

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

ABSTRACT

The dose-dependent preventive effect of methanol extract of *Carica papaya* seed was investigated on potassium bromate-induced nephrotoxicity in rats. The animals were concurrently administered a single oral dose of 100mg/kg body weight potassium bromate and varying dosages of 200mg/kg, 400mg/kg and 600mg/kg body weight methanol extract of *Carica papaya* seed obtained by maceration, and the effect examined 48hours after administration. The result showed significant ($P<0.05$) decreases in 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 when compared to control. Furthermore, the activities of biomarker enzymes of the renal brush border membrane namely γ -glutamyltransferase (GGT), alkaline phosphatase (ALP), maltase (Mal) and leucine aminopeptidase (LAP) in homogenates prepared from renal cortex and medulla were significantly ($P<0.05$) decreased with $KBrO_3$ administration but this was prevented by methanol extract of *Carica papaya* seed in a dose dependent fashion. Furthermore, there were significant changes in parameters of oxidative stress. While the activities of the antioxidant enzymes studied namely; catalase, superoxide dismutase and glutathione peroxidase as well as reduced glutathione were significantly decreased, the level of malondialdehyde significantly ($P<0.05$) increases in the renal homogenates following $KBrO_3$ administration but these were prevented by concurrent administration of $KBrO_3$ and methanol extract of *Carica papaya* seed in a dose dependent manner. It was suggested that *Carica papaya* seed extract can ameliorate potassium bromate- induced renal toxicity and oxidative stress by preventing the changes in all the kidney function parameters studied and improving the antioxidant defense system respectively.

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

1. INTRODUCTION

Potassium bromate 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 waving 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 bromated [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

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

47 *Carica papaya* is a fast growing tree-like herbaceous plant in the family *caricaceae* with four genera. The
48 genus *Carica linn* is the most common of the *Carica papaya* and is the most widely cultivated and best
49 known species [7]. Economically, *Carica papaya* is the most important species within the *caricaceae*
50 being widely cultivated for consumption as fresh drinks, jams and candies or as dried and crystallized
51 fruit. The green fruit leaves and flowers are also cooked as vegetable [8]. Several workers have reported
52 the therapeutic activities of *Carica papaya* seed to include anti-helminthic action [9], antibacterial [10],
53 antifungal [11] and female anti fertility [10] among others. Seeds of *carica papaya* have also been used
54 for centuries in folk medicine for the treatment of poison-related renal disorders among some ethnic
55 groups in Nigeria and therefore could be considered a potential candidate for chemoprevention of the
56 kidney against $KBrO_3$ -induced nephrotoxicity hence the need for the present study.

57 2. MATERIALS AND METHODS

58 2.1 Chemical and Assay Kits

59 Epinephrine, reduced glutathione, tris (hydroxymethyl) aminomethane (Tris), [2-[4-(2-hydroxyethyl)-1-
60 piperazinyl]ethanesulfonic acid], HEPES, Tricholoacetic acid (TCA), hydrogen peroxide, H_2O_2 ,
61 Thiobarbituric Acid (TBA). Potassium bromate was supplied by Labtech Chemicals Lagos, Nigeria. The
62 assay kits for urea, creatinine, uric acid, sodium, potassium, chloride and bicarbonate were all obtained
63 from Randox laboratories Ltd UK. All other chemicals used were of analytical grade

64 2.2 Plant Sample and Extraction

65 65 matured unripe *Carica papaya* was bought from Na'ibawa fruit market Kano, Nigeria and identified at
66 the plant Herbarium, Department of Plant Biology, Bayero University Kano, Nigeria with an accession
67 number BUKHAN 0012. Each of the samples was cut to remove the seeds which was washed with tap
68 water, shade-dried and ground into fine powder with an electric blender. Maceration as described by [12]
69 was used. 500 g of the powdered dried *Carica papaya seed* was suspended in 1200 ml of methanol for
70 24 hours and shaken at regular intervals. The extract was then sieved first with cheese cloth and then
71 with Watmann filter paper No 1. The filtrate was concentrated to dryness in a water bath preset at $50^\circ C$
72 and was kept in the refrigerator at $4^\circ C$ until required.

73 2.3 Experimental Animal

74 Thirty (30) apparently healthy young male Albino Wister rats, each weighing between 120-150g were
75 used for the study. The study was carried out at the animal house unit of the department of Biological
76 Sciences, Bayero University Kano, Nigeria. All animal procedures were performed according to the guide
77 for the care and use of laboratory animals of the National Institute of Health as well as the Animal Welfare
78 Act. Prior to the experiment, the animals were allowed to acclimatize to the animal house for one week
79 and were maintained on standard pellet rat diet with free access to water.

80 2.4 Experimental Design

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

87 Group one, normal control: given distilled water

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

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

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

97 Group five, treatment given 400mg/kg bw CPS + 100mg/ kg bw KBrO₃
98 Group six, treatment: given 600mg/kg bw CPS + 100mg/ kg bw KBrO₃
99

100 **2.4.1 Collection of blood sample**

101 All the animals were sacrificed by decapitation, 48 hours after the respective treatment and blood
102 samples were collected in lithium heparin tubes and centrifuged at 4000 rpm for 5 minutes to collect the
103 serum which is stored at 4C

104 **2.4.2 Preparation of renal homogenates**

105 The renal homogenates were prepared using the method of [4]. After the animal sacrifice, the kidneys
106 were excised, bisected and kept in ice-cold 154mM NaCl and 5 mM Tris-HEPES buffer, pH 7.5. The
107 cortex and medulla were carefully separated using a sharp scalpel and homogenized separately in a
108 glass Teflon homogenizer in 2 mM Tris-HCl, 50mM mannitol buffer, pH 7.0, to get a 10% (w/v)
109 homogenate. These homogenates were diluted to 5% with Tris-mannitol buffer followed by high speed
110 homogenization (20,000 rpm) in an Ultra Turrex Kunkel homogenizer. The renal homogenates were
111 divided into aliquots and frozen immediately pending analysis.

112 **2.4.3 Determination of biochemical Parameters**

113 **Urea, creatinine and uric acid**

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

119 **Electrolytes**

120 Na⁺, K⁺, Cl⁻ and HCO₃⁻ were all estimated in serum by spectrophotometric measurement using kits from
121 Teco Diagnostics Anaheim, USA

122 **Renal brush border membrane enzymes**

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

129 **Parameters of oxidative stress**

130 The parameters that show the induction of oxidative stress determined include catalase (CAT) (EC
131 1.11.1.6), superoxide dismutase (SOD) (EC 1.15.1.1), glutathione peroxidase (GPx) (EC 1.11.1.9),
132 reduced glutathione (GSH) and malondialdehyde (MDA). All the parameters were determined in
133 homogenates prepared from renal cortex and medulla separately. CAT activity in renal tissues were
134 determined by the quantitation of chromic acetate formed at pH 7.0 according to the method of Singha
135 [13] while SOD activities were determined by the method of Misra and Fridovich [14] by inhibition of auto
136 oxidation of epinephrine at pH 10.2. GPx activities were determined by the splitting of H₂O₂ with oxidation
137 of GSH at pH 7.4 using the method of Rotruck *et. al.* [15] while the levels of GSH were quantified in
138 deproteinised samples by measurement of 5', 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) using the method

150 of Beutler *et. al.* [16]. Malondialdehyde was determined by the measurement of thiobarbituric acid
151 reactive substances (TBARS) as described by Varshney and Kale [17].

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153 2.5 Statistical Analysis

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

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158 2.6 Histological Examination

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

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163 3. RESULTS AND DISCUSSION

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165 3.1 Kidney Function Parameters

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167 Administration of KBrO₃ significantly (P<0.05) increases the serum levels of urea, creatinine and uric acid
168 however concurrent administration of KBrO₃ and *Carica papaya* seed extract resulted in significant
169 (P<0.05) decreases in these parameters towards normal control values in a dose-dependent fashion from
170 200mg/kg to 400mg/kg and 600mg/kg in that order. The parameter most affected by KBrO₃ was creatinine
171 and the least affected was uric acid. Administration of *Carica papaya* seed extract alone did not give any
172 significant change in all the kidney function parameters studied.

173

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

	Normal control	KBrO ₃ control	<i>Papaya</i> control	200mg/kg	400mg/kg	600mg/kg
Urea (mmol/l)	8.44 \pm 0.56	14.82 \pm 0.53*	8.52 \pm 0.33	8.31 \pm 0.31	8.47 \pm 0.09	8.24 \pm 0.51*
Creatinine(mmol/l)	3.80 \pm 0.57	7.07 \pm 0.25*	3.59 \pm 0.33	3.52 \pm 0.42	3.21 \pm 0.24	3.20 \pm 0.25*
Uric acid (mmol/l)	5.49 \pm 0.21	5.94 \pm 0.19*	5.56 \pm 0.18	5.81 \pm 0.11	5.69 \pm 0.73	5.62 \pm 0.21

176 n = mean \pm SD of five different samples; * significantly different (P<0.05) from normal control

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180 3.2 Serum Electrolytes

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182 Potassium bromate administration resulted in significant (P<0.05) increases in serum levels of all the
183 electrolytes studied with HCO₃⁻ being the most adversely affected followed by K⁺ and Cl⁻. Na⁺ was the
184 least affected electrolyte by KBrO₃ administration. However concurrent administration of KBrO₃ and
185 methanol extract of *Carica papaya* seed led to significant decreases in the serum levels of all the
186 electrolytes studied in a dose-dependent fashion with 600mg/kg being the most active dosage.
187 Administration of *Carica papaya* seed extract alone did not significantly affect any of the electrolytes
188 studied.

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

	Normal control	KBrO ₃ control	<i>Papaya</i> 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

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

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196 3.3 BBM Marker Enzymes

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198 Administration of KBrO₃ significantly (P<0.05) decreases the activities of all the BBM marker enzymes,
 199 namely γ-glutamyltransferase, alkaline phosphatase, maltase and leucine aminopeptidase. The effect
 200 was observed in both in cortex and medulla, with the cortex being more extensively affected than medulla
 201 for all the enzymes. The BBM enzyme most affected by KBrO₃ was ALP followed by maltase and LAP.
 202 GGT was least affected. However concurrent administration of KBrO₃ and methanol extract of *Carica*
 203 *papaya* seed resulted in significant (P<0.05) increases in activities of all the BBM marker enzymes toward
 204 normal control values in a dose-dependent fashion. There was no any change in the *papaya* control
 205 group

206

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

	Normal control	KBrO ₃ control	<i>Papaya</i> 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

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

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213 3.4 Parameters of Oxidative Stress

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215 Administration of KBrO₃ resulted in significant (P<0.05) increase in MDA concentration in the
 216 homogenates of both renal cortex and medulla of rats as compared to control values however
 217 concurrent administration of KBrO₃ and methanol extract of *Carica papaya* seed resulted in significant
 218 (P<0.05) decrease in the level of MDA toward normal control values in both the cortex and medulla in
 219 a dose-dependent fashion.

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221 The result of the antioxidant enzymes studied (CAT, SOD, GPx) and GSH also showed significant
 222 decreases in all the enzyme activities and the level of GSH in both cortex and medulla with the cortex
 223 being extensively affected than the medulla. However concurrent administration of KBrO₃ and
 224 methanol extract of *Carica papaya* seed resulted in significant (P<0.05) increases in activities of all
 225 the AO enzymes studied and GSH in a dose dependent manner. Administration of *Carica papaya*
 226 seed extract alone did not significantly affect any of the parameters of OS studied

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

	Normal control	KBrO ₃ control	<i>Papaya</i> 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*

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

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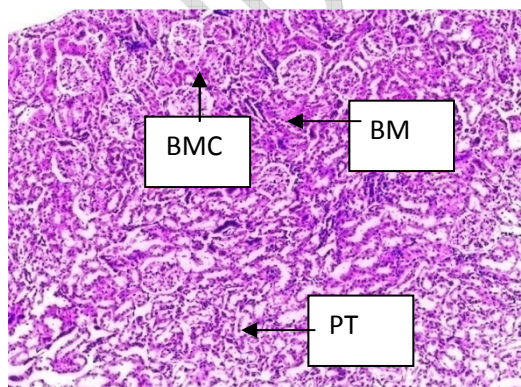


Plate 1: micrograph of rat kidney administered with distilled water (Mag. x100)
 Showing normal architecture with intact Bowman's capsule, tubular epithelium and basement membrane

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

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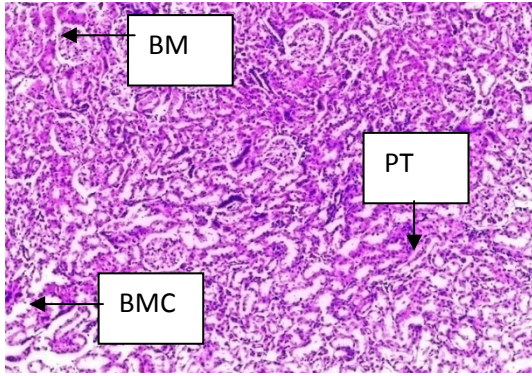


Plate 2: micrograph of rat kidney administered with *Papaya* (Mag. x100) Showing normal architecture similar to normal control with intact Bowman's capsule, tubular epithelium and basement membrane

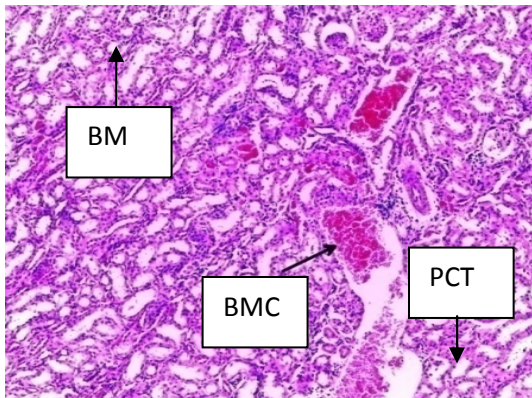


Plate 3: micrograph of rat kidney administered with $KBrO_3$ (Mag. x100) Showing changes in glomerulus such as irregular dilatation of tubules, and distortions on the distal and proximal tubules and damaged basement membrane

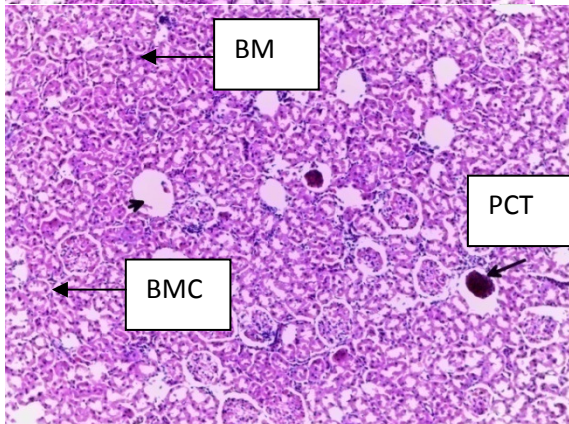


Plate 4: micrograph of rat kidney administered with $KBrO_3 + 200mg$ (Mag. x100) showing nearly normal architecture with intact Bowman's capsule, and tubular epithelium but basement membrane has not returned to normal

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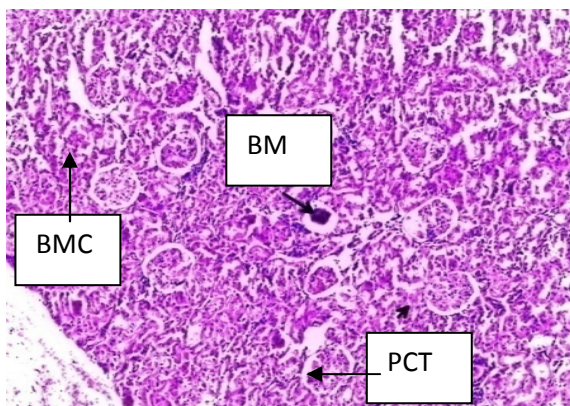


Plate 5: micrograph of rat kidney administered with $\text{KBrO}_3 + 400\text{mg}$ (Mag. x100) showing nearly normal architecture with intact Bowman's capsule, and tubular epithelium but basement membrane has not returned to normal

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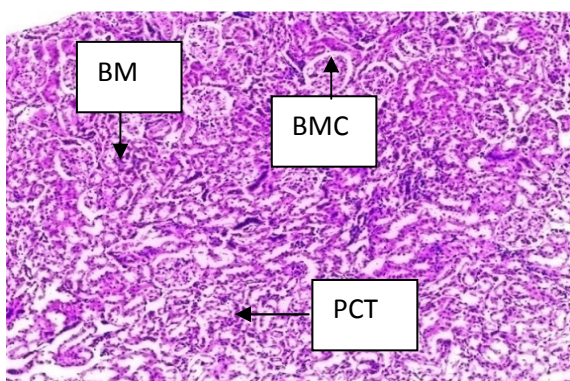


Plate 6: micrograph of rat kidney administered with $\text{KBrO}_3 + 600\text{mg}$ (Mag. x100) showing nearly normal architecture with intact Bowman's capsule, and tubular epithelium but basement membrane has not returned to normal

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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 generation of ROS and have outlined some key events that are involved in renal damage and antioxidant defense mechanism [21, 22, 23, 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 nephrotoxicity 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

302 KBrO₃. Histological findings showed alterations in the glomerulus such as irregular dilatation of the
303 tubules and necrosis leading to the distortion of the glomerular basement in KBrO₃ administered rats
304 (plate 3). These could alter the normal physiology of the kidney and lead to changes such as alteration in
305 intraglomerular hemodynamics [25, 26], decrease in renal blood flow and glomerular filtration rate (GFR)
306 and therefore leading to reduced uric acid and creatinine clearance [26] hence the accumulation of the
307 kidney function parameters observed however, concurrent administration of KBrO₃ and methanol extract
308 of *Carica papaya* seed prevented these changes and restored the values towards normal control.
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310 The increased levels of serum Na⁺, K⁺, HCO₃⁻ and Cl⁻ corroborated the observed increases in the serum
311 levels of urea, creatinine and uric acid. The kidney is responsible for the regulation of various electrolytes
312 and maintenance of homeostasis [27], for example Na⁺ and K⁺ are major components of extracellular and
313 intracellular fluids respectively and these physiological state are regulated by the kidney, therefore the
314 elevated levels of these electrolytes could indicate renal dysfunction particularly at glomerular and tubular
315 levels. Also, HCO₃⁻ and Cl⁻ are reabsorbed in the proximal tubule and therefore the elevated level of these
316 electrolytes could imply renal damage at the tubular level. However the serum levels of all the electrolytes
317 studied were restored towards normal with concurrent administration of KBrO₃ and methanol extract of
318 *Carica papaya* seed
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320 The significant decreases (P<0.05) in the activities of all the brush border membrane (BBM) biomarker
321 enzymes (GGT, ALP, Maltase, LAP) in the homogenates of both cortex and medulla of the kidney of rats
322 following the administration of KBrO₃ could be due to KBrO₃ damaging effect on the structure and function
323 of the BBM. Two likely reasons could be responsible for these suggestions:

- 324 (1) There could be direct loss of BBM or enzyme molecules into the lumen of the tubule following the
325 toxic attacks by KBrO₃, as it was reported for some nephrotoxicants by other researchers [28, 29]
326 and
- 327 (2) There could be enzymes inactivation due to conformational changes in the molecular structure of
328 the BBM enzymes. Earlier researchers have reported oxidative modification of amino acid side
329 chains of enzyme protein by reactive oxygen species that was generated by KBrO₃ toxicity and
330 consequent inactivation of the BBM enzymes [30, 31, 32]

331 BBM forms the major lining of the epithelial cells of the proximal tubule of the kidney and it was
332 reported to be the first barrier for various solutes during absorption in the kidney. Other workers have
333 reported the BBM as major target of renal injury due to ischemia and nephrotoxic agents [4, 28, 33]. This
334 of course could explain either of the two suggestions given above on why there was a significant (P<0.05)
335 decline in the activities of all the BBM marker enzymes observed in this research. However, concurrent
336 administration of KBrO₃ and methanol extract of *Carica papaya* seed was able to attenuate the decreases
337 in activities of all the BBM marker enzymes studied in a dose dependent fashion and restored the values
338 towards normal control.
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340 The significant decreases (P<0.05) in the activities of antioxidant enzymes studied (CAT, SOD, GPx) in
341 homogenates of both cortex and medulla of the kidney of rats following administration of KBrO₃ could be
342 due to the KBrO₃-induced reactive oxygen species production that could have caused oxidative stress.
343 ROS and oxidative stress have been reported to be important mediators of KBrO₃ nephropathy [34].
344 Similarly, higher levels of ROS increases oxidative modification of cellular components in protein, lipids
345 and nucleic acids, and causes damage to organelles such as the plasma membranes, mitochondria in the
346 kidney and other tissues [35, 36].
347

348 The significant decrease (P<0.05) in GSH level in renal cortex and medulla of rats following
349 administration of KBrO₃ could be due to oxidative stress occasioned by ROS. GSH, a valuable
350 physiological tri-peptide is reported to be a vital extracellular and intracellular protective antioxidant
351 against oxidative stress. It reduces H₂O₂ and hydroperoxides by its redox and detoxification reactions and
352 protects protein thiols groups from oxidation [19]. Literature reported that decrease in the level of GSH will
353 increase OS which will subsequently lead to cell damage [37].
354

355 Free radical and ROS is key to the initiating of peroxidation of polyunsaturated fatty acid (PUFA). This
356 reaction cascade which could lead to formation of lipid peroxide can be prevented by enzymatic and non
357 enzymatic controlled antioxidant [19]. In the present study, the significant increase (P<0.05) in MDA

358 concentration of kidney tissues in KBrO₃ administered rats could be the consequence of an increment in
359 the formation of oxygen free radicals generated by KBrO₃ and in fact indicates a compromised antioxidant
360 defense system. MDA is one of the potent metabolites of peroxidized PUFA [19, 38] and is considered a
361 biomarker of oxidative stress [39, 40]. Presence of MDA can induce cellular and functional damage [41].
362

363 However, concurrent administration of KBrO₃ and methanol extract of *Carica papaya* seed prevented all
364 the changes in the parameters of oxidative stress studied in both cortex and medulla in a dose dependent
365 manner
366

367 4. CONCLUSION

368 Methanol extract of *Carica papaya* seed possess ameliorative effect against potassium bromate- induced
369 nephrotoxicity and the most effective dose was 600mg/kg body weight. The effect of the extract was
370 experienced in both the renal cortex and medulla. Since KBrO₃ is known to induces oxidative stress in the
371 cell which in part is considered responsible for its toxicity, the preventive role of *Carica papaya* seed
372 extract could be due to its ability to act as an antioxidant and a scavenger of reactive oxygen species.
373 Thus *Carica papaya* seed extracts can be considered a potential preventive agent against renal damage
374 caused by KBrO₃ and other structurally related compounds.
375

376 377 COMPETING INTERESTS

378 Authors have declared that no competing interests exist.
379
380

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