELE, D. P. (2003). Antimicrobial activity of *Gymnema sylvestre* leaf extract. *Fitoterapia*, 74(7-8): 699 – 701.

SHANMUGAVEL, V., SANTHI, K. K., KURUP, A. H., KALAKANDAN, S., ANANDHARAJ, A. and RAWSON, A. (2020). Potassium bromate: Effects on bread components, health, environment and method of analysis: A review. *Food Chemistry*, 311: 125964. https://doi.org/10.1016/j.foodchem.2019.125964

SHUKLA, A., MUHAMMED, I. K., SHESHALA, R., MHAISKER, I. U. and RAMPAL, K. (2020). Acute toxicity evaluation of homeopathic preparation of *Gymnema sylvestre* and analysis of its chemical constituents. *Journal of Applied Biology and Biotechnology*, 8(4): 33 – 37.

TURNER, S., DIAKO, C., KRUGER, R., WONG, M., WOOD, W., RUTHERFURD-MARKWICK, K. and ALI, A. (2020). Consuming *Gymnema sylvestre* reduces the desire for high-sugar sweet foods. *Nutrients*, 12(4): 1046. https://doi.org/10.3390/nu12041046

UCHIDA, H. A., SUGIYAMA, H., KANEHISA, S., HARADA, K., FUJIWARA, K., ONO, T., YAMAKIDO, M. and MAKINO, H. (2006). An elderly patient with severe acute renal failure due to sodium bromate intoxication. *Internal Medicine*, 45(3): 151 – 154.

UTTERIDGE, T. and BRAMLEY, G. (2016). *Kew Tropical Plant Families Identification Handbook*. Royal Botanic Gardens, Kew.

WHO (2023). *Food Additives – Key Facts*. World Health Organization, Geneva, Switzerland. https://www.who.int/news-room/fact-sheets/detail/food-additives Accessed January 7, 2024.

YAKUBU, M. T. and AKANJI, M. A. (2011). Effect of aqueous extract of *Massularia acuminata* stem on sexual behaviour of male Wistar rats. *Evidence-Based Complementary and Alternative Medicine*, 2011: 738103. https://doi.org/10.1155/2011/738103