Research Article
ISSN: 0974-6943

Khatib Nayeem et al. / Journal of Pharmacy Research 2010, 3(11),2772-2775

Available online through
www.jpronline.info

Antiurolithiatic potential of the fruit extracts of *Carica papaya* on ethylene glycol induced urolithiatic rats

Khatib Nayeem*[a], Dhaval Gupta[a], Hashilkar Nayana[b], Rajesh K. Joshi[c]
[a] *Department of Pharmacology, KLE College Of Pharmacy, KLE University Belgaum-590 010, Karnataka, India.*
[b] *Department of Pharmacology and Pharmacotherapeutics, J. N. Medical College, KLE University Belgaum-590 010 Karnataka, INDIA.*
[c] *Department of Phytochemistry, Regional Medical Research Centre, Indian Council of Medical Research, Nehru Nagar, Belgaum-590 010, Karnataka, India*

Received on: 16-06-2010; Revised on: 15-08-2010; Accepted on:27-09-2010

ABSTRACT

The fruit of *Carica papaya* Linn (Caricaceae) used in traditional medicine for the treatment of urinary stones. The present study was undertaken to evaluate the antiurolithatic effects of the aqueous and alcoholic extracts of the fruit of *C. Papaya* on ethylene glycol (EG) induced urolithiatic rats. EG administration resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. Treatment with aqueous and alcoholic extracts of *C. papaya* fruit significantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. The increased deposition of stone forming constituents in the kidneys of calculogenic rats was also significantly lowered by curative and preventive treatment using aqueous and alcoholic extracts of the fruits of *C. papaya*. The results indicate that the fruit of *C. papaya* is endowed with antiurolithiatic activity and scientifically valid the traditional use of the fruit of *C. Papaya* in the treatment of urinary calculi.

Key words: Hyperoxaluria, Kidney stone, Microcrystal, Renal damage

INTRODUCTION

Urinary stone diseases (urolithiasis) is one of the most common afflictions of modern society that has been described since antiquity [1]. Presently, urolithiasis is the third most common disorder of the urinary tract, next too urinary tract infections and benign prostatic hyperplasia [2]. It is a consequence of complex physiochemical process and the major contributory factors being urinary supersaturation, crystallization, calculogenesis and matrix formation[3]. The pathogenesis of the lithiasis seems to be multifactorial and complex [4]. Surgical management of urolithiasis includes lithotripsy and other surgical that have certain drawback which includes renal injury, decreased renal function and increase incidence in stone recurrence along with possibility of infection [5]. In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi [2]. A variety of remedies have been used to treat urinary stones throughout history, most of which were taken from plants and have been proven to be useful.

Carica papaya L. of the family Caricaceae is commonly known as Papaya (fruit). Its food and nutritional values are popular throughout the world. The medicinal properties of papaya fruit and other parts of the plants are well known in traditional system of medicine. In Indian Materia Medica describes the traditional uses of *C. papaya* as carminative, diuretic, laxative, stomachic, treatment of urinary calculus, bleeding piles and injuries of urinary tract, abortifacient and antiobase. The dried fruits is known to be helpful in spleenomagaly, hepatomagly, dysentery and chronic diarrhea, ringworm and skin diseases like psoriasis, well known expectorant, sedative and tonic [6-9]. The extracts of fruit, leaves, seeds and roots of *C. papaya* have been extensively studied for many potential uses including, antioxidant [10], diuretic [11], wound healing [12-14], anti-inflammatory [15], antihypertensive [16], antiulcer [17,18], hypoglycaemic and hypolipidemic [19].

The phytoconstituents of the fruit of *C. Papaya* have been reported to possess vitamin A, vitamin C, thiamine, riboflavin, niacin, carotene, linalool, benzylisothiocyanate, a-carpaine, benzyl ß-D glucoside, 2-phenylethyl-ß-D-glucoside and phenolics like citric and malic acids [20,21].

In the traditional system of medicine, the fruit is reported to be useful in the treatment of urinary stones. However, there are no records of systematic pharmacological studies that support the claim of traditional use of *C. Papaya* fruit for treatment of urinary stone. The present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of aqueous and alcoholic extracts of *C. papaya* fruit using EG induced hyperoxaluria model in rats.

***Corresponding author.**
Khatib Nayeem
Department of Pharmacology, KLE College Of Pharmacy, KLE University Belgaum-590 010, Karnataka, India.
Tel.:+91831-2471399;
fax: +91831-2472387
E-mail:*khatibnayeem@hotmail.com*

MATERIAL AND METHODS

Plant Material

The ripen fruits of *C. papaya* were collected from local market of Belgaum, Karnataka, India. The fruits were authenticated by Dr. Harsha Hegde, Research scientist, Regional Medical Research Centre (RMRC), ICMR, Belgaum. A voucher specimen (No. RMRC-479) was deposited in the museum of RMRC for future references.

Preparation of aqueous extract

The fruits of *C. Papaya* were cut into small pieces, shade dried and reduced to coarse powder by mechanical grinding. The powdered material 100 g was subjected to cold maceration in distilled water for seven days with 2 ml chloroform to avoid any fungal and bacterial contamination. The flask was securely plugged with absorbent cotton and shaken periodically. The extract was filtered through muslin cloth and marc was pressed. The filtrate was again filtered through whatmann filter paper to get the clear extract. The filtrate was then lyophilized using freeze dryer to get powder form. The extractive value of the aqueous dry extract was 12% w/w. The powdered extract was stored in deep freezer at -20ºC for the experimental use. The extract was solubilised in distilled water and used for studying antiurolithiatic activity.

Preparation of alcoholic extract

The fruits of *C. Papaya* were cut into small pieces, shade dried and reduced to coarse powder by mechanical grinding. The powdered material 100 g was subjected to continue hot extraction in soxhlet by using ethanol (95% v/v). The extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was filtered and solvent was removed by distillation under reduced pressure. The extractive value of the alcoholic dry extract was 10% w/w. The extract was stored in deep freezer at -20ºC for the experimental use. The extract was solubilised in distilled water and used for studying antiurolithiatic activity.

Selection of the dose

The LD_{50} value of aqueous and alcoholic extracts of *C. Papaya* fruits has been reported [22]. Hence, one 10^{th} of LD_{50} dose was taken as a therapeutic dose for determination of antiurolithiatic effect.

Animals

Male Wistar rats weighing between 150-200g were used in the study. They were housed in well ventilated polypropylene cages at 25±2ºC under 12 h dark / light cycles and provided with standard pellet diet and water *ad libitum*. The

animal care and experimental protocol approved by Institutional Animal Ethical Committee (IAEC).

Antiurolithiatic activity: ethylene glycol induced urolithiatic model

The method of Atmani et al [23] was used to assess the antiurolithiatic activity in rats. Animals were divided into seven groups, each containing six animals. Group I served as normal control and maintained on regular laboratory diet and water *ad libitum*. Group II to VII were fed with 0.75% ethylene glycol (EG) in water for induction of renal calculi till 28th day. Group III received standard antiurolithiatic drug cystone (750 mg/kg body weight) from 15th to 28th day. Group IV and V served as curative regimen, received aqueous and alcoholic extracts of the fruit of *C. Papaya* at a dose of 250 mg/kg body weight from 15th day to 28th day respectively. Groups VI and VII served as preventive regimen received aqueous and alcoholic extracts of the fruit of *C. Papaya* at a dose of 250 mg/kg body weight from 1st day to 28th day respectively. Both the extracts were administered once daily by oral route.

Collection and analysis of urine

Urine samples were collected on 28th day for 24 h by keeping the animals in individual propylene metabolic cages. Animals had free access to drinking water during the urine collection period. The collected urine was analyzed for calcium (Erba calcium kit: OCPC method), magnesium (Coral magnesium kit: Calmagite method), oxalate and phosphate (Coral phosphorous kit: Mod. Gomorri's method) using standard methods. The volume of urine collected from all groups was recorded. Further microscopy of the urine was performed.

Serum analysis

After the experimental period, rats were anaesthetized and blood was collected from the retro orbital puncture. Serum was separated by centrifugation at 10,000 g for 10 minutes and analyzed for creatinine (Erba creatinine kit: Jaffe's method), uric acid (Erba uric acid kit: Modified trinder method) and BUN (Erba BUN kit: GLDH- Urease method).

Kidney analysis

The animals were anaesthetized and sacrificed by cervical decapitation and the abdomen was cut open to remove both the kidneys from each animal. The isolated kidneys were cleaned off extraneous tissues and weighted. The left kidney from each animal was then dried at 80 ºC in a hot air oven. About 100 mg of dried kidney was boiled in 10 ml of 1 N hydrochloric acid for 30 minutes and homogenized. The homogenate was centrifuged at 2000 g for 10 minutes and supernatant was separated. The collected supernatant was analyzed for calcium (Erba calcium kit: OCPC method), phosphate (Coral phosphorous kit: Mod. Gomorri's method) and oxalate [24].

Histopathology

Isolated right kidney from each animal was cleaned off extraneous tissue and transferred to 10% neutralized formalin (pH 7.4). Sections of kidney was fixed in paraffin, stained with hematoxylin and eosin and observed for histopathological studies.

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was carried out using one-way ANOVA followed by Dunnett's Multiple Comparison test. Differences between the data were considered significant at $P<0.05$.

RESULTS

The concentration of stone forming constituents namely calcium, phosphorous and oxalate was significantly elevated in renal tissue and urine of animals in Group II (EG induced) are shown in Table 1. However, these elevated levels were significantly (P<0.001) decreased by the treatment with aqueous and alcoholic extracts of fruit of *C. papaya* in both the curative and preventive regimens. Contradictorily, the urinary excretion of magnesium was decreased in Group II animals indicating hypomagnesuria. However, treatment with aqueous and alcoholic extracts of fruit of *C. papaya* in both regimens showed a significant (P<0.001) enhanced excretion of magnesium.

Table 1. Effect of *C. papaya* fruit extracts on urinary, kidney and serum parameters in control and experimental urolithiasis

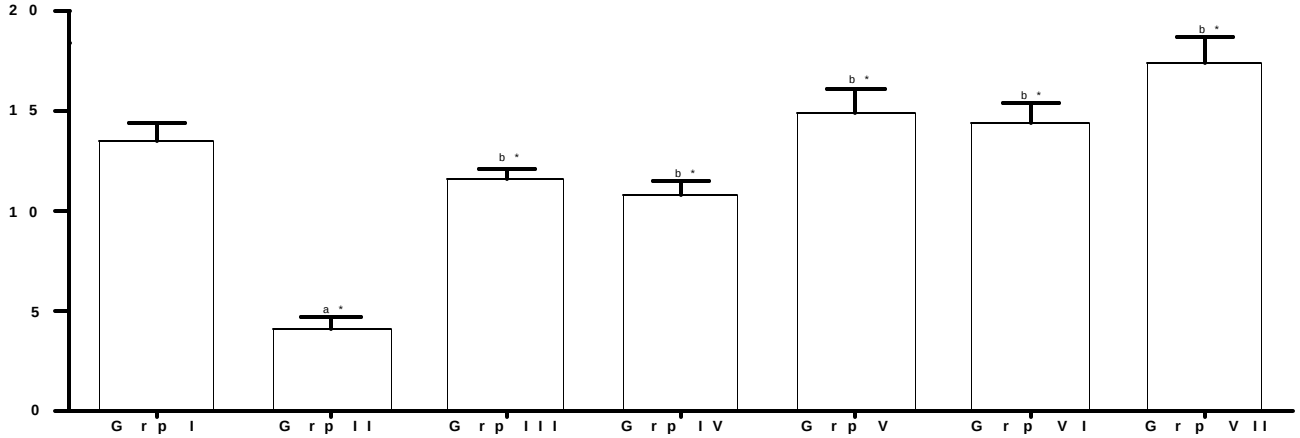
Parameters	Group I Normal Control	Group II EG treated	Group III Cystone treated	Curative regimen Group IV 250 mg/kg CR 1	Group V 250 mg/kg CR 2	Preventive regimen Group VI 250 mg/kg PR 1	Group VII 250 mg/kg PR 2
Urine (mg/dl)							
Calcium	1.71 ± 0.05	4.72 ± 0.12^{a*}	2.08 ± 0.06^{b*}	4.42 ± 0.05^{b‡}	2.34 ± 0.03^{b*}	2.55 ± 0.04^{b*}	2.12 ± 0.04^{b*}
Oxalate	0.66 ± 0.02	3.75 ± 0.15^{a*}	1.02 ± 0.07^{b*}	1.91 ± 0.09^{b*}	1.76 ± 0.12^{b*}	1.25 ± 0.19^{b*}	1.12 ± 0.11^{b*}
Magnesium	1.43 ± 0.05	0.74 ± 0.04^{a*}	1.12 ± 0.07^{b*}	0.98 ± 0.07^{b‡}	1.47 ± 0.02^{b*}	1.08 ± 0.06$^{b#}$	1.62 ± 0.07^{b*}
Phosphate	4.65 ± 0.06	7.54 ± 0.07^{a*}	4.91 ± 0.10^{b*}	7.24 ± 0.06^{b‡}	5.27 ± 0.06^{b*}	5.38 ± 0.05^{b*}	5.07 ± 0.06^{b*}
Kidney(mg/g)							
Calcium	4.17 ± 0.05	7.61 ± 0.07^{a*}	4.74 ± 0.06^{b*}	7.37 ± 0.06^{b‡}	5.25 ± 0.05^{b*}	5.33 ± 0.06^{b*}	5.04 ± 0.05^{b*}
Oxalate	1.94 ± 0.18	5.89 ± 0.37^{a*}	2.24 ± 0.24^{b*}	4.79 ± 0.21^{b‡}	4.53 ± 0.21$^{b#}$	2.81 ± 0.12^{b*}	2.34 ± 0.23^{b*}
Phosphate	2.58 ± 0.05	4.06 ± 0.05^{a*}	2.76 ± 0.06^{b*}	3.76 ± 0.05$^{b#}$	3.14 ± 0.04^{b*}	3.03 ± 0.06^{b*}	2.97 ± 0.06^{b*}
Serum(mg/dl)							
BUN	23.05 ± 0.36	36.27 ± 0.77^{a*}	26.61 ± 0.59^{b*}	31.23 ± 0.92^{b*}	27.31 ± 0.76^{b*}	28.22 ± 0.43^{b*}	25.6 ± 062^{b*}
Creatinine	0.52 ± 0.05	2.14 ± 0.06^{a*}	0.86 ± 0.06^{b*}	1.89 ± 0.05^{b‡}	1.84 ± 0.04$^{b#}$	1.30 ± 0.05^{b*}	0.92 ± 0.06^{b*}
Uric acid	1.94 ± 0.06	4.08 ± 0.06^{a*}	2.21 ± 0.04^{b*}	3.85 ± 0.05^{b‡}	2.84 ± 0.06^{b*}	2.44 ± 0.06^{b*}	2.28 ± 0.04^{b*}

CR1= Aqueous extract treated; CR2= Alcoholic extract treated; PR1= Aqueous extract treated; PR2= Alcoholic extract treated;
Values are expressed as mean ± SEM; [a] comparison made with Group I; [b] comparison made with Group II
[*] statistically significant at P<0.001
[#] statistically significant at P<0.01
[‡] statistically significant at P<0.05

Table 2. Effect of *C. papaya* fruit extracts on the body weight and kidney weight

Parameters	Group I Normal control	Group II EG treated	Group III Cystone treated	Curative regimen Group IV 250mg/kg CR 1	Group V 250mg/kg CR 2	Preventive regimen Group VI 250mg/kg PR 1	Group VII 250mg/kg PR 2
Body weight, gain (gms)	127.8 ± 7.47	75.83 ± 5.18^{a*}	105.2 ± 3.51$^{b#}$	84.83 ± 4.84^{+}	98.33 ± 3.67^{b‡}	108.7 ± 5.67^{b*}	119.0 ± 6.70^{b*}
kidney weight (g/100gm b.w.)	0.35 ± 0.004	0.47 ± 0.006^{a*}	0.38 ± 0.005^{b*}	0.45 ± 0.004^{b‡}	0.44 ± 0.009$^{b#}$	0.41 ± 0.006^{b*}	0.39 ± 0.003^{b*}

CR1= Aqueous extract treated; CR2= Alcoholic extract treated; PR1= Aqueous extract treated; PR2= Alcoholic extract treated;
Values are expressed as mean ± SEM; [a] comparison made with Group I; [b] comparison made with Group II
[*] statistically significant at P<0.001
[#] statistically significant at P<0.01
[‡] statistically significant at P<0.05



Values are expressed as mean ± SEM; [a] comparison made with Group I; [b] comparison made with Group II
[*] statistically significant at P<0.001
[#] statistically significant at P<0.01
[‡] statistically significant at P<0.05

Serum analysis showed that blood urine nitrogen (BUN), creatinine and uric acid level were higher in Group II compared to Group I, indicating marked renal damage in EG induced rats. However, aqueous and alcoholic extracts of fruit of *C. papaya* treated groups significantly lowered the elevated levels of BUN, creatinine and uric acid in curative and preventive regimens (Table 1). The histopathological finding of the kidney of EG induced rats shows deposition of microcrystals, marked dilation of tubules, tubular damage and infiltration of inflammatory cells in to the interstial space. Treatment with aqueous and alcoholic extracts of the fruits of *C. papaya* significantly showed marked improvement in the damages caused by the EG to the kidney and reduction in the crystals deposition (Table 1).

The body and kidney weight of the normal and experimental rats are represented in Table 2. After treatment with aqueous and alcoholic extracts of the fruit of *C. Papaya* in Group II gained the least significant body weight as compared to normal control and extracts treated groups. In addition, the wet weight of kidneys were taken and compared between the groups. There was a significant increase in the kidney weight of the animals in Group II, which was almost normalized in the extracts treated groups.

The urinary output of the normal control and experimental rats on 28th day are shown in Figure 1. The urine volume of the normal control rats (Group I) was 13.45 ± 0.92 ml/24 h, while in EG induced rats was reduced to 4.1 ± 0.61 ml/24 h. However, the urinary output of the aqueous and ethanolic extracts of the fruits of *C. papaya* treated groups (Group III to VI) increased significantly (P<0.001) when compared with Group II and normal control group.

DISCUSSION

Urolithiasis can be produced in rats by induction of acute or chronic hyperoxaluria by using a variety of agents such as ethylene glycol, sodium oxalate, ammonium oxalate, hydroxyl-L-proline and glycolic acid [25]. Kidney being the principal target for EG induced toxicity. Its administration to the experimental animals for 28 days resulted in substantial excretion of oxalate and deposition of microcrystals in kidney. Therefore in the present study, EG was preferred to induce lithiasis [26]. EG is broken down in vivo into four organic acids viz., glycolaldehyde, glycolic acid, glycooxalic acid and oxalic acid leading to hyperoxaluria which is the main initiative factor for lithiasis. In addition, oxalate precipitates as a calcium oxalate crystals in kidney. Oxalate metabolism is considered almost identical between rats and humans. Hence, rats are the most frequently used animals in models of calcium oxalate deposition in the kidneys, a process that mimics the etiology of kidney stone formation in human.

In this study EG induced rats gained substantially less body weight than the control group. Interestingly, treatment of aqueous and alcoholic extracts of the fruit of *C. papaya* exhibited a protective effect on body weight gain (Table 2).

Calcium, phosphate and oxalate play a vital role in renal calculogenesis. In EG induced rats, the urinary excretion and renal retention of calcium, phosphate and oxalate was significantly increased. The increase in calcium and phosphate excretion could be due to defective tubular re absorption in the kidneys [27]. While treatment with *C. papaya* markedly reduced the levels of these ions, suggested protective effect of *C. papaya* against urolithiasis. On the contrary *C. papaya* treatment significantly increased the urinary magnesium levels. Review of literature suggest that inhibitor effect of magnesium on crystallization in urine [28] and formation of calcium oxalate crystals by virtue of ability of magnesium to form a complex with free oxalate in the urine thereby forming a soluble complex thus reducing the availability of free oxalate to complex with Calcium. Accordingly in group II, the Magnesium level in urine was decreased which is common feature in urolithiasis.

In urolithiasis, the calculi formed in the renal tissue cause on obstruction in the urinary system that decreases the glomerular filtration rate (GFR) and cause an accumulation of certain waste products like nitrogenous substances e.g., BUN, creatinine and uric acid in the blood. Also increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented to calculi producing diet [29]. In this context, oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane [30]. Marked renal damage was seen in EG induced rats indicated by an elevated serum level of BUN, creatinine, and uric acid. However treatment with aqueous and alcoholic extracts of *C. papaya* fruit extracts in both the regimens caused diuresis and also decreased the serum level of BUN, creatinine and urea. Enhanced GFR and anti-lipid per oxidation property of *C. papaya* fruit extracts can be attributed for protective effect against uriolithiasis [31].

The present data indicate that, treatment with the aqueous and alcoholic extracts of the fruit of *C. papaya* significantly reduced the incidence and prevented the formation of urinary calculi in rats. The alcoholic extract was more effective than aqueous extract of the fruit of *C. papaya* in both curative and preventive regimens.

CONCLUSION

In conclusion, the findings of the present study support the folk information of use of *C. papaya* fruit in urolithiasis. However, the exact mechanism of action of antiurolithiatic activity of *C. papaya* cannot be proposed from the present study but could be attributed to diuretic and depletion of urinary calculi forming constituents. The present study scientifically valid the fruits of *C. Papaya* has antiurolithiatic activity, and could be extrapolated to human beings as an alternative therapy. Clinical studies in this regard are worthwhile.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal Dr. F. V. Manvi and Vice Principal Prof. A. D. Taranalli, KLE College of Pharmacy, Belgaum, Karnataka, India, for the support and constant encouragement.

REFERENCES

1. Eknoyan G. History of Urolithiasis, Clinic Rev Bone Miner Metab, 2004, 2, 117-185.
2. Hiatt R, Friedman GD, The frequency of kidney and urinary tract diseases in a defined population, Kidney Int, 1982, 22, 63.
3. Balaji KC, Menon M, Mechanism of stone formation. Urol Clin North Am, 1997, 24, 1-11.
4. Miller NL, Evan AP, Lingeman JE, Pathogenesis of renal calculi, Urol Clin North Am, 2007, 34, 287-293.
5. Ruml LA, Pearle MS, Charles YC, Calcium oxalate urolithiasis, Urol Clin North Am, 1997, 24, 117-133.
6. Bhattacharjee SK, Handbook of Medicinal plants, Pointer Publishers, Jaipur, 1998, pp. 71-72.
7. Nadkarni KM, Indian Materia Medica, Popular Prakashan Pvt. Ltd, Bombay, 1st edn, 1954, pp. 273-277.
8. Kirtikar KR, Basu BD, Indian Medicinal Plants, International Book Distributors, Dehradun. 2nd edn, 1998, pp. 1097-1099.
9. Vaidyaratnam PSV, Indian Medicinal Plants- A Compendium of 500 Species, Orient Longman Ltd; Chennai, 1994, vol 2, pp. 383.
10. Mehdipour S, Yasa N, Dehghan G, Khorasani R, Mohammadirad A, Rahimi R, Abdollahi M, Antioxidant potentials of Iranian *Carica papaya* juice in vitro and in vivo are comparable to a-tocopherol, Phytother Res, 2006, 20, 591-594.
11. Sripanidkulchai B, Wongpanich V, Laupattarakasem P, Suwansaksri J, Jirakulsomchok D, Diuretic effects of selected Thai indigenous medicinal plants in Rats, J Ethnopharmacol, 2001, 75, 185-190.
12. Nayak BS, Pereira LP, Maharah D, Wound healing activity of *Carica papaya* L. in experimentally induced diabetic rats, Indian J Exp Biol, 2007, 45, 739-743.
13. Gurung S, Basnet NS, Wound healing properties of *Carica papaya* latex: In vivo evaluation in mice burn model, J Ethnopharmacol, 2009, 121, 338-341.
14. Mahmood AA, Sidik K, Salmah I, Wound healing activity of *Carica papaya* L. aqueous leaf extract in rats, Int J Mol Med Adv Sci, 2005, 1, 398-401.
15. Owoyele BV, Adebukola OM, Funmilayo AA, Soladoye AO, Anti-inflammatory activities of ethanolic extract of *Carica papaya* Leaves, Inflammopharmacol, 2008, 16, 168-173.
16. Eno AE, Owo OI, Itam EH, Konya RS, Blood pressure depression by the fruit juice of *Carica papaya* (L.) in renal and DOCA-induced hypertension in the rat, Phytother Res, 2000, 14, 235-239.
17. Ologundudu A, Lawal AO, Ololade IA, Omonkhua AA, Obi FO, The Anti-ulcerogenic activity of aqueous extract of *Carica papaya* fruit on aspirin – induced ulcer in rats, Internet J Toxicol, 2008, 5.
18. Rajkapoor B, Jayakar B, Anandan R, Murugesh N, Antiulcer effect of dried fruits of *Carica papaya* linn in rats, Indian J Pharm Sci, 2003, 65, 638-639.
19. Adeneyea AA, Olagunjub JA, Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of *Carica papaya* Linn. in Wistar rats, Biol Med, 2009, 1, 1-10.
20. Krishna KL, Paridhavi M, Patel JA, Review on nutritional, medicinal and pharmacological properties of Papaya, Nat Prod Radian, 2008, 7, 364-373.
21. A. Ross, *Medicinal Plants of the world*, Humana Press Inc., Totowa, NJ. pp. 87-100.
22. Rajkapoor B, Jayakar B, Kavimani S, Murugesh N, Effect of dried fruits of *Carica papaya* Linn on hepatotoxicity, Biol Pharm Bull, 2002, 25, 1645-1646.
23. Atmani F, Slimani Y, Mimouni M, Hacht B, Prophylaxis of calcium oxalate stones by *Herniaria hirsute* on experimentally induced nephrolithiasis in rats, British J Urol Int, 2003, 92,137-140.
24. Hodgkinson A, William ANN, An improved colorimetric procedure for urine oxalate, Clin Chim Acta, 1972, 36, 127-132.
25. Khan SR, Animal models of kidney stone formation: an analysis, World J Urol, 1997, 15, 236-243.
26. Schladt L, Ivens I, Karbe E, Ruhl-Fehlert C, Bomhard E, Sub acute oral toxicity of tetra ethylene glycol and ethylene glycol administered to Wistar rats, Exp Toxicol Pathol, 1998, 50, 257-265.
27. Varalakshmi P, Shamila Y, Latha E, Effect of *Crataeva nurvala* in experimental urolithiasis, J Elhnopharmacol, 1990, 28, 313-321.
28. Kohri K, Garside J, Blacklock J, The Role of magnesium in calcium oxalate urolithiasis, British J Urol Int, 1988, 61, 107-115.
29. Sumathi R, Jayanthi S, Kalpanadevi V, Varalakshmi P, Effect of DL-a-lipoic acid on tissue lipid peroxidation and antioxidant systems in normal and glycolate treated rats, Pharmacol Res, 1993, 27, 1-10.
30. Ernster L, Nordenbrand K, *Methods in Enzymology*, Academic Press, New York, 1967, pp. 574-580.
31. Bhattacharjee S, Rana T, Sengupta A, Inhibition of lipid peroxidation and enhancement of GST activity by cardamom and cinnamon during chemically induced colon carcinogenesis in swiss albino mice, Asian Pac J Cancer Prevent, 2007, 8, 578-582.

Source of support: Nil, Conflict of interest: None Declared