Original Research Article

DOSE-DEPENDENT CHEMOPREVENTIVE EFFECT OF METHANOL EXTRACT OF *CARICA PAPAYA* SEED ON POTASSIUM BROMATE- INDUCED NEPHROTOXICITY IN RATS

ABSTRACT

Aim: To investigate the effect of *Carica papaya* seed extract on $KBrO_3$ - induced nephrotoxicity in rats. Renal toxicity was induced by a single oral dose of 100 mg/kg body weight of $KBrO_3$.

Study design: Thirty (30) male albino rats were divided into six groups, five rats per group; normal control, $KBrO_3$ control, *papaya* control and $KBrO_3$ group administered with methanol seed extract of 200 mg/kg, 400 mg/kg and 600 mg/kg body weight for 48 hours.

Place and Duration of Study: Department of Biochemistry Laboratory, Faculty of Basic Medical Sciences, Bayero University Kano, Nigeria, from April 2018 to August 2018.

Methodology: Serum urea, creatinine, uric acid and electrolytes were determined using kits from randox laboratories. Furthermore, activities of renal brush border membrane marker enzymes namely γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), maltase (Mal) and leucine aminopeptidase (LAP) and some parameters of oxidative stress including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH) and malondialdehyde (MDA) were determined in homogenates prepared from renal cortex and medulla of the kidney of rats using colorimetric methods.

Results: Administration of $KBrO_3$ significantly ($P<0.05$) increases the serum levels of urea, creatinine, uric acid and all electrolytes studied in a dose-dependent fashion from 200mg/kg to 400mg/kg and 600mg/kg in that order. Furthermore, the activities of GGT, ALP, Mal and LAP decrease in renal homogenates with $KBrO_3$ administration. Also the activities of CAT, SOD, GPx and level of GSH decreases while the level of MDA significantly ($P<0.05$) increases however concurrent administration of *Carica papaya* seed extract prevented all the $KBrO_3$- induced changes in the biochemical parameters studied .

Conclusion: It was suggested that methanol seed extract of *Carica papaya* possess nephroprotective effect against $KBrO_3$ –induced renal toxicity and oxidative stress, and the most effective dose was 600 mg/kg body weight.

Keywords: Chemopreventive, *Carica papaya* seed, Potassium bromate, Nephrotoxicity

1. INTRODUCTION

Potassium bromate ($KBrO_3$) is a white crystalline substance that is used as food additive in bread, as improver and a dough conditioner in flour. The cosmetics industry also uses bromate as component of permanent hair weaving solutions. Potable water disinfection by ozonation, which has been preferred as a better method of sterilization vis-à-vis chlorination because it does not precipitate hazardous substances such as trihalomethanes also generates bromate [1]. Ingestion of $KBrO_3$ can cause toxicity of several organs with the kidney being the primary target. $KBrO_3$ causes mutation in renal tissues and ingestion of higher doses of the substance over a long period induces carcinomas in rats, hamsters and mice [2]. The International Agency for Research on Cancer, IARC has classified bromate as a probable human carcinogen and a complete carcinogen in animals [3]. Researchers have suggested upsurge in production of reactive oxygen species (ROS) and free radicals as fundamental in mediating $KBrO_3$-induced toxicity [4]. Free radicals can cause tissue damage when they attack macromolecules such as proteins, nucleic acids and membrane lipids and leads to imbalance in homeostasis thus causing tissue injury [5]. Lethal oral doses of bromate in humans range from 154 and 385 mg/kg body weight while serious poisoning occurs at doses of 46–92 mg/kg body weight [6]. Oral doses of 185–385 mg/kg body weight can cause irreversible

toxic effects like renal failure and deafness in humans while lower doses are associated with vomiting, diarrhea, nausea and abdominal pain [6].

Carica papaya is a fast growing tree-like herbaceous plant in the family *caricaceae* with four genera. The genus *Carica linn* is the most common of the *Carica papaya* and is the most widely cultivated and best known species [7]. Economically, *Carica papaya* is the most important species within the *caricaceae* being widely cultivated for consumption as fresh drinks, jams and candies or as dried and crystallized fruit. The green fruit leaves and flowers are also cooked as vegetable [8]. Several workers have reported the therapeutic activities of *Carica papaya* seed: in a study to determine the anthelmintic activity of *Carica papaya* seed, Sapaat and co-workers reported that over 90% efficacy percentage against *Hymenolepis diminuta* in rats was observed following administration of1.2g/kg body weight of *Carica papaya* seed [9]. Aqueous extract of *Carica papaya* seed at 100mg/ml concentration was reported to have significantly inhibited bacterial activity against *Salmonella typhi* and other bacteria [10]. He and co workers reported the antifungal activity of *Carica papaya* seed essential oils (Eos). The workers revealed that the EO showed inhibitory effect against some *Candida strains* including *C. albicans, C. glabrata, C. crusei, C. parapsilosis and C. tropical* with inhibition zone diameters in the range of 14.2- 33.2 mm and minimal inhibitory concentrations in the range of 4.0 – 16.0 µg/ml [11]. Seeds of *carica papaya* have been used for centuries in folk medicine for the treatment of poison-related renal disorders among some ethnic groups in Nigeria and therefore could be considered a potential candidate for chemoprevention of the kidney against $KBrO_3$-induced nephrotoxicity hence the need for the present study.

2. MATERIALS AND METHODS

2.1 Chemical and Assay Kits

Epinephrine, reduced glutathione, tris (hydroxymethyl) aminomethane (Tris), [2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid] HEPES, Tricholoacetic acid (TCA), hydrogen peroxide, H_2O_2, Thiobarbituric Acid (TBA). Potassium bromate was supplied by Labtech Chemicals Lagos, Nigeria. The assay kits for urea, creatinine, uric acid, sodium, potassium, chloride and bicarbonate were all obtained from Randox laboratories Ltd UK. All other chemicals used meet the requirements of the American Chemical Society Committee on Analytical reagents

2.2 Plant Sample and Extraction

65 matured unripe *Carica papaya* was bought from Na'ibawa fruit market Kano, Nigeria and identified at the plant Herbarium, Department of Plant Biology, Bayero University Kano, Nigeria with an accession number BUKHAN 0012. Each of the samples was cut to remove the seeds which was washed with tap water, shade-dried and ground into fine powder with an electric blender. Maceration as described by [12] was used. 500 g of the powdered dried *Carica papaya seed* was suspended in 1200 ml of methanol for 24 hours and shaken at regular intervals. The extract was then sieved first with cheese cloth and then with Whatman filter paper No 1. The filtrate was concentrated to dryness in a water bath preset at 50^{o}C and was kept in the refrigerator at 4^{o}C until required.

2.3 Experimental Animal

Thirty apparently healthy young male Albino Wister rats, each weighing between 120-150g were used for the study. The study was carried out at the animal house unit of the department of Biological Sciences, Bayero University Kano, Nigeria. All animal procedures were performed according to the guide for the care and use of laboratory animals of the National Institute of Health as well as the Animal Welfare Act. Prior to 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.

2.4 Experimental Design

By the end of the seven days acclimatization period, the animals were randomly divided into six groups as shown below. Each group contains five rats. Solution of potassium bromate was administered orally as a single dose of 100mg/kg body weight to rats in the tests and $KBrO_3$ control groups. Methanol extract of *Carica papaya* seed was reconstituted in distilled water and administered concurrently to animals in the test*s* groups in dosages of 200mg, 400mg and 600mg/kg body weight of rats while animals in the normal control group was administered equivalent volume of distilled water

Group one, normal control: given distilled water

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

Group three, *papaya* control: given 600mg/kg bw CPS

Group four, treatment: given 200mg/kg bw CPS + 100mg/ kg bw $KBrO_3$

Group five, treatment given 400mg/kg bw CPS + 100mg/ kg bw $KBrO_3$

Group six, treatment: given 600mg/kg bw CPS + 100mg/ kg bw $KBrO_3$

2.4.1 Collection of blood sample

All the animals were sacrificed by decapitation, 48 hours after the respective treatment 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^{o}C

2.4.2 Preparation of renal homogenates

The renal homogenates were prepared as follows; after the animal sacrifice, the kidneys were excised, 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 [4].

2.4.3 Determination of biochemical Parameters

Urea, creatinine and uric acid

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

Electrolytes

Na^+, K^+, Cl^- and HCO_3^- were all estimated in serum by spectrophotometric measurement using kits from Teco Diagnostics Anaheim, USA

Renal brush border membrane enzymes

GGT (EC 2.3.2.2) was determined in the homogenates of renal cortex and medulla by colorimetric method using kit from Spectrum Diagnostic, Germany. The reaction is based on the measurement of chromogen ρ-nitroanilide at wavelength of 418nm. ALP (EC 3.1.3.1) was determined by colorimetric method by measuring of an intense yellow colour complex, p-nitrophenol using kit from Dialab Production Neudorf, Austria while maltase (3.2.1.20) and LAP (3.4.11.1) were determined using kits from Elabscience Biotechnology Inc, USA and Bioway Nanjiang, China respectively

Parameters of oxidative stress

The parameters that show the induction of oxidative stress determined include 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 separately. CAT activity in renal tissues were determined by the quantitation of chromic acetate formed at pH 7.0 according to the method of Singha [13] while SOD activities were determined by the method of Misra and Fridovich [14] 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.* [15] 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.* [16]. Malondialdehyde was determined by the measurement of thiobarbituric acid reactive substances (TBARS) as described by Varshney and Kale [17].

2.5 Statistical Analysis

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

2.6 Histological Examination

The method of Krause [18] was employed in the examination of the kidney tissues. The photomicrographs were observed using Leitz, DIALUX research microscope at x100 magnification.

3. RESULTS

3.1 Kidney Function Parameters

Administration of $KBrO_3$ significantly ($P<0.05$) increases the serum levels of urea, creatinine and uric acid however concurrent administration of $KBrO_3$ and *Carica papaya* seed extract resulted in significant ($P<0.05$) decreases in these parameters towards normal control values in a dose-dependent fashion from 200mg/kg to 400mg/kg and 600mg/kg in that order. The parameter most affected by $KBrO_3$ was creatinine and the least affected was uric acid. Administration of *Carica papaya* seed extract alone did not give any significant change in all the kidney function parameters studied.

Table 1: Dose-dependent effect of concurrent administration of potassium bromate and methanol extract of *Carica papaya* seed on kidney function parameters of rats

	Normal control	$KBrO_3$ control	*Papaya* control	200mg/kg	400mg/kg	600mg/kg
Urea (mmol/l)	8.44±0.56	14.82±0.53*	8.52±0.33	8.31±0.31	8.47±0.09	8.24±0.51*
Creatinine(mmol/l)	3.80±0.57	7.07±0.25*	3.59±0.33	3.52±0.42	3.21±0.24	3.20±0.25*
Uric acid (mmol/l)	5.49±0.21	5.94±0.19*	5.56±0.18	5.81±0.11	5.69±0.73	5.62±0.21

n = mean ±SD of five different samples; * significantly different ($P<0.05$) from normal control

3.2 Serum Electrolytes

Potassium bromate administration resulted in significant ($P<0.05$) increases in serum levels of all the electrolytes studied with HCO_3^- being the most adversely affected followed by K^+ and Cl^-. Na^+ was the least affected electrolyte by $KBrO_3$ administration. However concurrent administration of $KBrO_3$ and methanol extract of *Carica papaya* seed led to significant decreases in the serum levels of all the electrolytes studied in a dose-dependent fashion with 600mg/kg being the most active dosage. Administration of *Carica papaya* seed extract alone did not significantly affect any of the electrolytes studied.

Table 2: Dose-dependent effect of concurrent administration of potassium bromate and methanol extract of *Carica papaya* seed on serum electrolytes of rats

	Normal control	$KBrO_3$ control	*Papaya* control	200mg/kg	400mg/kg	600mg/kg
Na^+ (mmol/l)	139.86±2.01	144.76±2.09*	138.48±2.35	142.02±2.34	141.77±1.59	140.75±1.67
K^+ (mmol/l)	8.97±0.28	24.89±0.43*	9.19±0.52	11.49±0.99	9.33±0.77*	9.05±0.28*
Cl^- (mg/dl)	103.83±3.50	143.60±3.11*	103.46±3.77	106.61±4.27	104.27±4.04	100.25±4.02*
HCO_3^- (mg/dl)	5.45±0.56	23.69±1.68*	5.15±0.52	5.67±0.89	5.32±0.73	5.27±0.46

n = mean ±SD of five different samples; * significantly different ($P<0.05$) from normal control

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3.3 Brush Border Membrane Marker Enzymes

Administration of $KBrO_3$ significantly ($P<0.05$) decreases the activities of all the BBM marker enzymes, namely γ-glutamyltransferase, alkaline phosphatase, maltase and leucine aminopeptidase. The effect was observed in both in cortex and medulla, with the cortex being more extensively affected than medulla for all the enzymes. The BBM enzyme most affected by $KBrO_3$ was ALP followed by maltase and LAP. GGT was least affected. However concurrent administration of $KBrO_3$ and methanol extract of *Carica papaya* seed resulted in significant ($P<0.05$) increases in activities of all the BBM marker enzymes toward normal control values in a dose-dependent fashion. There was no any change in the *papaya* control group

Table 3: Dose-dependent effect of concurrent administration of potassium bromate and methanol extract of *Carica papaya* seed on activities of marker enzymes in brush border membrane in homogenates of renal cortex and medulla of rats

	Normal control	$KBrO_3$ control	*Papaya* control	200mg/kg	400mg/kg	600mg/kg
GGT (U/L)						
Cortex	5.71±0.45	3.36±0.37*	5.52±0.52	4.50±0.52	5.54±0.42*	5.74±0.15*
Medulla	3.97±0.41	1.27±0.54*	3.78±0.15	3.73±0.11*	3.55±0.15*	3.62±0.48*
ALP (U/L)						
Cortex	6.48±0.77	2.32±0.62*	6.28±0.65	4.52±0.48	6.17±0.55	6.58±0.33*
Medulla	4.83±0.37	1.33±0.44*	4.69±0.80	3.04±0.11	4.03±0.12*	5.51±0.45*
Maltase (U/L)						
Cortex	25.87±0.87	11.91±0.95*	26.07±0.57	25.05±0.04	26.52±0.11	26.95±0.20*
Medulla	18.54±0.53	8.14±0.41*	18.64±0.92	16.08±0.10	18.16±0.69*	18.53±0.54*
LAP (U/L)						
Cortex	5.49±0.50	3.42±0.48*	5.30±0.34	4.36±0.35	4.86±0.18	5.11±0.34*
Medulla	3.92±0.30	2.46±0.50*	3.86±0.44	4.30±0.21	4.31±0.20	4.41±0.40

n = mean±SD of five different samples, ALP= Alkaline phosphatase, GGT= γ-glutamyltransferase, LAP = leucine aminopeptidase; *significantly different (P<0.05) from normal control

3.4 Parameters of Oxidative Stress

Administration of $KBrO_3$ resulted in significant (P<0.05) increase in MDA concentration in the homogenates of both renal cortex and medulla of rats as compared to control values however concurrent administration of $KBrO_3$ and methanol extract of *Carica papaya* seed resulted in significant (P<0.05) decrease in the level of MDA toward normal control values in both the cortex and medulla in a dose-dependent fashion.

The result of the antioxidant enzymes studied (CAT, SOD, GPx) and GSH also showed significant decreases in all the enzyme activities and the level of GSH in both cortex and medulla with the cortex being extensively affected than the medulla. However concurrent administration of $KBrO_3$ and methanol extract of *Carica papaya* seed resulted in significant (P<0.05) increases in activities of all the antioxidant enzymes studied and GSH in a dose dependent manner. Administration of *Carica papaya* seed extract alone did not significantly affect any of the parameters of oxidative stress studied

Table 4: Dose-dependent effect of concurrent administration of potassium bromate and methanol extract of *Carica papaya* seed on parameters of oxidative stress in homogenates of renal cortex and medulla of rats

	Normal control	$KBrO_3$ control	*Papaya* control	200mg/kg	400mg/kg	600mg/kg
CAT (units/mg prtn)						
Cortex	71.76±2.48	44.92±1.24*	72.76±1.46	56.56±1.51	63.06±1.15*	75.35±1.43*
Medulla	42.67±1.83	22.86±1.21*	43.56±1.14	36.01±0.73*	42.50±1.66*	43.38±0.30*
SOD (units/mg prtn/min)						
Cortex	21.16±1.70	13.58±0.56*	20.64±1.28	19.37±0.51	20.35±1.38	20.55±1.43*
Medulla	12.84±0.42	7.77±0.69*	12.74±0.61	11.81±0.43	11.42±1.69	11.44±1.57*
GPx(units/mg prtn)						
Cortex	49.49±1.11	24.89±0.41*	49.74±1.24	50.43±2.01	42.52±0.81	50.86±0.71*
Medulla	18.27±0.92	12.45±1.34*	19.63±0.94	19.14±1.27	18.32±1.27*	19.49±0.25*
GSH(µmol/min T)						
Cortex	3.15±0.57	0.54±0.09*	3.45±0.52	1.49±0.34	1.42±0.08	1.48±0.05*
Medulla	2.30±0.32	0.42±0.03*	2.55±0.63	0.56±0.23	1.37±0.11	1.52±0.21*
MDA(units/mg prtn)						
Cortex	15.41±1.01	32.50±0.71*	15.47±1.46	16.28±0.59	15.76±0.12*	15.29±0.63*
Medulla	8.18±0.63	23.39±1.11*	8.47±1.07	8.32±0.07	8.07±0.22	8.14±0.49*

n = mean±SD of five different samples, CAT = Catalase, SOD= Superoxide dismutase, GPx = glutathione peroxidise; *significantly different (P<0.05) from normal control

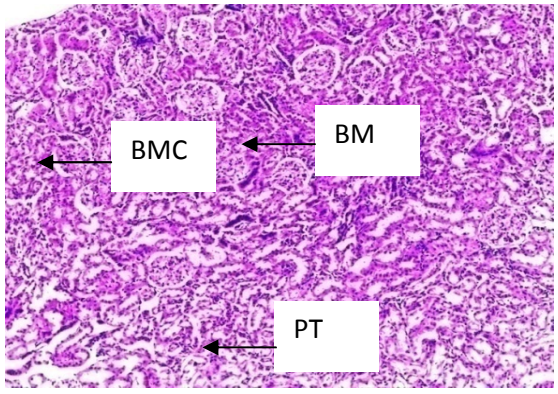

Plate A: micrograph of rat kidney administered with distilled water (Mag. x100)
Showing normal architecture with intact Bowman's capsule, tubular epithelium and basement membrane

BM = basement membrane, BMC = Bowmans capsule, PT = Proximal convoluted tubule

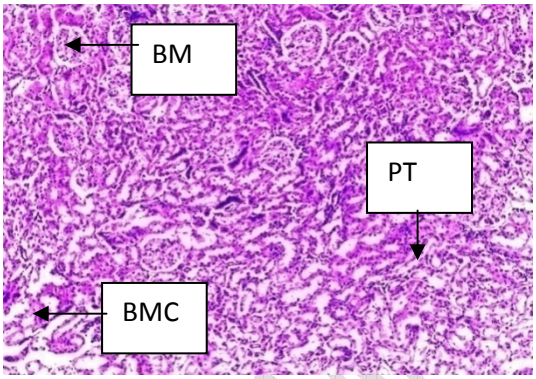

Plate B: micrograph of rat kidney administered with *Papaya* (Mag. x100)
Showing normal architecture similar to normal control with intact Bowman's capsule, tubular epithelium and basement membrane

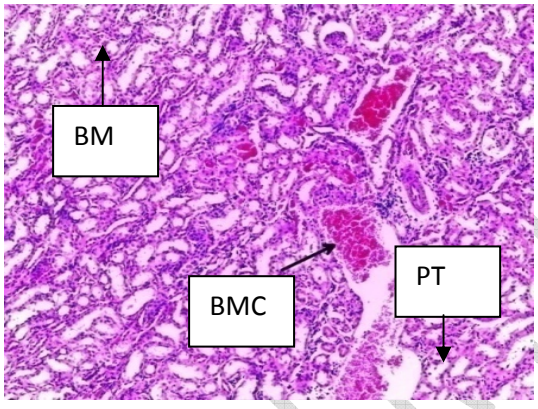

Plate C: micrograph of rat kidney administered with $KBrO_3$ (Mag. x100)
Showing changes in glomerulus such as irregular dilatation of tubules, and distortions on the distal and proximal tubules and damaged basement membrane

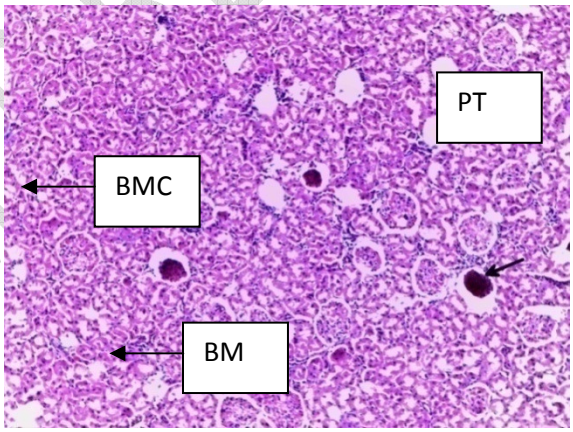

Plate D: micrograph of rat kidney administered with $KBrO_3$ + 200mg (Mag. x100)
showing nearly normal architecture with intact Bowman's capsule, and tubular epithelium but basement membrane has not returned to normal

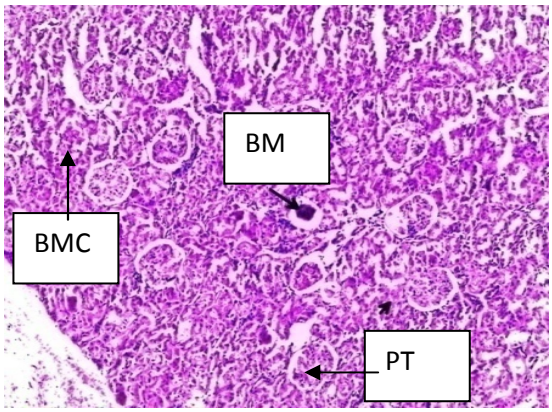

Plate E: micrograph of rat kidney administered with $KBrO_3$ + 400mg (Mag. x100)
showing nearly normal architecture with intact Bowman's capsule, and tubular epithelium but basement membrane has not returned to normal

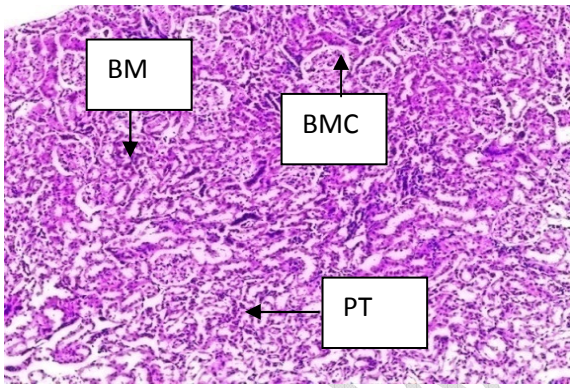

Plate F: micrograph of rat kidney administered with $KBrO_3$ + 600mg (Mag. x100)
showing nearly normal architecture with intact Bowman's capsule, and tubular epithelium but basement membrane has not returned to normal

4. DISCUSSION

The kidney is exposed regularly to high level of reactive oxygen species (ROS) and therefore requires a functional antioxidant defense system to protect its structure and function, and maintain its metabolic properties [19]. This is important because redox interruptions are reported to have devastating effect on body system because it can generate ROS which can attack and modify macromolecules such as proteins, lipids and DNA [20]. Researchers have linked several environmental pollutants, therapeutic drugs and certain food additives such as $KBrO_3$ with increase in generation of ROS and have outlined some key events that are involved in renal damage and antioxidant defense mechanism [21-24]. Thus, it is important to search for antioxidants that have ability to prevent the damaging effect of various toxicants' induced oxidative stress and the accompanying metabolic disorders. *Carica papaya* seed known for its uses in folk medicine for the treatment of poison-related renal disorders by several ethnic groups across Nigeria was used in the present study and its nephroprotective potential was investigated. A single oral dose of $KBrO_3$ was used to induce nephrotoxicty in rats and seeds from matured, unripe *Carica papaya* fruit were shade-dried, ground into fine powder, extracted with methanol and tested for potency against the $KBrO_3$-induced nephrotoxicity in rats. The organic extract of the dried seed was found to prevent the devastating effect of $KBrO_3$ on the kidney of rats studied

The observed significant ($P<0.05$) increases in serum urea, creatinine and uric acid levels in $KBrO_3$ administered rats could be due to the kidney's inability to carry out its functions of filtration and transport of metabolites as a result of the morphological changes on the kidney tissues following administration of $KBrO_3$. Histological findings showed alterations in the glomerulus such as irregular dilatation of the tubules and necrosis leading to the distortion of the glomerular basement in $KBrO_3$ administered rats (plate C). These could alter the normal physiology of the kidney and lead to changes such as alteration in intraglomerular hemodynamics [25, 26], decrease in renal blood flow and glomerular filtration rate (GFR) and therefore leading to reduced uric acid and creatinine clearance [26] hence the accumulation of the kidney function parameters observed however, concurrent administration of $KBrO_3$ and methanol extract of *Carica papaya* seed prevented these changes and restored the values towards normal control.

The increased levels of serum Na^+, K^+, HCO_3^- and Cl^- corroborated the observed increases in the serum levels of urea, creatinine and uric acid. The kidney is responsible for the regulation of various electrolytes and maintenance of homeostasis [27], for example Na^+ and K^+ are major components of extracellular and intracellular fluids respectively and these physiological state are regulated by the kidney, therefore the elevated levels of these electrolytes could indicate renal dysfunction particularly at glomerular and tubular levels. Also, HCO_3^- and Cl^- are reabsorbed in the proximal tubule and therefore the elevated level of these electrolytes could imply renal damage at the tubular level. However the serum levels of all the electrolytes

studied were restored towards normal with concurrent administration of $KBrO_3$ and methanol extract of *Carica papaya* seed

The significant decreases ($P<0.05$) in the activities of all the brush border membrane (BBM) biomarker enzymes (GGT, ALP, Maltase, LAP) in the homogenates of both cortex and medulla of the kidney of rats following the administration of $KBrO_3$ could be due to $KBrO_3$ damaging effect on the structure and function of the BBM. Two likely reasons could be responsible for these suggestions:

(1) There could be direct loss of BBM or enzyme molecules into the lumen of the tubule following the toxic attacks by $KBrO_3$, as it was reported for some nephrotoxicants by other researchers [28, 29] and

(2) There could be enzymes inactivation due to conformational changes in the molecular structure of the BBM enzymes. Earlier researchers have reported oxidative modification of amino acid side chains of enzyme protein by reactive oxygen species that was generated by $KBrO_3$ toxicity and consequent inactivation of the BBM enzymes [30, 31, 32]

BBM forms the major lining of the epithelial cells of the proximal tubule of the kidney and it was reported to be the first barrier for various solutes during absorption in the kidney. Other workers have reported the BBM as major target of renal injury due to ischemia and nephrotoxic agents [4, 28, 33]. This of course could explain either of the two suggestions given above on why there was a significant ($P<0.05$) decline in the activities of all the BBM marker enzymes observed in this research. However, concurrent administration of $KBrO_3$ and methanol extract of *Carica papaya* seed was able to attenuate the decreases in activities of all the BBM marker enzymes studied in a dose dependent fashion and restored the values towards normal control.

The significant decreases ($P<0.05$) in the activities of antioxidant enzymes studied (CAT, SOD, GPx) in homogenates of both cortex and medulla of the kidney of rats following administration of $KBrO_3$ could be due to the $KBrO_3$-induced reactive oxygen species production that could have caused oxidative stress. ROS and oxidative stress have been reported to be important mediators of $KBrO_3$ nephropathy [34]. Similarly, higher levels of ROS increases oxidative modification of cellular components in protein, lipids and nucleic acids, and causes damage to organelles such as the plasma membranes, mitochondria in the kidney and other tissues [35, 36].

The significant decrease ($P<0.05$) in GSH level in renal cortex and medulla of rats following administration of $KBrO_3$ could be due to oxidative stress occasioned by ROS. GSH, a valuable physiological tri-peptide is reported to be a vital extracellular and intracellular protective antioxidant against oxidative stress. It reduces H_2O_2 and hydroperoxides by its redox and detoxification reactions and protects protein thiols groups from oxidation [19]. Literature reported that decrease in the level of GSH will increase OS which will subsequently lead to cell damage [37].

Free radical and ROS is key to the initiating of peroxidation of polyunsaturated fatty acid (PUFA). This reaction cascade which could lead to formation of lipid peroxide can be prevented by enzymatic and non enzymatic controlled antioxidant [19]. In the present study, the significant increase ($P<0.05$) in MDA concentration of kidney tissues in $KBrO_3$ administered rats could be the consequence of an increment in the formation of oxygen free radicals generated by $KBrO_3$ and in fact indicates a compromised antioxidant defense system. MDA is one of the potent metabolites of peroxidized PUFA [19, 38] and is considered a biomarker of oxidative stress [39, 40]. Presence of MDA can induce cellular and functional damage [41].

However, concurrent administration of $KBrO_3$ and methanol extract of *Carica papaya* seed prevented all the changes in the parameters of oxidative stress studied in both cortex and medulla in a dose dependent manner

5. CONCLUSION

Methanol extract of *Carica papaya* seed possess ameliorative effect against potassium bromate- induced nephrotoxicity and the most effective dose was 600mg/kg body weight. The effect of the extract was experienced in both the renal cortex and medulla. Since $KBrO_3$ is known to induces oxidative stress in the cell which in part is considered responsible for its toxicity, the preventive role of *Carica papaya* seed extract could be due to its ability to act as an antioxidant and a scavenger of reactive oxygen species. Thus *Carica papaya* seed extracts can be considered a potential preventive agent against renal damage caused by $KBrO_3$ and other structurally related compounds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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