Journal of Cell and Tissue Research Vol. 17(3) 6223-6229 (2017)
(Available online at www.tcrjournals.com)
ISSN: 0973-0028; E-ISSN: 0974-0910

Original Article

RAPHNUS SATIVUS SEED EFFECT ON CALCIUM-OXALATE INDUCED NEPHROLITHIASIS IN MICE

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Raceived: October 20, 2017; Accepted: November 15, 2017

Abstract: Traditional herbal treatments is used for prevention and/or treatment of urolithiasis with less expense and perhaps fewer side effects. Urolithiasis is considered the most common urinary tract diseases. Raphanus sativus, (Brassicaceae) is consumed throughout the world. Traditionally, the plant has been recommended as an antiurolithic. The present study was undertaken to evaluate its effectiveness as an antiurolithic against expeimentally calcium oxalate induced nephrolithiasis in albino mice. Phytochemical and histopathological analysis were carried out. Raphanus sativus seeds extract was rich in saponins, anthraquinone glycosides, and phenolic compounds; while with limited amount of flavonoids, steroids, and alkaloids. Histological examination of kidney showed that, Raphanus sativus seeds extract has very potent antiurolithiatic activity. Antiurolithiatic effects of Raphnus sativus seed extract may be mediated possibly through a combination of calcium oxalate crystal inhibition, antioxidative, diuretic activity, antispasmodic, anti-inflammatory and analgesic properties. Further clinical studies are required to evaluate efficacy and safety of Raphnus sativus seed extract in human beings.

Key words: *Raphnus sativus* seed, Nephrolithiasis, Calcium-oxalate stone

INTRODUCTION

Kidney stone formation is the most common, costly, painful and recurent urologic disorder; it is formed due to the presence of foreign bodies in urinary tract, bacterial infections, increased urinary calcium levels and vitamin abnormalities [1]. Many traditional systems of medicine offer several effective antiurolithic herbal drugs [2]. It is needed to explore and provid experimental evidence of new drugs from herbal remedies for the treatment and prevention of kidney stones is highly recommended [3], considering the safety of the herbal use

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According to the WHO, about 80% of the world's population in developing countries depend on locally available plants as the main source in their primary medical care, since the modern medicines are often expensive or inaccessible [4].

Raphanus sativus, (Brassicaceae) generally known as Radish, is an annual herb, 20-100 cm long, It is grown and consumed throughout the world. Traditionally, the plant has been recommended as an antiurolithic [5]. The present study was undertaken to evaluate the effectiveness of Radish as an antiurolithic against expeimentally calcium oxalate induced nephrolithiasis in albino mice.

MATERIAL AND METHODS

Plant materials: Commercial radish seeds were

purchased from local market during January 2014, and identified as (*Raphanus sativus*) by a specialist in toxconomy, department of Botany, university of Tripoli.

Preparation of seed extract: Seeds of *Raphanus sativus* were washed thoroughly with distilled water and dried in open air at room temperature for 24 hours. The seeds were powdered mechanically. About 500g of powder was macerated twice with 2 litres of methanol (95%) for 24 hr at room temperature. The obtained extract was filtered and evaporated using rotatory evaporator at 40°C, the obtianed extract was weighed and stored in refrigirator at (20 °C) until used. Seeds weight 500gm yield 16.3 g of extract.

Phytochemical screening: Ethanolic extract of *Raphnus sativus* was subjected to phytochemical screening to detect different chemical groups of compounds [6,7].

1. **Test for saponins:** To 2ml of ethanolic extract, 5ml of distilled water was added and then shaken vigorisoly for 30 second, stable persistant frothing indicates saponin.
2. **Test for steroids:** To 2ml of ethanolic extract, 2ml of chloroform, few drops of acetic acid, and 1ml of concentrated H_2SO_4 was added, a greenish colour indicates the presence of steroids.
3. **Test for anthrquinone:** To 2ml of ethanolic extract, 2ml of 10% NH_4OH was added, a bright pink colour indicates the presence of anthra-quinone.
4. **Test for phenolic compounds:** To 2ml of ethanolic extract, one ml of ferric chlorid was added, blue black or greenish black precipitate indicate the presence of phenolic compounds.
5. **Test for glycosides:** To 1 ml of ethanolic extract, few drops of dilute sodium hydroxide was added, intense yellow coloure indicate the presence of glycosides especially flavonoid glycoside.
6. **Test for alkaloide:** 5ml of ethanolic extract was added to 2ml of HCl, then added 1ml dregendroffs reagent, orange or red precipitate indicate the presence of alkaloids.

Laboratory animals: Twenty five male albino mice weighing 30 ± 5g were maintained and habituated in standard cages at standard laboratory conditions for three days before use. Mice had free access to water and food. Experiments were done according to the internationally accepted guidlines for the use of animals.

Experimental design: Intraperitoneal administration of calcium oxalate (CaOx) (80mg/kg) and *Raphnus sativus* seed extract were adopted. Five groups of animals (n=5) were used. Group 1, received saline (5ml/kg) [8] for 6 days; group 2, received 80mg/kg CaOx [9] for 6 days; group 3, received CaOX for 6 days followed by administration of seed extract (500 mg/kg) for 3 days; group 4, received CaOx for 6 days, followed by administration of seed extract (200 mg/kg) for 6 days; group 5, received 80 mg/kg CaOX for 6 day, followed by 6 days without treatment. Administration of CaOx or extracts was once per day.

Histopathological analysis: At the end of the treatment peroid,animals were sacrificed, their kidneys removed, cleaned with tap water. The tissue was cut into small pieces, and fixed in 10 % formalin. In histopathological laboratory, the samples embedded in paraffin, then cut into 5μm thick section by microtome, the paraffin was removed by immersing the section in hot water for few seconds, then the slides were prepared and stained using hematoxylin-eosin dye [10] and examined under light microscope for histopathological analysis. Ca oxalate crystal present in each kidney tissue was examined by pizzolato staining methods [11]. Pathological analysis was examined with the help of a histopathologist.

RESULTS

Phytochemical investigation: The ethanolic extract of *Raphanus sativus* seeds contain bioactive compounds as saponins, flavonoid glycosides, steroids, alkaloids, and phenolic compounds (Table 1).

Histological examination of kidney Ca oxalate (Pizzolato) staining: Histopathological examination of kidney sections of group I, mice showed normal renal histological features; the cortex consisted of renal corpuscles, tubules, and minimal interstitial tissue

Table 1: The result of phytochemical screening. For ethanolic extract of R. sativus seeds

Test	Result
Saponins	+++
Anthraquinone glycosides	++
Phenolic compounds	++
Flavonoids	+
Steroids	+
alkaloids:	+

in between. The renal corpuscles were composed of glomeruli surrounded by Bowman's spaces. The proximal convoluted tubules (PCTs) appeared to be lined by acidophilic cuboidal epithelium with an apical brush border and enclosing a narrow lumen. The distal convoluted (DCTs) tubules were lined with acidophilic cuboidal epithelium surrounding a wider lumen (Figs. 1 a,b).

Examination of renal histology with pizzolato staining of mice in groups II revealed dilatation of the tubules with crystalline material within tubule lumens, and infiltrates of mononuclear cells in the interstitium, suggesting intense inflammation. A high content of CaOx crystal at the end of experimental period specially in PCTs. The second major histopathologic change found is a complete loss of adjacent lining cells at sites of crystalline (CaOx) deposition (Figs. 2 c,d).

While microscopic examination of paraffin kidney sections of group III, where the mice injected with high dose of seed extract, for 3 days, after induction of CaOx crystals, revealed that most of the renal tubules were more or less similar to those of the control group and preservation of a near to normal structure for many PCTs with regular brush border and no significant CaOx crystal were formed (Figs. 3 e,f).

Group IV, Light microscopic examination of kidney, treated withCaOX followed by low dose of seed extract for 6 days, the renal tubules showed significantly lower content of CaOx crystal deposition (Figs. 4 g,h).

Group V, Light microscopic examination of kidney, treated with CaOX followed by no treatment for 6 days, showed numerous intraluminal deposits of CaOx crystal aggregate within the tubular lumen, especially in medulla (black) (10x); with high magnification showed CaOx crystal block the lumen of renal tubules (Figs. 5 j,k).

DISCUSSION

Urolithiasis is considered the most common urinary tract diseases [12] viz., a common type is calcium oxalate stone [13]. Kidney stone is a painful disease. It is formed due to several factors like imbalance between promoters (sodium, urates) and inhibitors (citrate, glycosaminoglycans) [14].

Urine is normally a supersaturated solution, crystalluria is often observed in normal individuals. Such crystals remain apart from each other and washed away by urine flow. Under certain circumstances, they bind together due to chemical and electrical forces triggering the process of aggregation. The crystals then attach to the epithelium which allow them to grow further and form the stone [15].

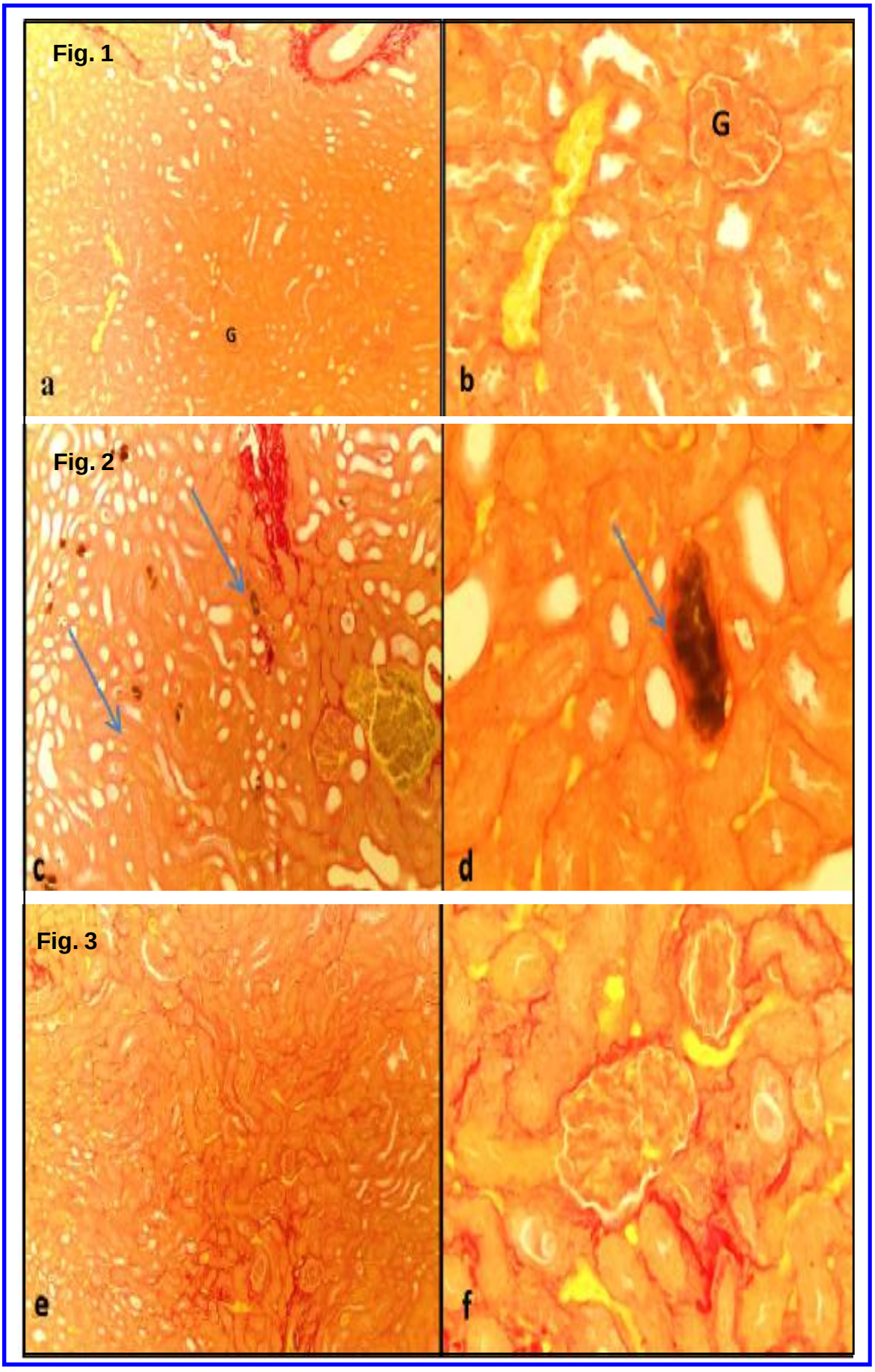
Traditional herbal treatments can be used for prevention and/or treatment of urolithiasis with less expense and perhaps fewer side effects [16]. Plant extract may contain some substances that inhibit the aggregation of oxalate crystals, which is the most critical step, as it occurs very rapidly and has a considerable effect on particle size [17]. Plant extract might contain some phytochemicals that inhibit the growth of oxalate crystals [18].

Kidney stone cause serious medical consequences, as obstruction, hydronephrosis, infection, and hemorrhage in the urinary tract system [19]. Therefore, a suitable herbal therapy for the treatment of urolithiasis is needed. Therefore, *Raphnus sativus* seeds were evaluated for antiurolithiatic effect using mice.

Table 1, represented the phytochemical constituents which found in the ethanolic extract of *Raphnus sativus* seed. The extract was rich in saponins, anthraquinone glycosides, and phenolic compounds; while with limited amount of flavonoids, alkaloids, and steroids were present.

Histopathological study using Hematoxylin and Eosin staining did not show clear CaOX stone; therefore, pizzolato staining was used for very clear black deposit of CaOX stone as seen. High dose of ethanolic extract of *Raphnus sativus* seed, produce the highest dissolution of calcium oxalate stones; the kidney was nearly normal. It was mentioned that CaOX stone could be disolved and disappear by time without treatment [20]; Group V showed that irriversable CaOX stone formation, and the dissolution of the stone in group III was due to the effect of *Raphnussativus* seed extract.

Raphnus sativus seed extract was rich of saponin, which may be responsible for antiurolithiatic activity. It is good inhibitor of calcium stone formation [21-26]. Saponins have anti-crystallization properties by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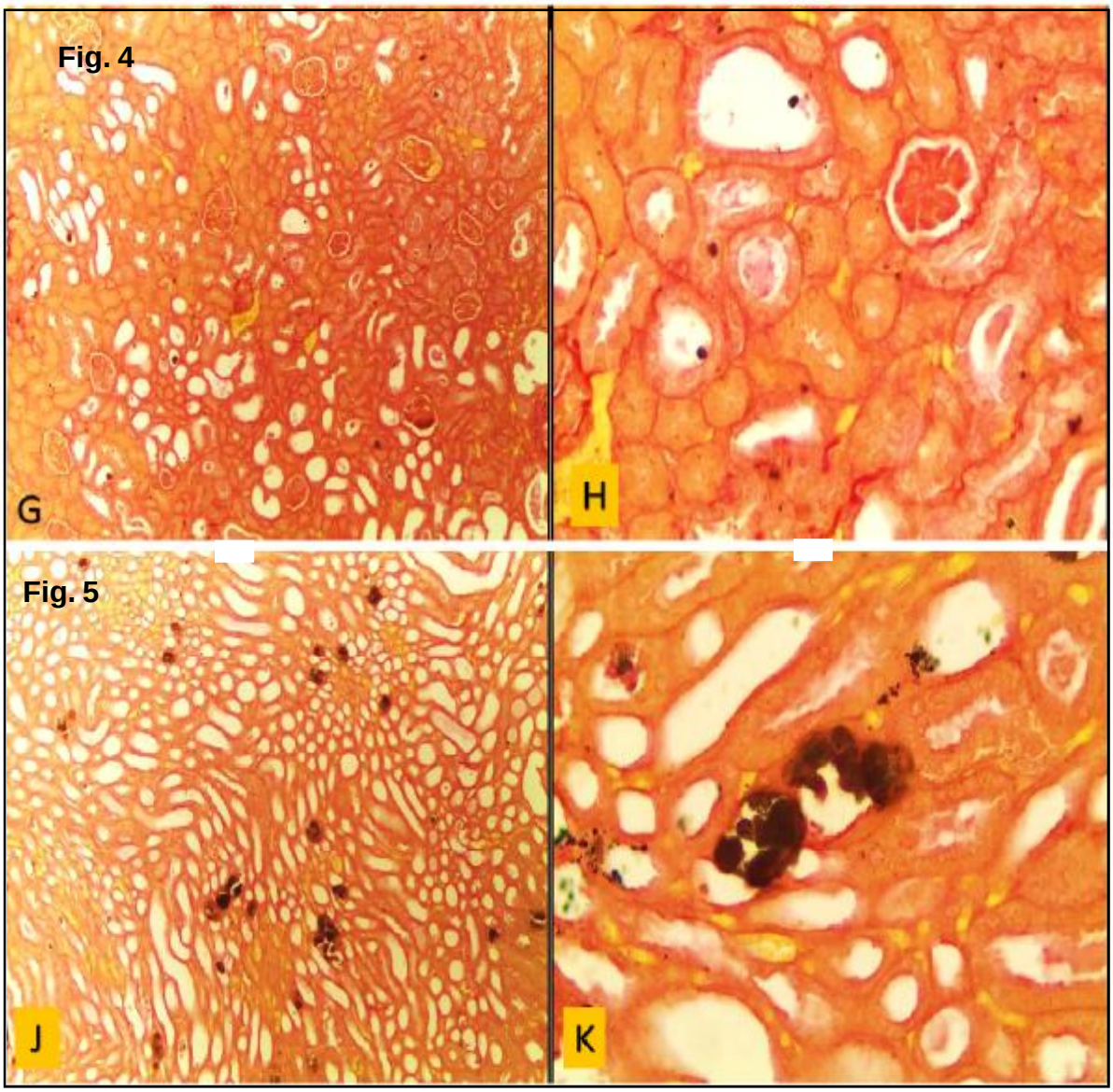

Fig. 1: Microscopy examinations of kidney tissue and CaOx crystals in experimental rat stained with Pizzolato stain showed; **a)** control kidney (Group I) showed the Glomeruli (G), proximal convoluted tubules (PCT), distal convoluted tubule (DCT) (10x). **b)** Higher magnification of same (40x). **Fig. 2**: Microscopy examinations of kidney tissue and CaOx crystals in experimental rat stained with Pizzolato stain showed; **c)** a section ofoxalate-treated kidney (Group II) with large CaOx crystal aggregate within the tubular lumen (black) (10x). **d)** a large CaOx crystal bock the lumen of PCT (40x). **Fig. 3:** Microscopy examinations of kidney tissue and CaOx crystals in experimental rat stained with Pizzolato stain showed; **e)** a section of extract-treated kidney (Group III) showed group of normal looked Glomeruli (G), PCT and DCT with normal appearance (10x). **f)** a section of the renal cortex of a mice treated with Extract (group III) showing preservation of a near to normal structure of Glomeruli (G) and other renal tubules (PCT), (40x). **Fig. 4:** Microscopy examinations of kidney tissue and CaOx crystals in experimental rat stained with Pizzolato stain; **g)** a section of extract-treated kidney (Group IV) showed scattered CaOx crystal aggregate within the tubular lumen (black) (10x). **h)** a High magnification of previous photo (40x)**. Fig. 5:** Microscopy examinations of kidney tissue and CaOx crystals in experimentalrat stained with Pizzolato stain showed; **j)** a section of CaOX treated kidney followed by no treatment for 6 days (Group VI) showed numerous intraluminal deposits of CaOx crystal aggregate within the tubular lumen , especially in medulla (black) (10x). **k)** a High magnification of previous photo showedCaOx crystal block the lumen of renal tubules .

disaggregating the mucoproteins, the promoters of crystallization [27,28].

Formation of calcium oxalate crystals increase lipid peroxidation and oxidative stress to the renal tissue and caused renal damage, leading to a reaction with polyunsaturated fatty acids in the cell membrane [29,30]. Medicinal plants with antioxidative properties showed preventive effects on kidney stones [19,31]. The phytochemical study showed that ***Raphnus sativus*** seed extract is rich of phenolic compound; Byahatti et al. [32], found that phenolic compound is effective in dissolving calcium oxalate urinary stones. It was found that, there was a positive correlation between phenolic compounds content and total radical scavenging capacity [33]. Also, flavonoids, saponins and steroids, that present in ***Raphnus sativus*** seed extract, have significant antioxidant property [34,35]. In present study, inhibition of crystallization may decrease the oxidative stress to the tissue, by decreasing lipid peroxidation–induced renal tubular damage.

Alkaloid [36-39], flavonoids and saponins [40] have potent antispasmodic effect; it may produce smooth muscle relaxation within the urinary tract, and diminished the size of calculi, facilitating the elimination of kidney calculi [41,42]. In the same time, Saponins [43], flavonoids [44] and phenolic contents [45] of ***Raphnus sativus*** seed extract may have diuretic effect. Diuretic activity may increase dissolving kidney stones, and prevent the formation of new stones [46]. Alkaloid, flavonoids and saponins present in ***Raphnus sativus*** seed extract may produce synergistic effects with other constituents as steroids, phenolics, and glycoside to have potent antiurolithiatic activity [39].

Raphnus sativus seed extract was rich of anthraquinone glycoside, it dissolves calcium oxalate stones and prevent their deposition in urinary tract [47,48]. Anthraquinone glycoside has good dissolving effect of calcium oxalate stone due to the hydroxyl groups and sugar fragments that has the complexing ability with calcium; also, it is a good inhibitor of calcium oxalate crystals formation [49].

Anti-inflammatory effect of herbs may have useful effect in urolithiasis, as inflammation is associated with urolithiasis process. Phytochemical test detects flavonoids in ***Raphnus sativus*** seed extract; flavonoids have potent anti-inflammatory and antioxidant effects [50,54]; it may prevent the formation of calcium oxalate urolithiasis, also may protects epithelial cell damage, and inhibit the inflammation induced by calcium oxalate crystals [55]. Flavonoid prevents supersaturation of calcium oxalate and prevent its deposition in renal tubules [56,57]. Flavonoids, and alkaloids showed analgesic effect [54]. Analgesic and anti-inflammatory effect of flavonoids may be through the inhibition of prostaglandins synthesis, through the inhibition of lipoxygenase, phospholipase, and cyclooxygenase [58].

Antiurolithiatic effects of ***Raphnus sativus*** seed extract may be mediated possibly through a combination of calcium oxalate crystal inhibition, antioxidative, diuretic activity, antispasmodic, anti-inflammatory and analgesic properties. Further clinical studies are required to evaluate efficacy and safety of ***Raphnus sativus*** seed extract in human beings.

REFERENCES

[1] Kaladhar, D.S.V.G.K., Krishna Apparao, R. and Varahalarao, V.: Open Access Scientific Reports, 1(12) doi:10.4172/scientificreports. 543 (2012).

[2] Joy, J.M., Prathyusha, S., Mohanalakshmi, S., Praveen Kumar, A.V.S. and Ashok Kumar, C.K.: Intern. J. Innov. Drug Discovery, 2(2): 66-75 (2012).

[3] Warrag, N.M., Tag Eldin, I.M. and Ahmed, E.M.: African J. Pharm. Pharmacol., 8(17): 443-450 (2014).

[4]World Health Organization, WHO. Traditional Medicine Strategy 2002-2005. Geneva, Switzerland

[5] Singh, P. and Singh, J.: Intern. J. Plant, Animal and Environ. Sci., 3(2): 103-105 (2013).

[6] Kumar, A.R., IIavarasan, T., Decaraman, J. M., Aravindhan, P., Padmanabhan, N. and Krishnan, M.R.V.: Pakistan J. Nutrition, 8(1): 83-85 (2009).

[7] Sumathy, V.S. Lachumy, J., Zakaria, Z. and Sasidharan, S.: Pharmacologyonline, 2: 118-127 (2011)

[8] Swayeh, A., Futter, L.E., Clifford, R.H. and Moore, P.K.: British J. Pharmacol., 130:1453-1456 (2000).

[9] Ghalayini, I.F., Al-Ghazo, M.A. and Harfeil, M.N.: Int. Braz. J. Urol., 37(2): 259-266 (2011).

[10] Bancroft, J.D. and Gamble M.: *Theory and Practice of Histological Techniques* 5th ed. Philadelphia: Churchill Livingstone, pp 125-138 (2002).

[11] Pizzolato, P.: J. Histochem. Cytochem., 12: 333-336 (1964).

[12] Aggarwal, A., Singla, S.K. and Tandon, C.: Indian J. Exp. Biol., 52(2): 103-111 (2014).

[13] Bensatal, A. and Ouahrani, M.R.: Urol. Res., 36: 283-287 (2008).

[14] Evan, A.P., Lingeman, J.E., Coe, F.L., Parks, J.H., Bledsoe, S.B., Shao, Y., Sommer, A.J., Paterson, R.F., Kuo, R.L. and Grynpas M.: J. Clin. Invest., 111: 607-616 (2003).
[15] Matlaga, B.R., Coe, F.L., Evan, A.P. and Lingeman, J.E.: J. Urol., 177: 31-38 (2007).
[16] Miyaoka, R. and Monga, M.: Int. Braz. J. Urol., 35: 396-405 (2009).
[17] Masao, T.: Int. J. Urol., 15: 115-120 (2008).
[18] Wesson, J.A., Worcester, E.M., Wiessner, J.H., Andel, N.S. and Kleinman, J.G.: Kidney Int., 53: 952-957 (1998).
[19] Hadjzadeh, M.A., Khoei, A. and Hadjzadeh, Z.: Urol. J., 4: 86-90 (2007).
[20] Okada, A., Nomura, S., Higashibata, Y., Hirose, M., Gao, B., Yoshimura, M., Itoh, Y., Yasui, T., Tozawa, K. and Kohri, K.: Urol Res., 35(2): 89-99 (2007).
[21] Arafat, O.M., Tham, S.Y., Sadikum, A., Zhari, I., Haughton, P.J. and Asmawi, M.Z.: J. Ethnopharm., 118: 354-360 (2008).
[22] Doddola, S., Pasupulati, H., Koganti, B., Koganti, V.S. and Prasad, R.G.: J. Nat. Med., 62: 300-307 (2008).
[23] Soundararajan, P., Mahesh, R., Ramesh, T. and Hazeena Begum, V.: Indian J. Exp. Bio., 44: 981-986 (2006).
[24] Fouada, A., Yamina, S., Nait, M.A., Mohammed, B. and Abdlekrim, R.: J. Bras. Nefrol., 28: 199-203 (2006).
[25] Chaudhary, A., Tandon, C. and Singla, S.K.: Indian J. Uro., 24: S111 (2008).
[26] Patel, P.T., Patel, M.A., Vyas, B.A., Shah, D.H. and Gandhi, T.R.: J. Ethano. Pharm., 44: 160-170 (2012).
[27] Gurocak, S. and Kupeli, B.: J. Urol., 176: 450-455 (2006).
[28] Saranya, R. and Geetha, N.: Intern. J. Pharm. Pharmaceut. Sci., 6(2): 361-365 (2014).
[29] Sumathi, R., Jayanthi, S., Kalpanadevi, V. and Varalakshmi, P.: Pharmacol. Res., 27: 309-318 (1993).
[30] Khan, S.R. and Thamilselvan, S.: Mol. Urol., 4: 305-312 (2000).
[31] Hadjzadeh, M., Khajavi-rad, A., Rajaei, Z., Tehranipour, M. and Monavar, N.: Pharmacogn. Mag., 7: 338-343 (2011).
[32] Byahatti, V.V., Pai, K.V. and D'Souza, M.G.: Anc. Sci. Life, 30: 14-17 (2010).
[33] NikoliČ, N.Č., StojanoviČ, J.S., Lazic, M.L., Karabegovic, I.T., Stojicevic, S.S., Stojanovic, G.S.: 6th Central European Congress on Food, CEFood2012. Proceedings of 6th Central European Congress on Food, pp 29-33, May 23-26, Novi Sad, Srbija (2012).
[34] Polterait, O.: Current Org. Chem., 1: 415-440 (1997).
[35] Mandal, P., Sinha Babu, S.P. and Mandal, N.C.: Fitoterapia, 76: 462-465 (2005)
[36] Calixto, J.B., Yunes, R.A., Neto, A.S., Valle, R.M. and Rae, G.A.: Braz. J. Med. Biol. Res., 17(3-4): 313-321 (1984).
[37] El-Shafae, A.M. and Die Pharmazie, 53(9): 640-643 (1998).
[38] Sadraei, H., Asghari, G., Hekmatti, A.A.: J. Ethnopharmacol., 86(2-3):187-190 (2003).
[39] Sethiya, N.K., Brahmbhat, K., Chauhan, B. and Mishra, S.H.: Indian J. Traditional Knowledge, 16(2): 303-309 (2017).
[40] Cimanga, R.K., Mukenyi, P.N.K., Kambu, O.K., Tona, G.L., Apers, S., Totté, J., Pieters, L. and Vlietinck, A.J.: Journal of Ethnopharmacology,127(2, 3): 215-220 (2010).
[41] Calixto, J.B., Santos, A.R., Cechinel Filho, V., Yunes, R.A.: 18: 225-258 (1998).
[42] Saha, S. and Verma, R.J.: Pharm. Biol., 53: 16-30 (2015).
[43] Garjani, A., Afrooziyan, A., Nazemiyeh, H., Najafi, M., Kharazmkia, A. and Maleki-dizaji, N.: BMC Complement Altern. Med., 9: 1-9 (2009).
[44] Khajavi-Rad, A., Hajzadeh, M., Rajaei, Z. and Sadeghian, M.H.: Avicenna J. Phytomed., 1: 14-23 (2011).
[45] Żlhan M., Ergene, B., Süntar, I., Özbilgin, S., Ēitošlu, G.S., Demirel, M.A, Keleț, H., Altun, L., and Akkol, E.K.: Evidence-Based Complementary Altern. Med., Volume 2014, Article ID 578103, 10 pages, http://dx.doi.org/10.1155/2014/578103 (2014).
[46] Ashok, P., Koti, B.K. and Vishwanathswamy, A.H.M.: Indian J. Pharmacol., 42(6): 380-383 (2010).
[47] Gebhardt, M.: Fortschr, Urol. Nephrol. 14(1979): 34-40 (1979).
[48] Dulce, H.J.: Urologe A 17 (4): 254-257 (1978).
[49] Anna, F., Przemys³aw, S., Wies³aw, G., Ewa, Z., Anna, C., Roman, G.: Eur. J. Med. Chem., 45, 1001. (2010).
[50]Ahmed, M.S., El Tanbouly, N.D., Islam, W.T., Sleem, A.A., El Senousy, A.S.: Phytother. Res., 19: 807-809 (2005).
[51] Xu, J., Li, X., Zhang, P., Li, Z.L., Wang, Y.: Arch. Pharm. Res.: 28: 395-399 (2005).
[52] Comalada, M., Ballester, I., Bailon, E., Sierra, S., Xaus, J., Gćlvez, J., de Medina, F.S. and Zarzuelo, A.: J. Biochem. Pharmacol., 72: 1010-1021 (2006).
[53] Nair, M.P., Mahajan, S., Reynolds, J.L., Aalinkeel, R., Nair, H., Schwartz, S.A. and Kandaswami, C.: Clin. Vaccine Immunol., 13: 319-328 (2006).
[54] Raihan, S.Z., Monir, M.M., Biswas, P., Biswas, S.K., Chowdhury, A., Das, J. and Das, AC.: J. Biological. Sci., 12(3): 202-205 (2012).
[55]Ghodasara, J., Pawar, A., Deshmukh, C. and Kuchekar, B.: Pharmacog. Res., 2(6): 388-392 (2010).
[56] Rad, A.K., Hajzadeh, M.A.R., Rajaei, Z., Sadeghian, M.H. and Keshavarzi, Z.: Avicenna J. Phytomed., 1: 14-23 (2011).
[57] Pareta, S.K., Patra, K.C., Mazumder, P.M. and Sasmal, D.: J. Pharmacol. Toxicol., 6: 321-332 (2011).
[58] Manthey, J.A.: Microcirculation, 7(6 Pt 2): S29- S34 (2000).