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Preventive Effect of L- Methionine on Potassium Bromate- Induced Nephrotoxicity in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAK designed the study, performed the statistical analyses, wrote the protocol and wrote the first draft of the manuscript. Authors RY and MPI managed the analyses of the study. Authors AIY and AN managed the literature searches. All the authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the preventive potential of L-methionine against potassium bromate –induced nephrotoxicity in rats.
Study Design: Twenty male Wistar rats were used. The rats were divided into four groups containing five rats per group as follows; normal control, $KBrO_3$ control, L- methionine control and $KBrO_3$ group administered with 100mg/kg body weight of L- methionine for 48 hours.
Place and Duration of Study: Department of Biochemistry, Faculty of Basic Medical sciences, Bayero University Kano, Nigeria.
Methodology: Serum levels of urea, creatinine, uric acid and electrolytes were assayed using standard methods. In addition, the following markers of oxidative stress were determined in homogenates of renal cortex and medulla: catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH) and malondialdehyde (MDA). Histopathological investigation was also carried out.
Results: Administration of $KBrO_3$ significantly ($P<0.05$) increases the serum levels of urea,

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creatinine, uric acid and electrolytes however co-administration of L-methionine resulted in decreases in concentrations of these kidney function parameters. Furthermore, there were significant ($P<0.05$) decreases in the activities of CAT, SOD, GPx and GSH level and a significant ($P<0.05$) increase in MDA concentration in renal homogenates following administration of $KBrO_3$ however concurrent administration of $KBrO_3$ and L-methionine prevented all these changes in oxidative stress markers. Histopathological studies revealed severe necrosis and changes in glomerulus.

Conclusion: L-methionine could prevent nephrotoxicity and oxidative stress caused by $KBrO_3$ and other structurally related compounds.

Keywords: L- methionine; potassium bromated; nephrotoxicity; wistar rats.

1. INTRODUCTION

Nephrotoxicity is that part of toxicology concerned with poisonous effect of some substances on the kidneys. It is one of the most common problems of the kidney and occurs when humans are exposed to certain drugs, environmental pollutant and some chemical food additives [1]. Nephrotoxicity caused by potassium bromate has well been documented by several researchers [2,3]. Oral ingestion of bromate by humans is a common phenomenon since the substance is used as food additive in bread to improve flour and condition dough [2]. $KBrO_3$ is also used in cosmetics as a component of hair weaving solution and is a by-product of water disinfection by ozonation [4]. The substance is used legally in controlled quantity in the United States and illegally in many countries including Nigeria to improve bread and cake.

L- methionine, a sulfur-containing essential amino acid is posited to possess antioxidant activity due to the presence of sulfhydryl group in its chemical structure [5]. Researchers have reported that the substance possesses preventive effect against nephrotoxicity caused by a number of nephrotoxicants [6, 7, 8]. Acetaminophen- induced increases in serum alanine aminotransferase, malondialdehyde and decrease GSH level in hepatic tissue of mice was also reported to be prevented by L-methionine [6]. The amino acid is also reported to attenuate gentamycin- induced increases in serum creatinine, blood urea nitrogen and urinary γ- glutamyl transpeptidase as well as the depleted renal cortical GSH in rats [7]. L-methionine is being used for more than a decade in clinical practice to decrease hepatic injuries due to acetaminophen poisoning [8]. However, despite this wide application, the effect of this amino acid has not been investigated on $KBrO_3$– induced nephrotoxicity hence the need for the present study.

2. MATERIALS AND METHODS

2.1 Experimental Animal

Twenty young male Albino Wister rats, each weighing between 120-150g were used for this study. The animal study was carried out at the Animal House Unit of the Department of Biological Sciences, Bayero University Kano, Nigeria. Before commencing the experiment, the animals were allowed to acclimatize to the Animal House for one week and were maintained on standard pellet rat diet with free access to water.

2.2 Experimental Design

Following the elapse of the seven days acclimatization period, the rats were randomly divided into four groups as shown below. Each group contains five rats. Animal in the various groups were assigned to receive the required oral dose of either $KBrO_3$ (Labtech Chemicals Lagos, Nigeria), L- methionine (Labtech Chemicals Lagos, Nigeria), a combination of both, administered concurrently or de-ionized water as normal control. Solution of $KBrO_3$ (100mg/kg body weight) was administered as a single dose to rats in the test and $KBrO_3$ control groups while L-methionine (100mg/kg body weight) and equivalent volume of de-ionized water were administered to animals in the L-methionine and normal control groups respectively. All the animals were observed for 48 hours.

Group one, normal control: given de-ionized water

Group two, $KBrO_3$ control: given $KBrO_3$, 100mg/kg bw

Group three, L-methionine control: given 100mg/kg bw L- methionine

Group four, treatment: given 100mg/kg bw L-methionine + 100mg/ kg bw $KBrO_3$

2.3 Collection of Blood Sample

At the end of the 48 hours experimental period, all the animals were sacrificed by decapitation and blood samples were collected in lithium heparin tubes and centrifuged at 4000 rpm for 5 minutes to collect the serum which is stored at 4^oC

2.4 Preparation of Renal Homogenates

Immediately after the animal sacrifice, kidneys of each rat were removed for the preparation of the renal homogenates. This was prepared as follows; Each of the kidneys was bisected and kept in ice-cold 154mM NaCl and 5 mM Tris-HEPES buffer, pH 7.5. The cortex and medulla were carefully separated using a sharp scalpel and homogenized separately in a glass Teflon homogenizer in 2 mM Tris-HCl, 50mM mannitol buffer, pH 7.0, to get a 10% (w/v) homogenate. These homogenates were diluted to 5% with Tris-mannitol buffer followed by high speed homogenization (20,000 rpm) in an Ultra Turrex Kunkel homogenizer. The renal homogenates were divided into aliquots and frozen immediately pending analysis [9].

2.5 Determination of Biochemical Parameters

2.5.1 Urea, creatinine and uric acid

These were all determined using kits from Randox Laboratories Ltd, UK. Urea was determined by colorimetric method in serum by diacetyl monoxime method. Creatinine level was determined in deproteinized serum based on its reaction with saturated picric acid to give a yellow-red complex while uric acid level was determined by the measurement of quinoneimine dye complex.

2.5.2 Serum electrolytes

Na^+, K^+, Cl^- and HCO_3^- were all estimated in serum by spectrophotometric measurement using kits from Teco Diagnostics Anaheim, USA. Na^+ determination was based on its reaction with excess uranium and ferrocyanide to produce a chromophore that is measured sphectrophotometrically. K^+ determination was based on the measurement of the turbidity formed when K^+ react with ferric ion to form a complex that is measured spectrophometrically while Cl^- determination was based on the formation of mercuric thiocyanate which then react with ferric ion to form a complex that is measured using spectrophotometer and HCO_3^- determination is based on the reaction catalyzed by phosphoenol pyruvate carboxylase to form oxaloacetate which undergoes further reactions to form a complex that is measured spectrophotometrically.

2.5.3 Markers of oxidative stress

The markers of oxidative stress determined are catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (GPx, EC 1.11.1.9), reduced glutathione (GSH) and malondialdehyde (MDA). All the parameters were determined in homogenates prepared from renal cortex and medulla. CAT activity in renal tissues were determined by the quantitation of chromic acetate formed at pH 7.0 according to the method of Singha [10] while SOD activities were determined by the method of Misra and Fridovich [11] by inhibition of auto oxidation of epinephrine at pH 10.2. GPx activities were determined by the splitting of H_2O_2 with oxidation of GSH at pH 7.4 using the method of Rotruck et al. [12] while the levels of GSH were quantified in deproteinised samples by measurement of 5', 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) using the method of Beutler et al. [13]. Malondialdehyde was determined by the measurement of thiobarbituric acid reactive substances (TBARS) as described by Varshney and Kale [14].

2.5.4 Histological examination

Kidney from the representative of each group was embedded in paraffin fixed in 10% neutral buffered formalin solution and dehydrated in graded anhydrous absolute ethanol and xylol. Sections of 5µm of each representative sample were stained by hematoxylin-eosin and examined using Leitz, DIALUX research microscope at x100 magnification [15].

2.6 Statistical Analysis

Results are expressed as mean ± SDM and n =5 for all readings. One-way analysis of variance (ANOVA) was used to analyzed data and a difference of ($P<0.05$) was considered significant.

3. RESULTS

3.1 Serum Urea Creatinine and Uric Acid

Administration of $KBrO_3$ significantly increases ($P<0.05$) serum levels of urea, creatinine and uric acid as compared with normal control however co-administration of L-methionine significantly decreased these effect while administration of L-methionine alone did not induce significant change ($P>0.05$) in the serum levels of these kidney function parameters. Result is shown on Table 1.

3.2 Serum Electrolytes

Administration of $KBrO_3$ resulted in significant increases ($P<0.05$) in serum concentrations of Na^+, K^+, Cl^- and HCO_3^- as compared with normal control however co-administration of L-methionine decreases these changes in rats receiving the dual treatment. There was no significant change ($P>0.05$) in the serum concentrations of these electrolytes in animals that were administered L- methionine alone. This is shown on Table 2.

3.3 Antioxidant Activity in Homogenates of Renal Cortex and Medulla

$KBrO_3$ administration induces significant decreases ($P<0.05$) in the activities of CAT, SOD, GPx and GSH level and significantly increases ($P<0.05$) MDA concentration in homogenates of renal cortex and medulla with the cortex being more adversely affected than medulla however concurrent administration of $KBrO_3$ and L-methionine resulted in significant increases ($P<0.05$) in activities of these antioxidant enzymes studied and the level of GSH, and also increases MDA concentration in the renal homogenates. There was no significant ($P>0.05$) change in these markers of oxidative stress in the L-methionine control group. The result is presented on Table 3.

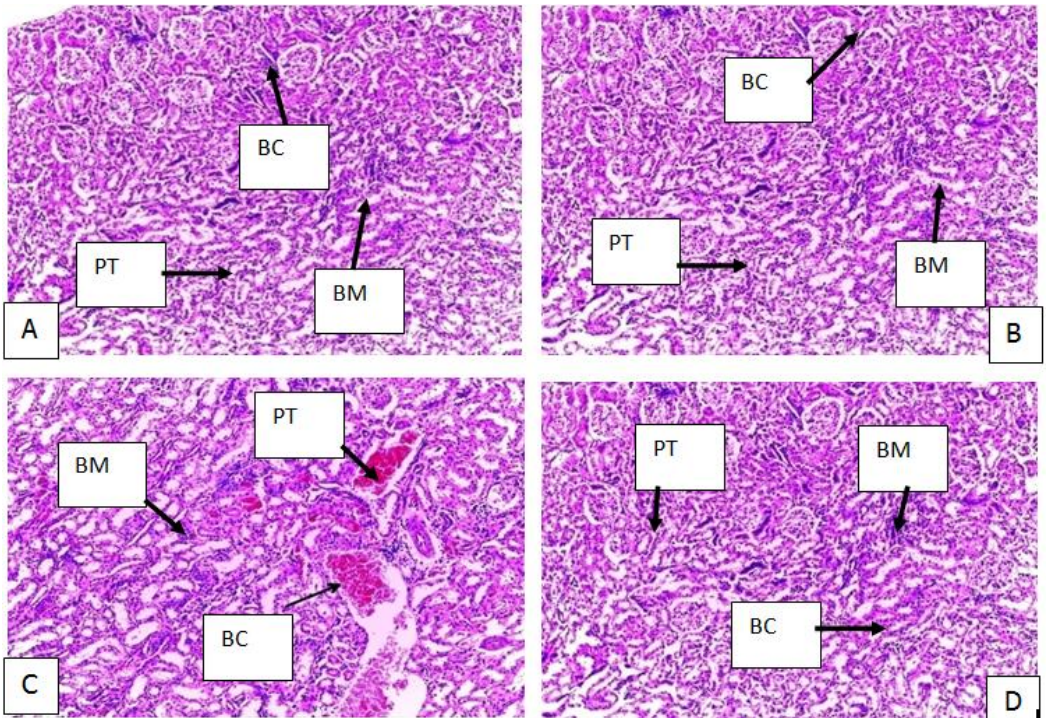

Fig. 1. Histopathological findings

(Mag x 100), BC = Bowman's capsule, PT = Proximal tubule, BM = Basement membrane

***A:** Micrograph of representative section of rat kidney administered de-ionized water only. It shows normal architecture with intact Bowman's capsule, tubular epithelium and basement membrane. **B:** Micrograph representative section of rat kidney administered $KBrO_3$ only. There were changes in glomerulus and tubules such as irregular dilatation of tubules, tubular cell necrosis. **C:** Micrograph representative section of rat kidney administered L- methionine only. It shows normal architecture with intact Bowman's capsule, tubular epithelium and basement membrane. **D:** Micrograph of representative section of rat kidney administered L- methionine + $KBrO_3$ showing tremendous preventive activity with intact Bowman's capsule, tubular epithelium and basement membrane*

Table 1. Effect of concurrent administration of potassium bromate and L- methionine on serum urea, creatinine and uric acid of rats

	Urea(mMol/l)	Creatinine(mg/dl)	Uric acid (mg/dl)
Normal control	8.44 ±0.56	3.80 ±0.57	5.49 ±0.21
$KBrO_3$control	14.82 ±0.53[a]	7.07 ±0.25[a]	6.63 ±0.30[a]
Met. Control	8.42 ±0.33	3.73 ±0.33	5.85 ±0.19
Met. + $KBrO_3$	8.74 ± 0.11[b]	3.22 ± 0,04[b]	5.55 ± 0.14

n= mean of five sample ± SDM, met = L- methionine
[a] is significant (P<0.05) from normal control, [b] is significant (P<0.05) from $KBrO_3$ control

Table 2. Effect of concurrent administration of potassium bromate and L- methionine on serum electrolytes

	Na^+(Mmol/l)	K^+(Mmol/l)	Cl^-(Mmol/l)	HCO_3^-(Mmol/l)
Normal control	139.86 ±2.01	8.97 ±0.30	103.83 ±5.02	5.45 ±0.56
$KBrO_3$control	144.76 ±2.09[a]	24.89 ±0.44[a]	143.60 ±5.11[a]	23.69 ±1.68[a]
Methionine control	137 .48 ±2.34	9.25 ±0.52	106.46 ± 5.77	5.25 ±0.54
Met. + $KBrO_3$	140.41 ± 2.01[b]	9.82 ±0.21[b]	107. 99 ± 1.78[b]	5.34 ± 0.07[b]

n= mean of five sample ± SDM, met = L- methionine
[a] is significant (P<0.05) from normal control, [b] is significant (P<0.05) from $KBrO_3$ control

Table 3. Effect of concurrent administration of potassium bromate and L- methionine on some parameters of oxidative stress in homogenates of renal cortex and medulla of rats

	CAT	SOD	GPx	GSH	MDA
Normal control					
Cortex	71.76±2.48	21.16±1.70	49.49±1.11	3.16±0.57	15.41±1.00
Medulla	42.67±1.83	12.84±0.41	18.27±0.92	1.36±0.53	8.18±0.63
Met. control					
Cortex	72.16±1.24	20.64±0.28	49.74±1.24	3.40±0.48	15.47±1.46
Medulla	43.56±1.21	12.74±0.61	19.63±0.94	1.42±0.32	8.74±1.07
$KBrO_3$ control					
Cortex	44.92±1.46[a]	13.58±0.56[a]	24.89±1.41[a]	0.54±0.19[a]	32.70±0.84[a]
Medulla	22.86±1.13[a]	7.77±0.69[a]	12.45±1.34[a]	0.21±0.02[a]	23.39±1.11[a]
Met. + $KBrO_3$					
Cortex	70.16 ± 1.08[b]	19.50 ± 1.18[b]	50.34 ±1. 66[b]	2.52 ±0.16[b]	14.73 ±0.35[b]
Medulla	43.24 ± 0.08[b]	10.28 ± 1.80	19.20 ±1.55[b]	1.27 ±0.32	7.95 ±0.50[b]

n= mean + SD for five different preparation;
CAT = Catalase; SOD= Superoxide dismutase; GPx = glutathione peroxidase, met = L- methionine
Activities of CAT and GPx are in units/mg protein, SOD activity is in units/mg protein/min, MDA concentration is in units/mg protein, GSH concentration is in µmol/min tissue
[a] is significant (P<0.05) from normal control, [b] is significant from $KBrO_3$ control

3.4 Histological Examination

Rats administered $KBrO_3$ showed severe changes in glomerulus such as irregular dilatation of tubules, distortions on proximal tubules and damaged membrane as compared to normal architecture observed on rat in normal control and L-methionine control groups. Rats in the treatment group, L- methionine + $KBrO_3$ shows considerable recovery with nearly normal kidney architecture as seen in plates A, B, C and D (Fig. 1).

4. DISCUSSION

The observed significant increases (P<0.05) in serum urea, creatinine and uric acid could be a consequence of decreased glomerular filtration following $KBrO_3$ toxic assault on the kidney. Previous workers have reported that the tasks of filtration and transport of metabolites by the kidney could be compromised in the event of any attack by toxic agent as a result of alteration in the kidney's physiology occasioned by such attacks [16]. The authors stated that these alterations could lead to changes in

intraglomerular hemodynamics, decreased in renal blood flow and glomerular filtration rate thus leading to accumulation of metabolites [16, 17]. Interestingly, the histological findings from the present study revealed several alterations such as severe cell necrosis, irregular dilatation of tubules and distortion on glomerular basement on kidney tissues of rat in $KBrO_3$ control group (Plate B).

The significant increases ($P<0.05$) in serum concentration of Na^+, K^+, Cl^- and HCO_3^- corroborates the increases in urea, creatinine and uric acid. The kidney is the organ that ensures the internal environment of a cell is kept constant by regulating the levels of various electrolytes. For instance, the physiological state wherein Na^+ and K^+ are predominantly found in extracellular and intracellular spaces, respectively is regulated by the kidney; therefore any changes in the levels of these electrolytes could imply renal dysfunction particularly at the glomerulus [18]. Furthermore, the absorption of various electrolytes including Cl^- and HCO_3^- readily takes place in the tubules [19]. Therefore changes in the levels of these electrolytes could indicate dysfunctional renal tubules.

The decreased activities of CAT, SOD and GPx could be due to high levels of reactive oxygen species generated by $KBrO_3$. Previous literature has reported that several toxicants including $KBrO_3$ can induce oxidative stress leading to a compromised antioxidant defense system [20]. The decreased in GSH level observed in this study is in agreement with previous findings [21]. The same workers also reported a direct proportional relationship between GSH concentration and renal function which they stated that, restoring GSH concentration is a strategy for therapy of impaired renal function [21]. Another reason for the decrease in GSH level is that GSH could be oxidized by GPx in the process of inactivating free radicals in the $KBrO_3$ administered group [20]. Finally, the trio of increased free radical production, decreased antioxidant enzymes activities and decreased GSH level could enhance peroxidation of polyunsaturated fatty acid leading to the significant ($P<0.05$) increase in MDA concentration and subsequent tissue injury [22]. These three have been implicated in the pathogenesis of renal injuries [20].

Although the exact mechanism by which $KBrO_3$ induces nephrotoxicity has not been elucidated, researchers have suggested several hypotheses among which oxidative stress has been drawing much attention [2, 3, 4]. It has been reported that reduction of $KBrO_3$ in the gut by intracellular reductants can generate reactive oxygen species and decrease the activities of antioxidant enzymes, consequently leading to lipid peroxidation [16, 23]. On the other hand, the preventive effect of L-methionine against $KBrO_3$ – induced renal toxicity could be traced to the compound's antioxidant activity. Literature has posited that L-methionine and cystein, the two sulphur- containing naturally occurring amino acids, could resist almost all forms of ROS thus making them antioxidative [24]. The same workers also reported that L- methionine and cystein are precursors of S- adenosylmethionine, hydrogen sulfide, taurine and glutathione and that these products are well known to alleviate oxidant stress and protect tissues from damage [24]. Finally, another likely mechanism in the antioxidant property of methionine is its activity as a metal chelator. Like most amino acid, methionine has metal chelating property and can chelate both essential and toxic radicals leading to its detoxification [25].

5. CONCLUSION

L- methionine possesses preventive effect against $KBrO_3$ –induced nephrotoxicity and therefore the clinical application of this amino acid to decrease hepatic injuries cause by acetaminophen and ameliorating some forms of renal injuries could be extended to the nephrotoxicity caused by $KBrO_3$ and other structurally similar compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was granted by the Research Ethics Committee, College of Health Sciences, Bayero University Kano, Nigeria with Reference No.: BUK/CHS/REC/98.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for

any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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