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Antihyperuricemic and Nephroprotective Effects of *Carica papaya* Aqueous Leaf Extract in a Murine Model of Hyperuricemia and Acute Renal Tissue Injury

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Abstract: This a pilot study on the effects of *Carica papaya* aqueous leaf extract (PLE) on blood uric acid levels (BUA) and kidney histomorphology of potassium bromated ($KBrO_3$)-treated adult male albino mice. PLE was prepared from dried papaya leaves and qualitative phytochemical analysis done showed the presence of alkaloids, flavonoids, 2-deoxysugars, and tannins. Sixty (60) mice were randomized to six groups (n=10): sham and negative controls (sterile water only); positive control (ascorbic acid 200 mg/kg BW); and three experimental groups: PLE1, PLE2 and PLE3 (1, 2, and 3 g/kg body weight [BW]). Baseline fasting BUA at days 0 and 7 were measured. Oral administration of PLE was done for 14 days via gastric gavage. On day 14, the subjects (except sham) were given $KBrO_3$ 200 mg/kg BW via gavage to induce hyperuricemia. BUA was measured pre-$KBrO_3$ (333 h) and post-KBrO3 (336 h). The subjects were then anesthetized and the kidneys were excised for histologic examination. The extent of renal tissue damage was scored using a standard numerical scheme. Results showed a significant rise ($p<0.001$) in the BUA of the negative control from 333 to 336 h, confirming the induction of hyperuricemia. In all PLE-treated groups, BUA levels were not significantly increased ($p>0.05$) from 333 h to 336 h. BUA response to KBrO3, however, was not dose-dependent ($p=0.80$) at PLE 1, 2, and 3 g/kg BW. Histologically, all PLE-treated groups showed significant attenuation of acute renal tissue damage. However, similar to the BUA response, the renal tissue response to injury was not dose-dependent ($p=0.66$) at PLE 1, 2, and 3 g/kg BW. In this study, BUA level was found to be directly correlated with the extent of renal tissue damage. These findings suggest that PLE may have antihyperuricemic and nephroprotective effects in this murine model of hyperuricemia and acute renal tissue injury.

Key Words: hyperuricemia; potassium bromate; *Carica papaya*; acute kidney injury

1. INTRODUCTION

Hyperuricemia is a cardinal precursor of gouty arthritis and renal disease. It is an important contributor in the increasing incidence of chronic, degenerative and preventable lifestyle illnesses. Hyperuricemia is prevalent among 37.8% adult males and 18% adult females. In the Philippines, mean blood uric acid in men is relatively higher in women. Interestingly, hyperuricemia and gout are both more prevalent among Filipinos living in the United States than those living in the Philippines (Azmi *et al.,* 2012; Mok *et al.*, 2012).). These pronounced differences may be attributed to diet, lifestyle, and underlying renal genetic defects (Emmerson, 1996).

Commonly prescribed antihyperuricemic medications, such as allopurinol and probenecid, are approved only in the treatment of gout, urate-associated urolithiasis, and for prevention of tumor lysis syndrome in cancer. These drugs, however, may pose greater risk of adverse drug reactions (Juraschek *et al.,* 2011). Currently, there is no indication for long-term administration of these drugs to maintain normal blood urate levels and to prevent associated chronic disease, including gouty uropathy. Two locally available medicinal plants with well established anti-hyperuricemic properties include onion *Allium cepa* and "pansit-pansitan" *Peperomia pellucida*. Treatment with onion has shown beneficial effects on hyperuricemia and oxidative stress, significantly inhibiting hepatic xanthine oxidase/xanthine dehydrogenase activity, with a great improvement in biomarkers of oxidative stress (Haidari *et al*, 2008). Interestingly, *Carica papaya* leaves have been shown to contain substance/s with potential xanthine oxidase inhibiting activity (Azmi *et al*, 2012).

Hence, in this study, the effects of *Carica papaya* aqueous leaf extract (PLE) on blood uric acid levels (BUA) and kidney histomorphology of potassium-bromate treated male albino mice were determined. Specifically, the study aimed to (1) determine the phytochemical components of PLE; (2) determine the effects of PLE at 1, 2, 3 g/kg body weight (BW) on BUA and kidney histomorphology; (3) describe the histomorphologic changes in the kidneys of potassium bromate-treated male albino mice pretreated with PLE; and (4) determine the relationship between the blood uric acid levels and kidney histomorphology.

2. METHODOLOGY

2.1. Procurement and Authentication of Plant Material

Fresh leaves of *Carica papaya* were collected from Angeles, Pampanga, Philippines (15° 9' 0" N, 120° 35' 0" E). The samples were sent to the Botany Division of the Philippine National Museum (Manila, Philippines) for identification and authentication.

2.2. Preparation of Plant Extract

The collected papaya leaves were washed with tap water and were air-dried for three days. The dried leaves were reduced to powder using an electric osterizer. About 300g (dry weight) papaya leaves were macerated with 2.4 L of distilled water. The mixture was allowed to stand for 48 hours. The solution was then filtered through a muslin cloth, and the resulting filtrate was filtered again using Whatman No. 1 filter paper. The filtrate was then collected and allowed to evaporate until a brown semisolid extract was obtained. The extract was stored in an airtight and waterproof container, was and kept at 4°C. The extract was reconstituted using sterile water for oral administration to the subjects (Owoyele, 2005).

2.3. Phytochemical Screening of the Plant Extract

The collected papaya leaf extract (PLE) was subjected to phytochemical screening for alkaloids, quaternary base and/or amine oxide, 2-deoxysugars, unsaturated steroids, anthraquinones, flavonoids, saponins, tannins and polyphenols following the methods of Limuaco *et al.* (2012).

2.4. Test Animals and Experimental Approach

Sixty (60) inbred male albino mice, about six weeks of age, each weighing 25-35 grams, were procured from the Food and Drug Administration (Muntinlupa City, Philippines). They were housed in individual cages and were given free access to standard commercial rodent food (pellet form) and drinking water. Feeding was performed by personnel who were blinded to the study. The animals were acclimatized for seven days. The mice were kept at the San Beda College of Medicine Animal Laboratory (Manila, Philippines) under the supervision of a

licensed veterinarian who was blinded to the study. Standard ethical guidelines for animal care were followed. The subjects were randomized to six groups (n=10 per group) using the Saranomy Random Generator (U.S.A.) (Table 1). Oral feedings using gastric gavage on all treatments were done for 14 consecutive days, at around 11 AM.

Table 1. Treatment Groups

GROUP	TREATMENT
Group 1 Sham group	Water[1]
Group 2 Negative Control	Water[2]
Group 3 Positive Control	Ascorbic acid[3]
Group 4 (1g/kg BW)	PLE1[4]
Group 5 (2/kg BW)	PLE2[5]
Group 6 (3g/kg BW)	PLE3[6]

[1]Sterile water (0.1mL/10g body weight, *per orem* by gastric gavage) once daily

[2] Sterile water (0.1mL/10g body weight, *per orem* by gastric gavage) once daily + KBrO3 (200mg/kg BW) on Day 14

[3] Ascorbic Acid (United Laboratories, Philippines) (200mg/kg body weight, *per Orem* by gastric gavage) once daily + KBrO3 (200mg/kg BW) on Day 14

[4] Papaya leaf extract (1g/kg body weight, *per orem* by gastric gavage) once daily + KBrO3 (200mg/kg BW) on Day 14

[5] Papaya leaf extract (2g/kg body weight, *per orem* by gastric gavage) once daily + KBrO3 (200mg/kg BW) on Day 14

[6] Papaya leavesextract (3g/kg body weight, *per orem* by gastric gavage) once daily + KBrO3 (200mg/kg BW) on Day 14

2.5. Induction of Hyperuricemia using Potassium Bromate

Seventy-five minutes after the final administration of PLE, groups 2-6were given potassium bromated ($KBrO_3$) (Sigma-Aldrich, Singapore) at a dose of 200 mg/kg BWdissolved in sterile water *per orem* by gastric gavage.

2.6. Blood Uric Acid (BUA) Determination

The mice were fasted (placed in *nothing per orem*) eight hours prior to phlebotomy. Blood was collected from the lateral tail vein and BUA was measured on day 0 (as baseline), day 7, and day 14 (333 h) immediately before and three hours post-induction (336 h) of $KBrO_3$ using *Easy Touch* Glucose Cholesterol Uric acid (GCU) meter (Bioptik Technology, Inc., Taiwan).

2.7. Surgical Harvest of Kidney Tissues

Three hours after $KBrO_3$ administration, the mice were bilaterally nephrectomized under inhalational ether anesthesia. Animal surgery was performed under the supervision of a veterinarian who was blinded to the study. The tissues were labeled and fixed in 10% neutral buffered formalin.

2.8. Kidney Histomorphology

Paraffin sections (4am thick) were prepared from each kidney, stained with hematoxylin and eosin (H & E) and examined under light miscroscope (Eclipse Ni-E, Nikon Instruments Inc., Japan). The specimens were evaluated by a licensed veterinary pathologist who was blinded to the study. The scoring scheme used to evaluate the kidneys was adapted from Pay *et al.* (2012) (Table 2).

Table 2. Scoring of Kidney Histology

SCORE	DESCRIPTION
0	Normal
1	Areas of focal granulovacuolar epithelial cell degeneration and granular debris in tubular lumens with or without evidence of tubular epithelial cell desquamation of small foci (<1% of total tubule population)
2	Tubular epithelial necrosis and desquamation easily seen but involving less than half of cortical tubules
3	More than half of proximal tubules showing desquamation of necrosis but involved tubules easily found
4	Complete or almost complete tubular necrosis

2.9. Statistical Treatment

Uric acid measurements were treated using one way ANOVA followed by Tukey's multiple comparison test. Using ANOVA, % change in blood uric acid levels between pre-induction and post-induction in all groups. Paired t-test was used to detect if there was a significant difference in the blood uric acid levels per point in time within every group. The renal histologic grading median scores were treated using Analysis of Variance (ANOVA) followed by Kruskall Wallis method. Pearson correlation was used to determine if there is a relationship between the blood uric acid levels of potassium bromate-treated male albino mice and the severity of kidney tissue damage.

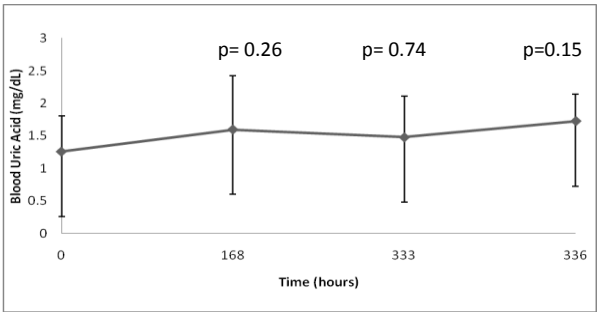

Figure 1. Mean BUA levels of sham group during the 14-day (336 hours) experiment. The p-values indicated were determined by paired t-test comparing the latter value with the immediate former.

3. RESULTS

3.1. Phytochemical Analysis of PLE

Phytochemical screening of *Carica papaya* aqueous leaf extract (PLE) showed the presence of alkaloids, 2-deoxysugars, flavonoids, and hydrolysable tannins. The presence of quaternary base and/or amine oxide, unsaturated steroids, anthraquinones, saponins, and polyphenols were not observed.

3.2. BUA Levels from Day 0 to Day 14 Pre-$KBrO_3$ Induction Were Stable

Serial measurements of BUA levels across all groups were performed on day 0 (0 hour), day 7 (168 hours) and day 14 pre-$KBrO_3$ induction (333 hours), in order to document any possible confounding effects of the placebo, Vitamin C, and even the PLE treatments on BUA levels prior to the induction of hyperuricemia. It appeared that across all the groups, there was no statistically significant difference among mean BUA levels (p= 0.22 on day 0; p= 0.16 on day 7; p= 0.21 on day 14 pre-$KBrO_3$ induction) prior to $KBrO_3$ administration. Treating the groups individually with either placebo (Fig. 2), Vitamin C (Fig. 3), and PLE at varying dosages (Figs. 4-6) did not yield any significant difference in BUA levels with respect to time, i.e., from day 0 to 7 to 14 pre-$KBrO_3$ induction (all p values >0.05).

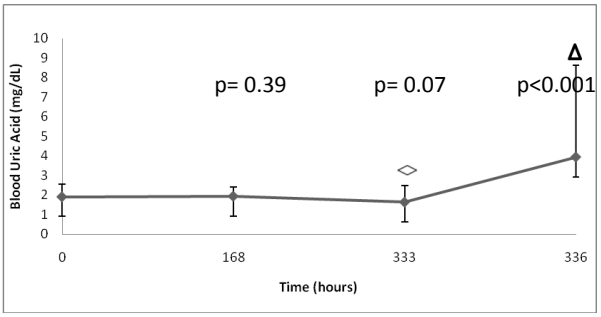

Figure 2. Mean BUA levels of negative control during the 14-day (336 hours) treatment. The p-values indicated were determined by paired t-test comparing the latter value with the immediate former.
◇Pre-induction; ΔPost-induction

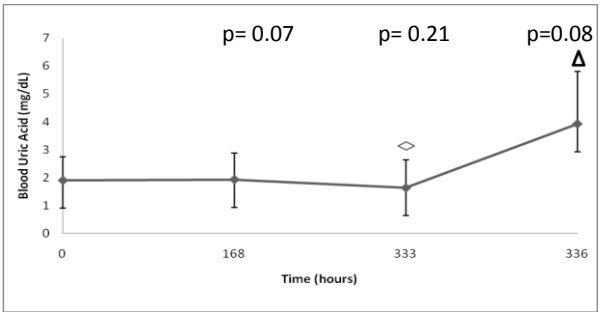

Figure 3. Mean BUA levels of vitamin C-treated group during the 14-day (336 hours) treatment. The p-values indicated were determined by paired t-test comparing the latter value with the immediate former.
◇Pre-induction; ΔPost-induction

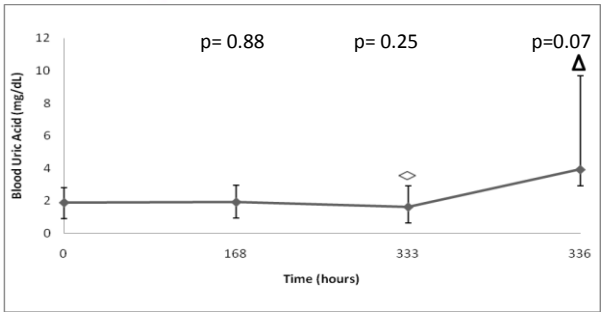

Figure 4. Mean BUA levels of PLE 1 during the 14-day (336 hours) treatment. The p-values indicated were determined by paired t-test comparing the latter value with the immediate former.
◇Pre-induction; ΔPost-induction

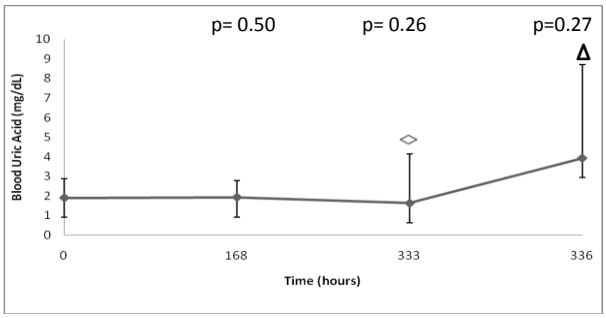

Figure 5. Mean BUA levels of PLE 2 during the 14-day (336 hours) treatment. The p-values indicated were determined by paired t-test comparing the latter value with the immediate former.
◇Pre-induction; ΔPost-induction

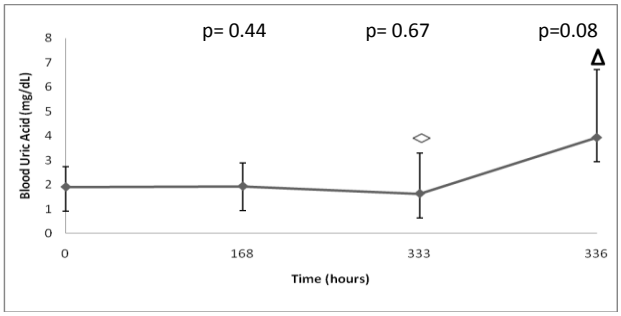

Figure 6. Mean BUA levels of PLE 3 during the 14-day (336 hours) treatment. The p-values indicated were determined by paired t-test comparing the latter value with the immediate former.
◇Pre-induction; ΔPost-induction

3.3. BUA Levels in PLE-treated Groups Were Not Significantly Increased After $KBrO_3$ Administration

There was a statistically significant increase in the BUA levels of the negative control three hours after treatment with $KBrO_3$ ($p<0.001$). This likewise confirmed the biochemical diagnosis of hyperuricemia (defined as mean BUA >6.7 mg/dL in mice) in the negative control. The Vitamin C group likewise did not show any statistically significant rise in the BUA levels post-$KBrO_3$ treatment ($p= 0.08$). Interestingly, BUA levels from 333 hours (pre- $KBrO_3$ induction) to 336 hours (post- $KBrO_3$ induction) were not significantly increased ($p>0.05$) in the PLE-treated groups (Fig. 7).

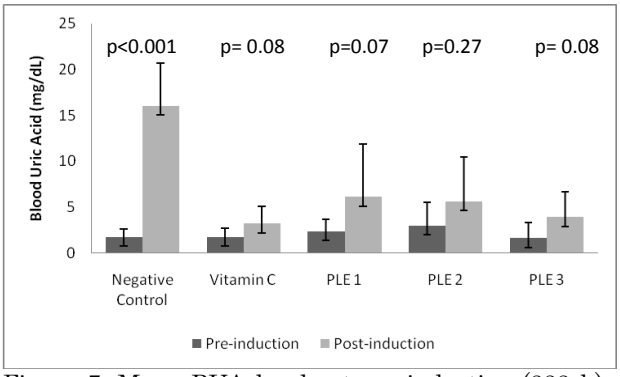

Figure 7. Mean BUA levels at pre-induction (333 h) and post-induction (336 h) with $KBrO_3$ on day 14. The p-values indicated were determined by paired t-test.

3.4. Attenuation of BUA Levels in PLE-treated Groups Was Not Dose-Dependent

There was no significant difference among Vitamin C, PLE1, PLE2, PLE3 in attenuating BUA increase ($p= 0.68$). Moreover, there was likewise no significant difference among the BUA responses among the PLE-treated groups ($p= 0.80$). These findings suggest that neither the positive control nor any one of the PLE treatments were superior in attenuating the increase in BUA levels as a response to $KBrO_3$ treatment. Further, the findings suggest that the BUA response to PLE treatment was not dose-dependent at 1, 2, 3 g/kg BW.

3.5. Kidney Histomorphology of PLE-treated Groups Revealed Less Severe Tissue Damage

Kidney histology of the Sham and Vitamin C-treated groups both revealed normal (score 0) results (Fig. 8-A). Extensive damage was revealed in the negative control (score 2) which, among others, showed areas of epithelial desquamation and necrosis. All the PLE-treated groups showed only focal and mild renal tissue degeneration (score 1).

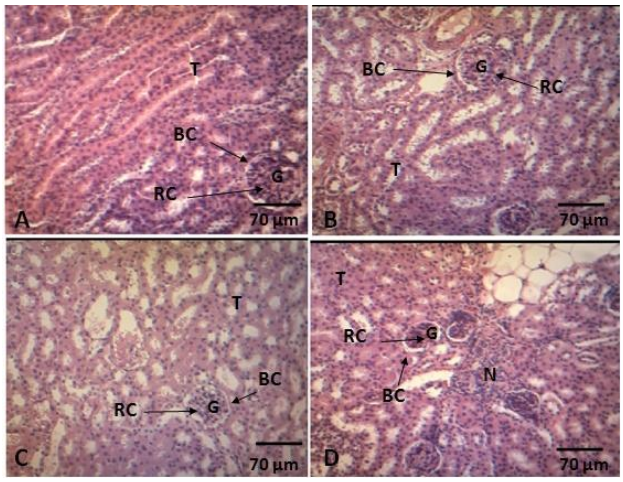

Figure 8. Histology of the kidney, H&E Stain, 400X. (A) Glomerulus surrounded by narrow Bowman spaces and tubules filling the bulk of parenchyma between the corpuscles seen in the sham and Vitamin C groups. (B) Areas of focal granulovacuolar epithelial cell degeneration and granular debris in tubular lumens with or without evidence of tubular epithelial cell desquamation of small foci seen in PLE groups. (C) Tubular epithelial desquamation seen in negative control; (D) Desquamation and necrosis seen in negative control. *Tubule (T) Renal corpuscle (RC) Bowman's capsule (BC), Necrosis (N).*

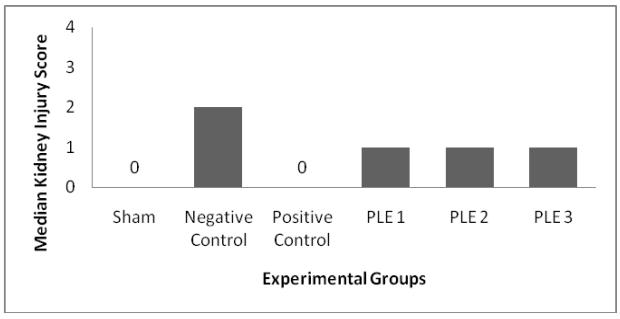

Figure 9. Median kidney injury scores of experimental groups after 14-day treatment.

By Kruskall-Wallis test, the median score of the negative control was different from the other groups ($p<0.001$). However, there was no significant difference in the median scores among PLE-treated groups ($p = 0.66$). These qualitative and quantitative findings on renal histomorphology both suggest that treatment with PLE may have attenuated the histotoxic effects of $KBrO_3$ administration to renal tissue. These findings likewise suggest that, similar to the BUA response, the renal response to injury was independent of the dosage of PLE at 1, 2, and 3g/kg BW.

3.6. BUA Level and Degree of Renal Tissue Injury Were Directly Correlated

Pearson correlation analysis of the relationship between post-induction BUA levels and kidney histomorphology injury scores showed strong positive correlation ($p= 0.03$) in the negative control at 95% confidence interval. However, there was no statistically significant correlation between post-induction BUA levels and kidney histomorphology injury scores in the Vitamin C ($p= 0.42$), PLE1 ($p= 0.33$), PLE2 ($p= 0.42$), PLE3 ($p=0.56$) groups. Note that among all the groups, only the negative control developed biochemical hyperuricemia (mean BUA >6.7mg/dL). Overall, there was a direct correlation between post-$KBrO_3$ induction BUA levels and the degree of kidney tissue damage.

4. DISCUSSION

On the day of *per orem* $KBrO_3$ administration there was a significant two fold increase in the blood uric acid level of the negative control group (Group 2) from pre-induction to post-induction. Uric acid elevation in this study appeared to be a response to $KBrO_3$-induced oxidative stress, which may have eventually reduced catalase activity and increased xanthine oxidase activity leading to elevated blood uric acid levels (Watanabe *et al.*, 2004; Azmi *et al.,* 2012). In our study, we have shown that there was attenuated increase in the blood uric acid levels in the PLE-treated groups, probably due to the xanthine oxidase inhibitory activity of one or more of its components (Azmi *et al.*, 2012). There was no significant difference in the BUA between and among PLE-treated groups and the positive control (ascorbic acid).

Preliminary screening of aqueous extract *C. papaya* leaf extract has shown potential activity xanthine oxidase inhibitory property. The most abundant components of papaya leaf were identified using HPLC quantitative analysis, including flavonoids (~30%), alkaloids (~15%) and saponins (~2%). Secondary metabolites, mainly phenolic compounds, flavonoids, alkaloids, tannins, saponins and xanthine alkaloids, anthracene-derivative glycosides and terpenoids were probably responsible for its xanthine oxidase inhibitory property (Azmi *et al.*, 2012; Yusha'u *et al.*, 2009). In this study, results of the phytochemical analysis PLE were consistent with previously published data.

Specific flavonoids such as myricetin, apigenin, quercetin and isovitexin were known to have xanthine oxidase inhibitory properties. Flavonoids inhibit xanthine oxidase activity by binding to the reactive site. Interestingly, apigenin was shown to be the most potent inhibitor among these flavonoids, probably due to hydrogen binding and electrostatic interactions between inhibitors and active site (Azmi *et al.*, 2012)

In this study, treatment with PLE may have attenuated renal damage by inhibiting a significant rise in BUA, which can possibly result in remarkable intrarenal oxidative damage and injury. PLE-treated groups showed less severe acute kidney injury by demonstration of significantly lower areas of focal tubular granulovacoular epithelial cell degeneration and granular debris in tubular lumens seen in all experimental groups. Treatment with PLE almost preserved the normal histoarchitecture, probably because of the action of free-radical scavenging activities of phenolics and flavonoids (Li-Bao *et al.,* 2008; Kensara, 2013).

Elevation of BUA may have a direct causal role in the development of kidney damage such as marked deleterious tubulointerstitial inflammation, sloughing of the tubular epithelial cells and predominant insterstitial cellular infiltration. These changes support the direct involvement of hyperuricemia in induction of renal damage. Hyperuricemia has direct pro-inflammatory effects by stimulating monocyte chemotaxis and release of pro-inflammatory mediators from vascular cells and causes glomerular hypertrophy by promoting oxidative stress (Kensara, 2013).

Oxidative stress plays an important role in the pathophysiology of KBrO3-mediated kidney injury by causing an increase in kidney xanthine oxidase and deleterious modifications in membranes, proteins, enzymes and DNA. Increased xanthine oxidase activity produces free radicals that further generate superoxide anions during the purine metabolism catalyzed by xanthine oxidase and accumulation of peroxynitrite (Watanabe *et al.*, 2004; Li-Bao *et al.,* 2008). Oxidative damage is one of the most important pathogenic mechanisms in the development of renal diseases by forming cellular injury such as inflammation, cell death, necrosis, and DNA damage. Kidney tissues are rich sources of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase derived ROS that can contribute to renal dysfunction and damage under pathological conditions (Kensara, 2013).

5. CONCLUSION

In this study, alkaloids, flavonoids, 2-deoxysugars, and tannins were present in the papaya leaf extract. Groups treated with PLE had significantly lower post-induction blood uric acid levels. However, the relationship is not dose-dependent at dosages of 1, 2, and 3g/kg BW. Papaya-treated groups showed less severe acute kidney injury by demonstration of significantly less areas of focal tubular granulovacuolar epithelial cell degeneration and granular debris in tubular lumens, but without dose dependency at dosages of 1, 2, and 3g/kg BW. There was a direct correlation between blood uric acid levels and the degree of kidney histomorphology damage seen in the potassium bromated-treated male albino mice.

6. ACKNOWLEDGMENTS

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