Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 1 pp. 129–135, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

DIURETIC ACTIVITY OF AQUEOUS EXTRACT OF *NIGELLA SATIVA* IN ALBINO RATS

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Abstract: The study aims to evaluate the diuretic effect and acute toxicity of a crude aqueous extract of *Nigella sativa* using animal models. To evaluate the diuretic activity of the plant, Albino rats were divided into five groups. The control group received normal saline (10 mL/kg), the reference group received furosemide (10 mg/kg) and the test groups were administered different doses (i.e., 10, 30 and 50 mg/kg) of the crude extract by intra-peritoneal route, respectively. Graph Pad Prism was used for the statistical analysis and p-values less than 0.05 were considered statistically significant. We observed significant diuretic, kaliuretic and natriuretic effects in the treated groups in a dose dependent manner. However, urinary pH remained unchanged during the course of the study. The diuretic index values showed good diuretic activity of the crude extract. The Lipschitz values demonstrated that the crude extract, at the dose of 50 mg/kg, showed 46% diuretic activity compared with furosemide. With regard to the acute toxicity study, no lethal effects were observed among Albino mice even at the higher dose of 5000 mg/kg. The extract of *Nigella sativa,* at the dose of 50 mg/kg, significantly increased the urinary volume and modified the concentration of urinary electrolytes, and there was observed no signs of acute toxicity associated with the crude extract. Further studies are encouraged to isolate the pure phytochemical responsible for diuresis.

Keywords: *Nigella sativa*, saliuretic, natriuretic, Lipschitz value, diuretic index, Na^+/K^+ ratio

Herbal plants as medicines are still the mainstay of health care in several developing as well as underdeveloping countries where people mostly rely on local herbs for food and cure of different ailments. The practice of traditional medicine is widespread in China, Japan, Pakistan, Sri Lanka and Thailand. In Pakistan, the local communities of different regions have centuries old knowledge and traditional practices of most of the plants occurring in their regions (1). This indigenous knowledge of plants has been transferred from generation to generation through oral communication and personal experiences (2). In early 1950's, up to 84% of Pakistani population relied on indigenous medicines for traditional health practices (3), but now this practice is only limited to the remote areas due to assorted reasons which are beyond the scope of this paper. *Nigella sativa* (family Ranunculaceae) (Ns) commonly known as Black Cumin is an annual herb that grows in the Mediterranean region and Western Asian countries including Pakistan. Black Cumin has been used as a natural healing aid in various cultures and civilizations around the world, as well as a supplement to help maintaining good health (4).

Seeds of Ns are traditionally used as aromatic, diuretic, diaphoretic, stomachic, carminative, condiment, liver tonic and digestive aid (5, 6). Many studies have been conducted to scientifically prove the effects of Ns on cardiovascular and renal system. The oil of Ns has profound cardiovascular depressant effects, potent centrally acting antihypertensive and cardioprotective properties (7). The seed extract of Ns has also been proved to have renoprotective effects in a number of animal models (8, 9). Additionally, it has been demonstrated to have antioxidant, free radical scavenger and antibacterial activities against Gram-positive and Gram-negative bacteria (10, 11).

The aim of our study was to evaluate the diuretic properties of the aqueous extract of Ns seed in normal Albino rats. We also aimed to study the effect of the crude drug extract on the excretion of urinary electrolytes. Finally, the acute toxicity of the aqueous crude extract was studied in Albino mice.

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EXPERIMENTAL

Collection of plant material

About 600 g of dried seeds of Ns were purchased from the local market of Bahawalpur, Pakistan, and the well identified sample was deposited in the herbarium of Pharmacology laboratory at the Pharmacy Department, the Islamia University of Bahawalpur (IUB), Pakistan, and a voucher no. NS-SD-06-10-002 was allotted to the seeds for future reference.

Aqueous extraction

After removing the foreign material, the seeds were ground with an electric grinder (National, MJ-176NR, China) into a coarse powder. Approximately, 0.5 kg of ground material was soaked in hot boiling water (1 L) at room temperature (23–25^{o}C) for 3 days with occasional shaking followed by filtration (muslin cloth and filter paper [Whatman, Grade 1]). This procedure was repeated three times. Finally, the resultant filtrate was evaporated in a rotary evaporator (Heidolph Laborota 4000 efficient, Germany) under reduced pressure (-760 mm Hg) to a thick, semi-solid pasty mass of dark greenish color (the crude extract). The yield of the crude extract of *Nigella sativa* (Ns.Cr.) was about 8%. Ns.Cr. was completely solubilized in distilled water and normal saline for use in *in vitro* and *in vivo* experiments (12).

Experimental controls

A loop diuretic (Furosemide; Lasix, Aventis Pharma, Pakistan), was used as positive control (reference drug) and 0.9% sodium chloride (Merck, Germany) was used as control drug, respectively.

Animal housing and treatment

Animals (Albino rats and mice) were kept in polycarbonate cages (Techniplast, Italy), and were housed under the standard conditions of temperature, humidity and dark/light cycles (12 h/12 h). The animals were given pelleted food and drinking water *ad libitum*. The bedding of the animal cages was changed after every 48 h. Ns.Cr. was given by intraperitoneal (*i.p.*) route for the diuretic activity, and by the oral route for the acute toxicity study (13). Three days prior to the experimentation, the animals were caged daily for 6 h in the metabolic cages for acclimatization to the experimental conditions. The animals were also kept in an isolated area away from the normal flow of the students to avoid the stress and other psychological effects which may influence diuresis.

Phytochemical screening

The standard procedures were used to test the presence of a variety of secondary metabolites (alkaloids, flavonoids, saponins, anthraquinones, coumarins, and tannins) in the crude aqueous extract (12–14).

Diuretic activity

Adult Albino rats of either sex, weighing 200–220 g, were divided into five groups of six animals each. Prior to experimentation, the animals were screened for any visible signs of disease, and only the healthy animals were selected for the study. The study was performed at a normal room temperature (25 ± 2^{o}C). Before the administration of the extract/controls, the bladder of the rat was emptied by gentle compression of pelvic area and pulling of tails. Group I (the control group) was given 10 mL/kg of normal saline, Group II (the reference group) was given 10 mg/kg of furosemide and the test groups (III, IV and V) were administered different doses of Ns.Cr., respectively. All the doses were prepared in the same volume of normal saline in order to ensure that each animal received the same volume of liquids. Owing to ease of administration and freedom to administer large volume of fluids, the *i.p.* route was used for the administration of extract/controls. Immediately after administration, the animals were placed in metabolic cages (one animal per cage), specially designed to separate urine and feces. The urine, collected in graduated vials, was measured at the end of 6 h and expressed as mL/100 g of body weight per 6 h (12, 15). The animal handling protocol was approved by board of advanced studies under the registration number of IU/125 M.Phil/2009.

Urinary parameters

The concentration of Na^{+} and K^{+} ions in the fresh urine samples was estimated using calibrated flame photometer (Corning 410, UK) and was expressed in parts per million (ppm). Before estimating the electrolyte levels, the samples were filtered to remove debris and shedding (16, 17).

A calibrated pH meter (WTW-Series pH-720) was used to measure the pH of the fresh urine samples (12).

Calculation of diuretic index, Lipschitz value, saliuretic index and Na^{+}/ K^{+} ratio

The following formulas were used for the calculation of different urinary parameters (16, 17):
diuretic index = Mean urine volume of the test group/Mean urine volume of the control group;

Lipschitz value = Mean urine volume of the test group/Mean urine volume of the reference group;
saliuretic index = Concentration of electrolyte in urine of the test group/Concentration of electrolyte in urine of the control group;
Na^+/K^+ ratio = Concentration of Na^+ in urine of a group/Concentration of K^+ in urine of the same group.

Acute toxicity

Albino mice of 18–25 g body weight were used to study acute toxicity of Ns.Cr. The animals were divided into five groups of five mice each. The control group of mice was administered normal saline (10 mL/kg), while other groups received increasing doses of extracts, up to 5000 mg/kg. All the treatments were administered by oral gavage.

After administration, the animals were observed closely for 2 h, and then at 30 min intervals for 6 h for any visible sign of toxicity (i.e., salivation, lachrymation, ptosis, squinted eyes, writhing, convulsions, tremors, yellowing of fur, loss of hair), stress (i.e., erection of fur and exopthalmia), behavioral abnormalities (i.e., impairment of spontaneous movement, climbing, cleaning of face and ataxia, and other postural changes), aggressive behavior (i.e., biting and scratching behavior, licking of tail, paw and penis, intense grooming behavior and vocalization) and diarrhea. Mortality of the animals was noted at the end of 24 h (12, 13).

Data analysis

The data were expressed as the mean ± standard error of the mean (S.E.M.). Student *t*-test was applied to test difference among the groups, and p-values less than 0.05 were considered significant. We used Graph Pad Prism (Graph PAD, San Diego, USA) for statistical analysis.

RESULTS

Phytochemical screening

The preliminary qualitative phytochemical analysis of Ns.Cr. revealed that it was positive for

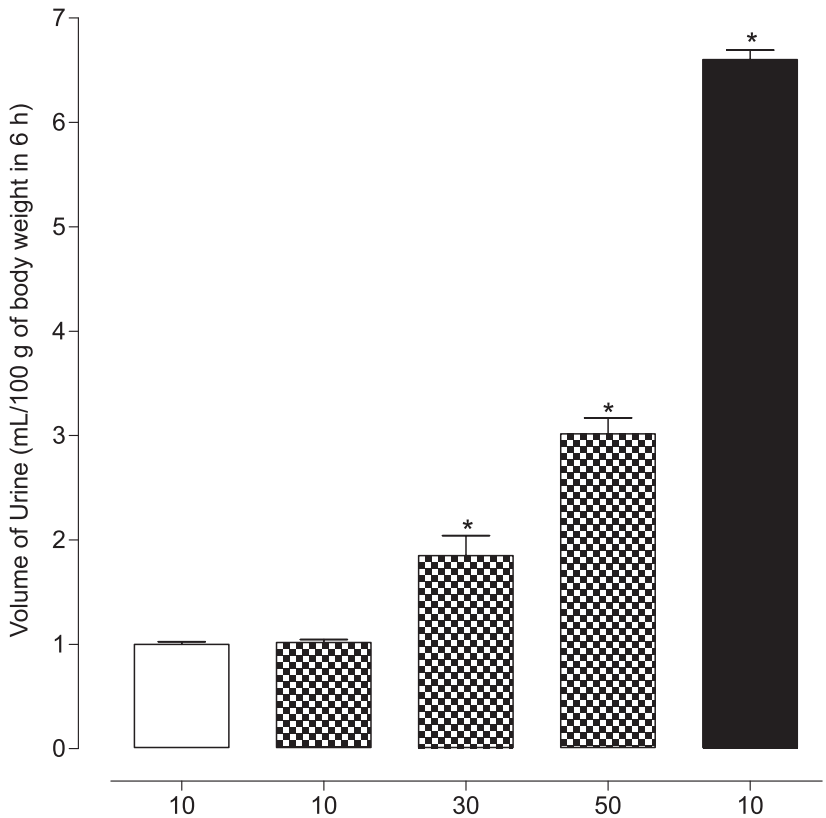

Figure 1. The effect of the Ns.Cr. on urination in Albino rats. Values shown are the mean ± SEM of six observations and the values are compared with control group and considered significant as *$p < 0.05$. ☐ = Normal saline [mL/kg]; ▩ = Ns.Cr [mg/kg]; ■ = Furosemide [mg/kg]

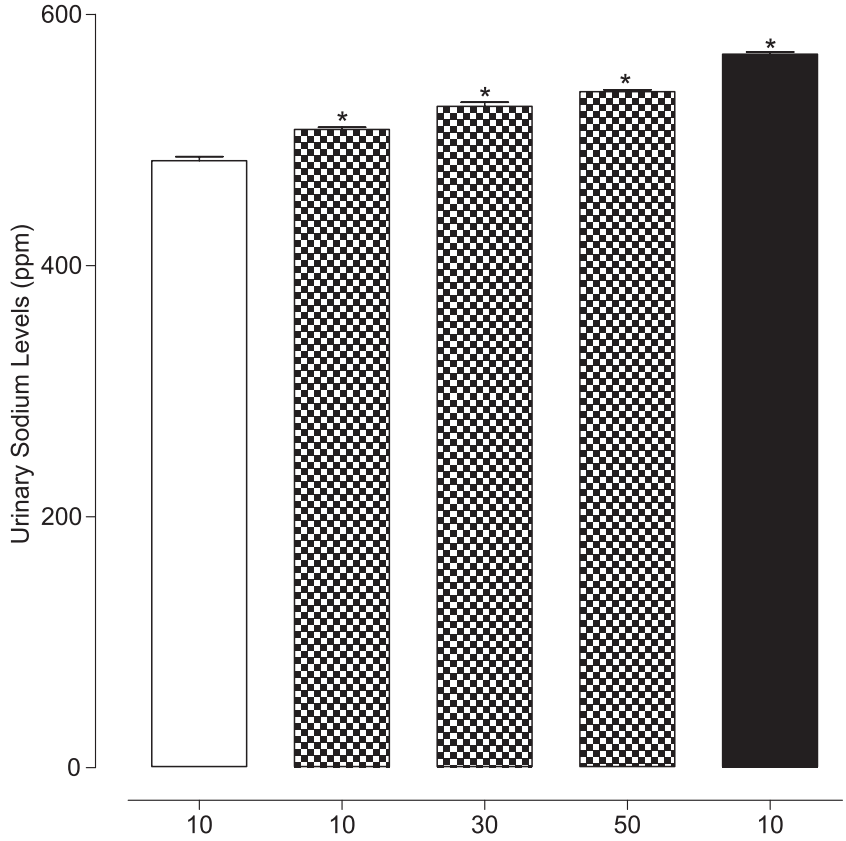

Figure 2. The effect of the NS.Cr. on urinary sodium levels in urine. The values shown are the mean ± SEM of six observations and the values are compared with the control group and considered significant at *p < 0.001. ▭ = Normal saline [mL/kg]; ▩ = Ns.Cr. [mg/kg]; ■ = furosemide [mg/kg]

Table 1. Effect of *Nigella sativa* aqueous extract on urinary volume and electrolyte concentration.

Group	Extract & dose [mg/kg]	Volume of urine [mL/6 h]	Urine sodium [ppm]	Urine potassium [ppm]	pH	Diuretic index	Lipschtiz value	Saliuretic index		Na^+/K^+
								Na	K	
1	**Normal saline 10 [mL/kg]**	1.0 ± 0.3	483.3 ± 3.3	26.3 ± 0.2	7.0	——-	——-	——-	——-	18.38
2	**Furosemide 10**	*6.5 ± 0.1	*568.3 ± 1.7	*48.8 ± 0.5	7.8	6.5	——-	1.18	1.86	11.64
3	**Ns.Cr. 10**	1.0 ± 0.1	*508.3 ± 1.6	*42.1 ± 0.1	6.9	1.0	0.15	1.05	1.60	12.07
4	**Ns.Cr. 30**	*1.9 ± 0.2	*526.7 ± 3.3	*47.1 ± 0.1	6.9	1.9	0.29	1.09	1.79	11.18
5	**Ns.Cr. 50**	*3.0 ± 0.1	*538.3 ± 1.7	*47.8 ± 0.1	7.0	3.0	0.46	1.11	1.81	11.26

Values given are the mean ± SEM of six observations. All values are compared with control group and considered significant at *p < 0.001.

alkaloids, anthraquinones, flavonoids, saponins and tannins, while negative for coumarins.

Diuretic effects

The *i.p.* administration of Ns.Cr. increased the urinary flow in a dose-dependent manner (Fig. 1). When compared with the control group, 1.9 and 3.0 fold increase in urine output was observed in the group IV and V, respectively.

The diuretic index values of the test groups (group III, IV and V) were 1.0, 1.9 and 3.0, respectively, which indicated a good diuretic activity at the dose of 50 mg/kg (Table 1). The Lipschitz values demonstrated that, at the doses of 10, 30 and 50

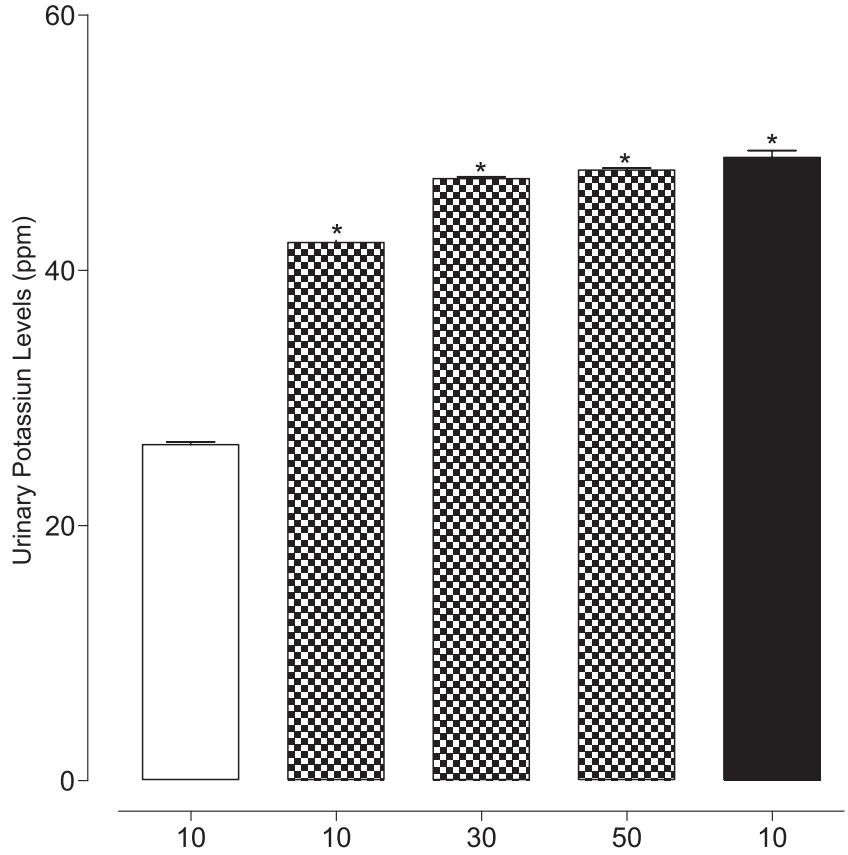

Figure 3. The effect of the NS.Cr. on urinary potassium levels in urine. The values shown are the mean ± SEM of six observations and the values are compared with the control group and considered significant at ***p < 0.001. ☐ = Normal saline [mL/kg]; ▩ = Ns.Cr. [mg/kg]; ■ = furosemide [mg/kg]

mg/kg, Ns.Cr. showed 15%, 29% and 46% of diuretic activity, respectively, compared with furosemide (Table 1).

Urinary electrolyte and pH

Ns.Cr. produced significant natriuretic effects in a dose dependent manner, especially at the dose of 50 mg/kg (Fig. 2).

A dose-dependent increase in the excretion of urinary potassium was observed after the *i.p.* administration of Ns.Cr. (Fig. 3). Especially, at the dose of 50 mg/kg, the concentration of potassium excretion was almost the same compared to the furosemide treated group. The saliuretic index values also showed a stepwise increase in the excretion of Na^+ and K^+ in the urine samples of the treated groups. The Na^+/K^+ ratio was also calculated to estimate the degree of excretion of the electrolytes with respect to each other. The values of Na^+/K^+ ratio showed that, with the increase in dose, K^+ excretion was relatively higher than Na^+.

The pH of fresh urine samples in control and treated groups was not significantly different (Table 1).

Acute toxicity

In the acute toxicity study, no visible signs of toxicity or any other abnormal behavior were observed in the test animals even, at the dose of 5000 mg/kg of body weight.

DISCUSSION

Due to a broad range of medicinal uses and extremely safe nature of Ns, it is studied extensively for a variety of secondary metabolites, and is scientifically proved to have proteins, carbohydrates, fats, mucilage, alkaloids, flavonoids, glycosidal saponins, tannins, resins, bitter principles and minerals (18). Our preliminary phytochemical investigation also confirmed the presence of alkaloids, anthraquinones, flavonoids, saponins and tannins in

the Ns.Cr, which was in accordance with the above mentioned studies. Alkaloids, flavonoids, saponins and organic acids have been shown to produce diuresis (13, 19), which indicates that, in our study, the diuretic activity of Ns.Cr. might be due to these secondary metabolites.

Herbal medicine, which is also called as botanical medicine, is the use of herbs as a medicine. Herbal plants produce and contain different classes of chemical substances eliciting a variety of pharmacological actions. Herbalists use the fruits, flowers, leaves, roots, stems and seeds of the plants for the prevention and treatment of various disorders. It is a fact that many well known treatments of the twentieth century were developed from the plants. Today, science has explored the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in the pharmaceutical preparations. For example, vincristine (an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator) were all originally discovered through research on plants (20).

In the developed as well as in developing countries, still the use of medicinal plants for the cure of different ailments is a common practice. In some cases, extracts of medicinal plants are used by the traditional healers for renal disorders (21). A number of different botanical medicines have shown the promise in the treatment of a variety of urologic disorders and around 85 species of plants belonging to diverse families have been demonstrated to have diuretic activity. In addition, the Commission E approves at least 12 plant extracts as diuretics, indicating the need of further detailed studies of natural herbs for the treatment of different ailments (22).

It is now evident through research that the renal dysfunction is a common comorbidity accompanying the uncontrolled hypertension. By controlling high blood pressure, progression of renal disease may be halted. Likewise, the antioxidants are scientifically proven to have renoprotective effects in numerous animal models and may be administered as an adjuvant therapy in hypertensive patients with compromised renal function.

Within this context, plants like *Nigella sativa,* which contains secondary metabolites with diuretic and antioxidant activities (23), are expected to be the ideal candidates for the treatment of hypertension associated with renal disorders. The results of our study showed that Ns.Cr. have dose dependent diuretic effects. Studies have shown that the crude herbal extracts have dual activity at different doses due to the presence of a variety of phytochemical constituents acting on different components of the biological system (24).

The diuretic activity of an extract is considered to be good if the diuretic index values of treated groups are greater than 1.50 (13, 19). In our study, the diuretic index values of the treated groups (III, IV and V) were 1.0, 1.9 and 3.0, respectively. This indicates 3-fold increase in the urine volume in the Group V. Lipschitz values also showed that, at the maximal dose (50 mg/kg), the plant showed 46% of diuretic activity compared with furosemide. The mild diuretic activity of crude extract, as compared to the standard drug, necessitates further fractionation and isolation of pure secondary metabolite(s) responsible for the diuretic activity of this plant extract.

An excess of Na^+ in body fluids is considered to be one of the important external factors in idiopathic hypertension and one of the main reasons for the deformities of arterial blood pressure (25). Our study showed that, compared with the saline treated group, the *i.p.* administration of Ns.Cr. produced significant natriuretic effects in a dose dependent manner. This finding highlights the medicinal use of Ns.Cr in the control of hypertension. However, at the dose of 50 mg/kg, the amount of Na^+ excretion was significantly lower than that of the reference standard indicating a lower probability of inducing hyponatremia. Likewise, the excretion of K^+ in the urine was also significantly increased in a dose-dependent manner but it is noteworthy that concentration of K^+ in the urine of the treated groups, especially at the dose of 50 mg/kg, was the same as that of the reference treated animals, reflecting the loop diuretic like activity of our extract.

CONCLUSION

Combined with published antioxidant and renoprotective effects of Ns (10, 11, 23), the present study indicates that Ns.Cr. has strong potential to be used as a diuretic agent. However, activity guided fractionation is required to separate the potent diuretic phytochemical constituents.

Acknowledgment

We would like to thank Professor Dr. Karamat Mehmood at Department of Chemistry, IUB for his valuable contribution in phytochemical analysis. We are extremely thankful to the laboratory staff at the Department of Pharmacy, IUB, for the supply of chemicals, reagents and animals. The bench work

was performed at the Department of Pharmacy, the Islamia University of Bahawalpur, Punjab, Pakistan.

Conflict of interest: Not declared.

REFERENCES

1. Alam N., Shinwari Z.K., Ilyas M., Zahid U.: Pak. J. Bot. 43, 773 (2011).
2. Shinwari Z.K.: J. Med. Plants Res. 4, 161 (2010).
3. Hocking G.M.: Pakistan Medicinal Plants. T. Qual. Plant. Mater. Veg. 5, 145 (1958).
4. Abu-Zinadah O.A.: J. King Abdulaziz Univ. Sci. 21, 335 (2009).
5. Usmanghani K., Saeed A., Alam, M.T.: Indusyunic Medicine: Traditional Medicine of Herbal, Animal and Mineral Origin in Pakistan. p. 310–311. B.C.C. and T. Press, University of Karachi, Pakistan 1997.
6. Gilani A.H., Jabeen Q., Khan A.U.: Pak. J. Biol. Sci. 7, 441 (2004).
7. Ebru U., Burak U., Yusuf S., Reyhan B., Arif K., Faruk T.H., Emin M. et al.: Basic Clin. Pharmacol. Toxicol. 103, 574 (2008).
8. Ragheb A., Attia A., Eldin W. S., Elbarbry F., Gazarin S., Shoker, A.: Saudi J. Kidney Dis. Transpl. 20, 741 (2009).
9. Yaman I., Balikci E.: Exp. Toxicol. Pathol. 62, 183 (2010).
10. Khan M. A., Ashfaq M. K., Zuberi H. S., Mahmood M. S., Gilani A.H.: Phytother. Res. 17, 183 (2003).
11. Sen N., Kar Y., Tekeli Y.: J. Food Biochem. 34, 105 (2010).
12. Asif M., Jabeen Q., Abdul Majid A.M.S., Atif M.: Pak. J. Pharm. Sci. 27, 1811 (2014).
13. Asif M., Atif M., Malik A.S.A., Dan Z.C., Ahmad I., Ahmad A.: Trop. J. Pharm. Res. 12, 967 (2013).
14. Tona L., Kambu K., Ngimbi N., Cimanga K., Vliellnck A.J.: J. Ethnopharmacol. 61, 57 (1998).
15. Asif M., Atif M., Sulaiman S.A.S., Hassali M.A., Shafie A.A., Haq N., Saleem F.: Value Health 15, A644 (2012).
16. Sathianarayanan S., Jose A., Rajasekaran A., George R.M., Chittethu A.B.: Phytomedicine 2, 7 (2011).
17. Danamma K.A.K., Jayasimha B.G., Basha S.N.: Int. J. Pharm. Biol. Sci. 1, 160 (2011).
18. Shabina I., Muhammad A., Muhammad Q.H., Mudassir A. Int. J. Agri. Bio. 15, 1151 (2013).
19. Patel U., Kulkarni M., Undale V., Bhosale A.: Trop. J. Pharm. Res. 8, 215 (2009).
20. Srichaikul B., Supachai S., Gordon B., Sunthorn D., Saksurn J.: Advan. Natur. Sci. 5, 11 (2012).
21. Yarnell E.: World J. Urol. 20, 285 (2002).
22. Clare B.A., Conroy R.S., Spelman K.: J. Altern. Complement. Med. 15, 929 (2009).
23. Rastogi L., Feroz S., Pandey B.N., Jagtap A., Mishra K.P.: Int. J. Radiat. Biol. 86, 719 (2010).
24. Mehmood M.H., Aziz N., Ghayur M.N., Gilani A.H.: Dig. Dis. Sci. 56, 1460 (2011).
25. Hoareau L., DaSilva E.J.: Electron. J. Biotechnol. 2, 56 (2009).

Received: 27. 11. 2013