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# Sub-fractions from *Carica Papaya* Seed Extracts Can Prevent Potassium Bromate- induced Changes in Activities of Renal Brush Border Membrane Enzymes and Some Enzymes of Carbohydrate Metabolism in the Kidney of Rats

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***Authors' contributions***

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

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## ABSTRACT

**Aim:** To investigate the effect of the chromatographic fractions of *Carica papaya* seed on $KBrO_3$ – induced reduction in the activities of renal brush border membrane (BBM) marker enzymes and the changes in activities of some enzymes of carbohydrate metabolism in the kidney of rats.
**Study Design:** twenty male Wistar rats were divided into four groups, five rats per group; normal control, $KBrO_3$ control, *papaya* fraction control and $KBrO_3$ group administered with 126mg/kg body weight of the most active fraction of partially purified methanol extract of *C. papaya* for 48 hours.
**Place and Duration of Study:** Department of Biochemistry Laboratory, Faculty of Basic Medical Sciences, Bayero University Kano, Nigeria.
**Methodology:** The activities of renal BBM marker enzymes: γ-glutamyl transferase, alkaline

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phosphatase, maltase and leucine aminopeptidase were assayed in homogenates of renal cortex and medulla, and in brush border membrane vesicle (BBMV) isolated from cortex using standard methods. Furthermore, activities of the following enzymes representing different pathways of carbohydrate metabolism were determined in renal homogenates: hexokinase (HK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), glucose 6-phosphatase (G6P), fructose 1,6-bisphosphatase (FBP), glucose 6-phosphate dehydrogenase (G6PD) and malic enzyme (ME).

**Results:** $KBrO_3$ administration significantly ($P<0.05$) decreases the activities of all the BBM marker enzymes in renal homogenates and BBMV. It also decreases the activities of MDH, G6P, FBP and G6PD, and significantly increases ($P<0.05$) that of HK, LDH and ME in renal homogenates however co-administration of most active fraction of *C. papaya* seed prevented all the $KBrO_3$ -induced changes in these biochemical parameters.

**Conclusion:** Chromatographic fractions of *C. papaya* seed extract possesses potent phytochemicals that could prevent $KBrO_3$ –induced reduction in activities of renal BBM marker enzymes and the changes in enzymes of carbohydrate metabolism studied and therefore could be analyzed further to isolate the bioactive compounds.

*Keywords: Carica papaya seed; potassium bromated; renal brush border membrane; carbohydrate metabolism.*

## 1. INTRODUCTION

Toxic effects of xenobiotics including certain drugs, environmental pollutants and some chemical food additives can affect the kidney and lead to acute or chronic renal injury [1-3]. Potassium bromate, a food additive used in bread to improve flour and condition dough has been reported as one of these chemicals with negative effect against the kidneys [4].

The kidney consists of several types of cells organized into a basic functional unit called nephron. Absorption and transport of metabolites, considered one of the major functions of the kidney is mediated by the nephron and these processes are dependent on the functional and structural integrity of the kidney as well as availability of energy in form of ATP [5]. Consequently, any stimuli that can induce loss in structure of these cells or its functions as well as the supply of ATP can induce renal toxicity [5,6]. Previous studies have reported that toxic attacks by $KBrO_3$ can affect both the structure and function of the kidney by impairing glomerular filtration and inhibiting tubular absorption in the kidney as well as depressing ATP supply by causing changes in activities of enzymes of carbohydrate metabolism [4,6] hence motivating researchers to seek possible protection for the kidney against the $KBrO_3$ –induced assault. We have previously reported that crude methanol extract of *C. papaya* seed possesses preventive activity against $KBrO_3$ –induced nephrotoxicity [7] and have also demonstrated the safety of same plant material which have $LD_{50}$ above 5000mg/kg body weight [8]. The present study goes further to investigate the effect of various fractions from methanol extract of *C. papaya* seed against $KBrO_3$ –induced reduction in activities of renal brush border membrane (BBM) marker enzymes and some enzymes of carbohydrate metabolism in renal tissues of rats. This is important because it will provide the necessary background for the isolation and characterization of the active principle in *C. papaya* seed against renal toxicity.

## 2. MATERIALS AND METHODS

### 2.1 Chemical and Assay Kits

Potassium bromate, tris (hydroxymethyl) aminomethane (Tris), [2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid], HEPES, pyruvate, glucose, ATP, $MgCl_2$, $NADP^+$, $MnCl_2$, $MgSO_4$, cystein, fructose 6-bisphosphate, serine, ammonium molybdate, glucose 6- phosphate were supplied by Labtech Chemicals Lagos, Nigeria. These and all other chemicals used meets the requirement of the American Chemical Society Committee on Analytical reagents.

### 2.2 Plant Sample and Preparation

25 matured *Carica papaya* was procured from Na'ibawa market Kano, Nigeria and the taxonomic identification was done by the department of Plant Biology, Bayero University Kano, Nigeria with an accession number, BUKHAN 0012. Each of the plant samples was bisected to remove the seeds which was washed with tap water, shade-dried and ground into fine powder using an electric blender.

## 2.3 Extraction and Fractionation Procedures

The sequential extraction of dried powdered *Carica papaya* seed using five different solvents of increasing polarity was carried out by maceration. 625g of powdered *C. papaya* seed was in turn suspended in 1000$cm^3$ of each solvent for 24 hours and shaken at interval. It was then filtered, first with cheese cloth and then Whatman No. 1 filter paper [9]. The fractionation of methanol extract of *C. papaya* seed being the most active extract was carried out by chromatographic techniques [10]. This is schematized in Fig. 1. (Note: only the most active fraction of methanol extract of *C. papaya* seed was tested against the $KBrO_3$ –induced reduction in BBM marker enzymes and the changes in activities of the selected enzymes of carbohydrate metabolism).

## 2.4 Experimental Animals

Twenty healthy young male Wistar rats, each weighing between 120-150g were used for the research. The study was carried out at the animal house unit of the department of Biological Sciences, Bayero University Kano, Nigeria. 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.

## 2.5 Experimental Design

After the seven days acclimatization period, the rats were randomly divided into four groups into metal-plastic cages as shown below. Each group contains five rats and solution of potassium bromate was administered orally as a single dose of 100mg/kg body weight of rats to the test and $KBrO_3$ control groups while animals in the normal control and *papaya* control groups were administered the equivalent volume of distilled water and the most active fraction of partially purified methanol extract of *Carica papaya* seed, F1 respectively. The experiment was carried out for 48 hours.

Group one, normal control: given distilled water

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

Group three, *papaya* fraction control: given 126mg/kg bw F1

Group four, treatment: given 126mg/kg bw F1 + 100mg/ kg bw $KBrO_3$

## 2.6 Harvesting of Kidney and Preparation of Homogenates of Renal Cortex and Medulla

Immediately after the animal sacrifice, the kidneys of each rat were removed, 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 homogenizer [11]. Brush border membrane vesicle (BBMV) was isolated from renal cortex at the elapsed of the experimental period [12]. The renal homogenates and the BBMV were frozen immediately after preparation pending analysis.

## 2.7 Determination of Biochemical Parameters

### 2.7.1 Renal brush border membrane marker enzymes

Γ- glutamyltransferase (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. Alkaline phosphatase ( 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 leucine aminopeptidase (LAP, 3.4.11.1) were determined using kits from Elabscience Biotechnology Inc, USA and Bioway Nanjiang, China respectively.

### 2.7.2 Selected enzymes of carbohydrate metabolism

Some selected enzymes from different pathways of carbohydrate metabolism were assayed namely hexokinase (HK, EC 2.7.1.1) and lactate dehydrogenase (LDH, EC 1.1.127 from glycolysis, Malate dehydrogenase (MDH, EC 1.1.1.37) from tricarboxylic acid cycle (TCA), glucose 6-phosphatase (G6P, EC 3.1.3.3) and fructose 1,6-bisphosphatase (FBP, EC 3.1.3.11) representing gluconeogenesis and glucose 6-phosphate dehydrogenase (G6DH, EC 11:1.49) was assayed for hexose monophosphate (HMP) shunt pathway while malic enzyme (ME, EC

1.1.1.40) was assayed for the generation of NADPH.

HK was determined by measuring the rate of formation of glucose -6-phosphate from glucose and ATP in the presence of $Mg^{2+}$ [13]. LDH was determined from the rate of formation of Lactate and $NAD^+$ [14] while MDH was assayed by colorimetric method using kit from Abcam Biotech Co. Cambridge, UK. G6P was determined spectrophotometrically by measuring the rate of liberation of inorganic phosphate from glucose 6-phosphate [15] while FBP was determined spectrophotometrically by measuring the rate of liberation of inorganic phosphate from dephosphorylation of fructose-1,6-bisphosphate [16]. G6PD was determined in renal tissues using kit from Gen way Biotech Inc. San Diego, USA and malic enzyme was assayed spectrophotometrically from the rate of formation of NADPH from $NADP^+$ [17].

### 2.8 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 Extraction and Fractionation Processes

The crude extraction and chromatographic fractionation of *C. papaya* seed is schematized on Fig. 1. Crude methanol extract fractionation yielded 267 aliquots of 50$cm^3$ each which were later pooled to eight fractions according to their chemical profiles analyzed by thin layer chromatography. Bioassay of the eight fractions shows that F1 was the most potent fraction.

### 3.2 Brush Border Membrane Marker Enzymes

Administration of $KBrO_3$ resulted in significant ($P<0.05$) decreases in the activities of all the renal BBM marker enzymes namely; GGT, ALP, maltase and LAP in homogenates of renal cortex and medulla as well as in BBMV isolated from cortex. The effect was severe in cortex than medulla for all the BBM marker enzymes and the enzyme most affected by $KBrO_3$ was ALP followed by maltase and LAP. GGT was least affected. However concurrent administration of $KBrO_3$ and F1 resulted in significant ($P<0.05$) increases in activities of all the BBM marker enzymes toward normal control values in both the renal homogenates (Table 1), and BBMV isolated from cortex (Table 2). Administration of F1 alone did not show any significant change ($P>0.05$) as compared to normal control group.

### 3.3 Percentage Losses and the Subsequent Recovery in the Activities of Renal BBM Marker Enzymes in BBMV Isolated from Cortex of rat's Kidney Following $KBrO_3$ Administration and after Concurrent Administration of $KBrO_3$ and F1

Analysis of the percentage losses in the activities of BBM biomarker enzymes in the isolated BBMV following potassium bromate administration and the preventive effect of F1 shows that the renal BBM marker enzyme most affected by $KBrO_3$ toxicity is maltase followed by GGT and LAP in that order. ALP is the least affected enzyme. All the BBM enzymes lost more than half of their activities upon $KBrO_3$ administration. However concurrent administration of F1 prevented these $KBrO_3$-induced BBM marker enzymes decreases with up to 93.32% recovery in activity for maltase, 85.72% for GGT, 82.62% for ALP and 81.36% for LAP.

### 3.4 Enzymes of Carbohydrate Metabolism in Rats

Administration of $KBrO_3$ resulted in significant ($P<0.05$) increases in activities of LDH, HK and ME, and significant decreases ($P<0.05$) in activities of MDH, FBP, G6P and G6PD in homogenates of renal cortex and medulla. The changes in activities of these enzymes in either cortex or medulla did not follow any particular trend: it was noted that enzyme activities for HK, LDH, MDH and G6PD were higher in the cortex as compared to medulla, the reverse exist in the enzyme activities of ME, FBP and G6P where activities in medulla were higher than in cortex. However, concurrent administration of F1 and $KBrO_3$ resulted in significant ($P<0.05$) prevention of all the changes observed in these enzymes in renal homogenates. Administration of F1 alone did not significantly ($P>0.05$) affect the activities of any of these enzymes.

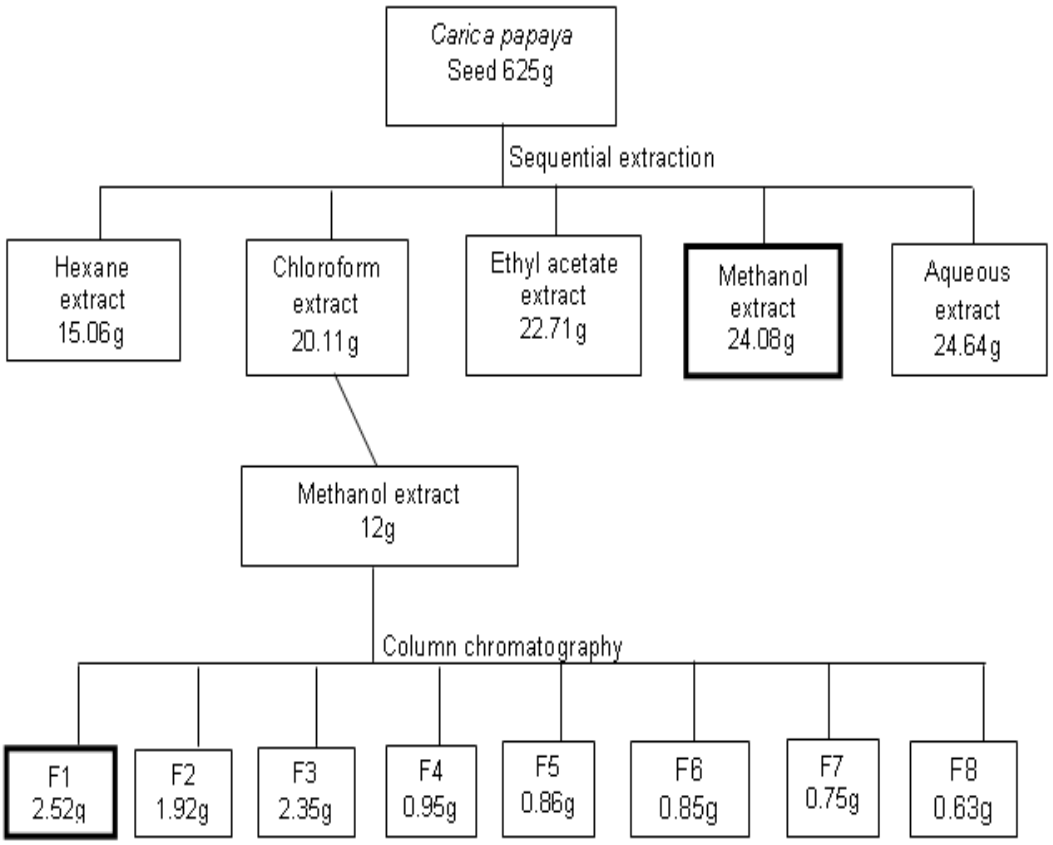


**Fig. 1. Extraction and fractionation process of the powdered *Carica papaya* seed**
*Bold boxes marks most active fractions and sub-fractions*

**Table 1. Effect of concurrent administration of most active fraction of partially purified methanol extract of *Carica papaya* seed and potassium bromate on the activities of renal brush border membrane marker enzymes in homogenates of renal cortex and medulla of rats**

| | GGT (U/L) | ALP (U/L) | Maltase (U/L) | LAP (U/L) |
|---|---|---|---|---|
| Normal Control | | | | |
| Cortex | 5.51±0.31 | 6.48±0.77 | 25.87±0.87 | 5.49±0.50 |
| Medulla | 3.97±0.41 | 4.83±0.37 | 18.54±0.53 | 3.92±0.30 |
| $KBrO_3$ control | | | | |
| Cortex | 3.36±0.37[a] | 2.32±0.61[a] | 11.91±0.94[a] | 3.42±0.48[a] |
| Medulla | 1.27±0.54[a] | 1.33±0.44[a] | 8.14±0.40[a] | 2.46±0.50[a] |
| *Papaya* control | | | | |
| Cortex | 5.28±0.65 | 6.28±0.65 | 26.07±0.57 | 5.30±0.34 |
| Medulla | 3.78±0.15 | 4.69±0.80 | 18.64±0.91 | 3.86±0.44 |
| F1 + $KBrO_3$ | | | | |
| Cortex | 4.88±0.82[b] | 5.47±0.51[b] | 24.51±1.61[b] | 4.85±0.37 |
| Medulla | 3.42±0.45[b] | 3.21±1.38[b] | 17.27±1.72[b] | 3.76±0.41[b] |

*ALP= Alkaline phosphatase; GGT= γ-glutamyltransferase, LAP = leucine aminopeptidase*
*[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 most active fraction of partially purified methanol extract of *Carica papaya* seed and potassium bromate on the activities of renal brush border membrane marker enzymes in isolated renal brush border membrane vesicle of rats**

| | GGT (U/L) | ALP (U/L) | Maltase (U/L) | LAP (U/L) |
|---|---|---|---|---|
| Normal Ctrl | 41.38±2.39 | 47.35±1.54 | 62.10±2.00 | 43.50±0.69 |
| $KBrO_3$ control | 27.37±1.14[a] | 27.60±1.16[a] | 49.16±4.71[a] | 25.34±0.46[a] |
| *Papaya* control | 41.42±1.87 | 45.42±1.27 | 58.34±2.56 | 41.53±1.45 |
| F1+ $KBrO_3$ | 35.47±2.62[b] | 39.15±2.29[b] | 57.95±3.03[b] | 35.39±2.28[b] |

*n= mean ± SDM for five different preparation*
*ALP= Alkaline phosphatase; GGT= γ- glutamyltransferase; LAP = leucine aminopeptidase*
*[a] is significant (P<0.05) from normal control, [b] is significant (P<0.05)from $KBrO_3$ control*

**Table 3. Percentage losses and subsequent recovery in the activities of renal brush border membrane marker enzymes following administration of partially purified fraction of methanol extract of *Carica papaya s*eed (F1) in the brush border membrane vesicle of potassium bromate administered rats**

| | Normal Control | $KBrO_3$ Control | F1 + $KBrO_3$ | % loss in enzyme activity | % recovery in enzyme activity |
|---|---|---|---|---|---|
| GGT | 41.38±2.39 | 25.37±1.14 | 35.47±2.26 | 66.14±1.23 | 85.72± 068 |
| ALP | 47.35±1.54 | 27.60±1.16 | 39.15±2.29 | 58.29±2.04 | 82.62±1.45 |
| Maltase | 62.10±2.00 | 49.16±4.71 | 57.95±3.03 | 79.16±3.04 | 93.32±0.94 |
| LAP | 43.50± 0.69 | 28.34±0.46 | 35.39±1.45 | 65.15±1.34 | 81.36±1.45 |

*n= mean ± SDM*

**Table 4. Effect of concurrent administration of most active fraction of partially purified methanol extract of *Carica papaya* seed and potassium bromate on the activities of some enzymes of carbohydrate metabolism in homogenates of renal cortex and medulla of rats**

| | Normal control | *Papaya control* | $KBrO_3$control | FI + $KBrO_3$ |
|---|---|---|---|---|
| LDH | | | | |
| Cortex | 20.53± 0.71 | 21.04± 0.73 | 28.84± 0.42[a] | 23.53± 0.46[b] |
| Medulla | 22.77± 1.08 | 22.59 ± 1.08 | 50.83± 1.11[a] | 24.65± 2.9 |
| HK | | | | |
| Cortex | 8.22 ± 0.19 | 9.00 ± 0.32 | 20.01 ± 0.78[a] | 10.16± 0.64[b] |
| Medulla | 7.43 ± 0.61 | 8.22 ± 0.45 | 18.42 ± 0.75[a] | 8.81 ± 0.49 |
| MDH | | | | |
| Cortex | 17.29 ± 0.40 | 17.27 ± 0.26 | 5.81 ± 0.66[a] | 13.59 ± 0.65[b] |
| Medulla | 15.05 ± 0.76 | 14.66 ± 0.28 | 4.74 ± 0.36[a] | 13.79 ± 1.37 |
| ME | | | | |
| Cortex | 2.68 ± 0.87 | 2.58 ± 0.75 | 18.42 ± 0.91[a] | 6.65 ± 0.45[b] |
| Medulla | 5.74 ± 0.38 | 5.77 ± 0.44 | 27.30 ± 1.99[a] | 13.91 ± 0.43[b] |
| FBP | | | | |
| Cortex | 2.55 ± 0.58 | 2.42 ± 0.86 | 0.74 ± 0.24[a] | 1.40 ± 0.40[b] |
| Medulla | 2.96 ± 0.55 | 2.74 ± 0.79 | 1.15 ± 0.24[a] | 2.20 ± 0.33[b] |
| G6P | | | | |
| Cortex | 0.81 ± 0.17 | 1.01 ± 0.14 | 0.32 ± 0.13[a] | 0.45 ± 0.13[b] |
| Medulla | 0.87 ± 0.13 | 1.20 ± 0.24 | 0.63 ± 0.26[a] | 0.67 ± 0.08 |
| G6PD | | | | |
| Cortex | 15.48 ± 0.93 | 15.79 ± 1.07 | 5.66 ± 0.42[a] | 14.53 ± 0.51[b] |
| Medulla | 13.66 ± 1.08 | 13.84 ± 1.32 | 4.37 ± 0.52[a] | 13.42 ± 1.17 |

*n= mean ± SD for five different preparation; LDH = lactate dehydrogenase; HK= Hexokinase; MDH = Malate dehydrogenase; ME = Malic enzyme, FBP = Fructose 1,6-bisphosphatatse; G6P = Glucose 6-phosphatase; G6PD = Glucose 6-phosphate dehydrogenase; Activities of HK is in mmol/min/mg protein, LDH is in units/mg protein, MDH is in nmol/min/µl, ME is in nmol/mg protein/min, G6PD is in nmol/min/ml, FBP and G6P are in µmol/mg protein/h*
*[a] is significant (P<0.05) from normal control, [b] is significant (P<0.05) from $KBrO_3$ control*

## 4. DISCUSSION

The use of plant material to prevent cell, tissue and organ toxicity appears to be gaining increasing acceptance in recent time notably because plant is considered safe and has proven to be effective against many kinds of illnesses [18]. *C. papaya* seed is one of such plants material that has been found to reduce the risk of many kinds of ailments including anti-helminthic, anti-bacteria, anti-fungi and female anti fertility [19] among others. Crude methanol seed extract of *C. papaya* seed has been reported to possess potent activity against $KBrO_3$ –induced nephrotoxicity in rats [7]. In the present study, the most active chromatographic fraction from methanol extract of *C. papaya* seed was tested against $KBrO_3$ –induced reduction in activities of renal brush border membrane marker enzymes and some enzymes of carbohydrate metabolism in renal tissues of rats.

The observed significant decreases ($P<0.05$) in the activities of renal BBM marker enzymes namely GGT, ALP, Maltase and LAP in homogenates of renal cortex and medulla as well as in the BBMV isolated from cortex 48 hours after $KBrO_3$ administration could be due to the toxic effect of $KBrO_3$ on the structure of the kidney which in turn could have affected the function of the BBM. Previous workers have reported that these could be due to direct loss of BBM or enzyme molecules into the lumen of the proximal tubule following the toxic attacks by $KBrO_3$, as it was reported for some nephrotoxicants since the anatomical position of the BBM is such that, it faces the lumen directly [20,21]. Furthermore, BBM marker enzymes could be inactivated due to conformational changes in the molecular structure of enzymes protein. This is in agreement with earlier studies which reported oxidative modification of amino acid side chains of enzyme protein by reactive oxygen species that was generated following $KBrO_3$ administration [22]. However, concurrent administration of $KBrO_3$ and the most active fraction of partially purified methanol extract of *C. papaya* seed significantly ($P<0.05$) attenuated the decreases in activities of all the BBM marker enzymes in both the homogenates and the BBMV.

It could be noticed that the reduction in renal BBM enzymes activities following administration of$KBrO_3$ was greater in BBMV than the homogenates of renal cortex. This suggests that the membrane bound enzymes are more responsive to $KBrO_3$ toxicity than the soluble enzymes. Analysis of the percentage losses in activities of the BBM marker enzymes in the BBMV following $KBrO_3$ administration shows that the BBM marker enzyme most affected is maltase followed by GGT and ALP in that order. LAP was the least affected. However the preventive potential of F1 followed a different trend as follows; ALP > maltase > LAP > GGT.

The observed significant increases ($P<0.05$) in the activities of HK and LDH as well as significant decrease in MDH activity in homogenates of renal cortex and medulla as compared to normal control following $KBrO_3$ administration may suggest an increase in glycolysis with corresponding decrease in oxidative utilization of glucose and this could cause substantial reduction in ATP production leading to poor transport and re-absorptive property of the kidney. The observed increases in the activities of these enzymes of glycolysis studied and the corresponding decrease in the activity of the TCA cycle enzyme studied, notwithstanding the fact that the actual rates of these pathways were not calculated, could suggest a shift in energy production from aerobic metabolism of glucose to anaerobic state. These could be supported by the previously reported damage of mitochondria (major site for the aerobic metabolism of glucose) following $KBrO_3$ administration [23]. These workers reported an up-regulation in several glycolytic genes and loss of mitochondrial function following $KBrO_3$ administration. The increases in activities of the glycolytic enzymes studied could also be a cellular adaptation strategy to ensure continuous production of energy to meet critical physiological needs after mitochondrial damage. The significant ($P<0.05$) decreases in the activities of G6P and FBP could be due to the decrease in the activity of TCA cycle enzymes particularly MDH. This is because, even though reduced MDH activity mainly affects the production of TCA cycle intermediates particularly oxaloacetate (OA) from malate for the continuation of TCA cycle, however OA is also a substrate for gluconeogensis [24]. Finally, the observed significant ($P<0.05$) decrease in activity of G6PD, the first enzyme in the HMP shunt pathway for the production of NADPH in response to $KBrO_3$ administration, may not likely affect reductive biosynthesis due to the observed increase in the activity of NADP-malic enzyme which could have compensated for the decrease in G6PD activity.

However concurrent administration of $KBrO_3$ and the most active fraction of the partially purified methanol extract of *C. papaya* seed prevented the $KBrO_3$ –induced changes in all the enzymes of carbohydrate metabolism studied. From the foregoing, it can be stated that the $KBrO_3$ - induced depression on energy production in renal tissues are significantly prevented by concurrent administration of the most active fraction of the partially purified methanol extract of *C. papaya* seed to experimental rats.

## 5. CONCLUSION

The results of the present study indicate that the nephrotoxic effect of $KBrO_3$, as shown by the reduced activities of renal brush border membrane marker enzymes and changes in activities of the enzymes of carbohydrate metabolism studied was prevented by concurrent administration of the most active fraction of *C. papaya* seed. The data suggests that these enzymes could serve as biomarkers of renal toxicity and therefore should be investigated in urine and serum samples. Also, the changes in the activities of the enzymes of carbohydrate metabolism studied may not imply net changes of direction in their respective pathways since the isolated activities of the enzymes have not been studied to know whether it can support that. Thus for the meantime, the mechanism involved in the changes is yet to be understood. Nevertheless, the preventive effect of *C. papaya* seed on renal BBM marker enzymes and the enzymes of carbohydrate metabolism that was studied is clear and therefore the active ingredients of *C. papaya* seed needs to be isolated and characterized for further studies and likely consideration in clinical practice.

## ETHICAL APPROVAL

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

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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