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## Assessment of the Effect of *Pycnocycla spinosa* Hydroalcoholic Extract on Rat Renal Function

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**Abstract:** Previous studies show that the hydroalcoholic extract of *Pycnocycla spinosa* is a relaxant of rat ileum and at the dose of 1 mg kg<sup>-1</sup> inhibits diarrhoea in mice. Other studies indicate that *P. spinosa* extract has a selective pharmacological action to inhibit diarrhea. To use the *P. spinosa* extract as antidiarrhoeal, it is necessary to know the toxic effect on the kidney or live functions. Therefore, the aim of this study was to investigate the effect of oral administration of *P. spinosa* extract on renal function. Three groups of rats were orally given *P. spinosa* extract (1 and 10 mg kg<sup>-1</sup> and vehicle) and then creatinine, uric acid, urea nitrogen and total protein concentration was measure in the serum and urine 24 h after treatment. Another three groups of rats were treated daily with similar doses of the extract for 14 successive dosing days and the same parameters were assessed. The results show that *P. spinosa* extract with dose of 1 mg kg<sup>-1</sup> has no effect on serum and urine biochemical parameters such as urine creatinine, uric acid, urine nitrogen and total protein concentration after 24 h or 14 successive dosing days. At the dose of 10 mg kg<sup>-1</sup> of *P. spinosa* extract, which is ten-times more potent than antidiarrhoeal dose, the urine total protein likely increased while no effects on other biochemical parameters were found. Therefore, from this study it could be concluded that the oral administration of *P. spinosa* extract at the antidiarrhoeal dose has no effect on rat renal function.

**Key words:** *Pycnocycla spinosa*, urea nitrogen, creatinine, renal function

### INTRODUCTION

*Pycnocycla spinosa* Decne. exBoiss. var. *spinosa* (Fam. Umbelliferae) is a wild plant growing in Iran (Asghari, 2001, 2002). It is shown that the hydroalcoholic extract of *P. spinosa* is a potent relaxant (IC<sub>50</sub> = 40±7.3 µg mL<sup>-1</sup>) of isolated ileum (Sadraei, 2003a, b). In addition, *P. spinosa* extract was shown to have antidiarrhoeal action at doses of 250 to 1 mg kg<sup>-1</sup> in mice (Asghari, 2001). The anti-spasmodic action of *P. spinosa* extract is very similar to that of dicyclomine (Hajhashemi, 2000) and its antidiarrhoeal dose on castor oil induced diarrhoea is very close to that of loperamide (Sairam *et al.*, 2003; Sadraei *et al.*, 2006a) and dephenoxylate (Adzu *et al.*, 2003). Therefore, *P. spinosa* extract could be an alternative remedy for treatment of gastrointestinal spasm and diarrhoea. Herbal medicine is the use of drugs found in plants for prevention and cure of disease, many plants are highly toxic and probably

presents a greater risk of adverse effects. However, if this extract is considered for clinical use, hence its possible action on other organ should be investigated to understand the toxic effects. Previous studies on blood pressure and heart rate central nervous system and behavioral activity (Sadraei *et al.*, 2006a) have shown that *P. spinosa* extract has no toxic effect on those organs. The objective of this research was to investigate the effect of *P. spinosa* extract on rat renal function by measuring a number of known renal parameters reflecting organ damage.

### MATERIALS AND METHODS

**Plant material:** The whole aerial parts of *P. spinosa* were collected from Isfahan University campus, situated on the base of *Sofeh* mountain in north of Isfahan city, Iran. A voucher specimen (A24) was authenticated and then deposited in the herbarium of Faculty of Pharmacy and

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Pharmaceutical Sciences, Isfahan, Iran. The plant was dried in shade and then grounded. Hydroalcoholic extract was obtained with 70% ethanol by percolation for 24 h at room temperature (Samuelsson, 1999). The extract was filtered and solvent was evaporated at 40°C and percentage of extract yield was calculated. Dried extract was made up as 10 mg mL<sup>-1</sup> stock solution in distilled water and further diluted with distilled water to make 1 mg mL<sup>-1</sup> solution. These solutions of *P. spinosa* hydroalcoholic extract were used for oral administration of rats.

**Animal procedure:** As a general rule, plants toxic to animals are also toxic to humans. Male Wistar rats were procured from the Pasture Institute of Iran. Animals were used in all experiments are bred in the university animal house with weighing between 180-250 g (4-5 months old). Six groups of rats were used in this study. The first and second groups were, respectively treated per orally (p.o.) with 1 mg kg<sup>-1</sup>, as a potent relaxant (IC<sub>50</sub> = 40±7.3 µg mL<sup>-1</sup>) and 10 mg kg<sup>-1</sup>, as a potent toxic, of *P. spinosa* extract by a feeding tube. The third group was used as control and received equivalent volume of the vehicle. Following administration of the extract each rat was separately placed in a metabolic cage for 24 h with free access to food and water. The urine was collected over 24 h (1-1.5 mL) and urine output was measured and then centrifuged at 1000 grv over 15 min. An aliquot of the clear supernatant was frozen for biochemical analysis. Twenty four hours after treatment, animals were anaesthetized by ether and blood samples were taken followed exsanguinations. The blood sample was kept in a test tube for 15 min for clotting and then its serum was separated and centrifuged at 1000 grv over 20 min. An aliquot of the clear supernatant was frozen for biochemical analysis. Other groups of rats were treated p.o. with *P. spinosa* extract (1 and 10 mg kg<sup>-1</sup> or vehicle) every day for 14 successive days. During this period each animal was kept separately in a cage with free access to food and water. On the last day following extract administration the animal was put into a metabolic cage for 24 h and urine and blood samples were collected as describe above (Klaassen, 1996). Animal experimental procedures were approved by the University animal ethics committee.

**Biochemical analysis:** The following parameters were measure in both urine and blood samples: creatinine, urea nitrogen, uric acid and total protein. Creatinine, urea nitrogen, uric acid and total protein were measured by standard Kit purchased from Ziest Chem diagnostic (Iran). Blood total protein measurement was based on Biuret

method (Kingsley, 1942). Urine (1-1.5 mL) total protein was measured by Bradford method (Bradford, 1976) using Bio-RAD Kit set (Germany). Uric acid measurement was based on Phosphotungstate method (Reece and Hobbie, 1972). Urea nitrogen measurement was based on Berthelot reaction. The method for determination of urea nitrogen is an indirect method and based on preliminary hydrolysis of urea with urease enzyme followed by some process that quantities the ammonium ion product. Spectrophotometric approach to ammonium quantitation includes the Bethelot reaction and the enzymatic assay with glutamate dehydrogenase (Burtis and Ashwood, 1994). Creatinine measurement was based on Jaffe method (Butler, 1976). The method most widely used today, is based on the Jaffe reaction, which occurs between ceatenine and the picrate ion in alkaline medium, a red-orange color develops. Creatinine clearance was calculated by measuring urine creatinine concentration in 24 h divided by plasma creatinine concentration multiplied by urine volume over 24 h.

**Statistical analysis:** Mean and Standard Error of Mean (SEM) values were calculated for each group of results and significance of differences between the means was calculated by two-tailed unpaired Student's t-test. Differences between test and control group were considered statistically significant when p<0.05.

## RESULTS AND DISCUSSION

Dried extract looks dark green color and the yield of hydroalcoholic extract was 8% (w/w). Following administration of *P. spinosa* extracts (1 and 10 mg kg<sup>-1</sup>) there were no observable changes in general rat behavior, activity, irritability, during the course of study in comparison with the control groups. The measured biochemical parameters (creatinine, urea nitrogen, uric acid, total protein) in the control group were very close with the reference data (Loeb and Quimby, 1999). In single dose study *P. spinosa* extract (1 mg kg<sup>-1</sup>) had no significant effect on above measured parameters in blood serum, or in the urine. With dose of 10 mg kg<sup>-1</sup> *P. spinosa* extract, again there was no significant changes in the measured parameters in blood serum or urine in compared with the control group (Table 1). Chronic treatment of *P. spinosa* extract at a dose of 1 or 10 mg kg<sup>-1</sup> had no effect on concentration of serum or urine creatinine, urea nitrogen and uric acid or total protein after 14 days of dosing (Table 2). In contrast to single dose study the total protein of urine was likely increased with repeated doses of 10 mg kg<sup>-1</sup> *P. spinosa* extract (Table 2). There was no significant change in other measured urine parameters (Table 1, 2).

Table 1: The effect of single dose *P. spinosa* extract on serum and urine biochemical parameters in rat reflecting organ damage

Biochemical parameters	Creatinine	Urea nitrogen	Uric acid	Protein
	----- (mg dL <sup>-1</sup> ) -----			(g dL <sup>-1</sup> )
<b>Serum</b>				
Control	0.69±0.05	9.4±0.45	3.6±0.42	7.8±0.38
<i>P. spinosa</i> extract (1 mg kg <sup>-1</sup> )	0.51±0.06	9.6±0.26	4.4±0.52	7.2±0.30
<i>P. spinosa</i> extract (10 mg kg <sup>-1</sup> )	0.49±0.05	9.7±0.75	4.2±0.13	7.3±0.30
<b>Urine</b>				
Control	43.20±3.90	7.5±0.38	49.8±0.53	9.8±0.65
<i>P. spinosa</i> extract (1 mg kg <sup>-1</sup> )	35.60±2.20	7.1±0.37	48.9±0.57	8.2±0.82
<i>P. spinosa</i> extract (10 mg kg <sup>-1</sup> )	35.00±13.7	7.3±0.29	50.9±0.30	10.5±1.90

Rats were orally treated with single dose of *P. spinosa* extract (1 and 10 mg kg<sup>-1</sup>). Urine and serum biochemical analysis was performed after 24 h. Each data is expressed as mean±SEM (n = 6). There is no significant difference in animal treated with the *P. spinosa* extract in comparison with the control group (Student's t-test)

Table 2: The effect of repeated dosing of *P. spinosa* extract on serum and urine biochemical parameters in rat reflecting organ damage

Biochemical parameters	Creatinine	Urea nitrogen	Uric acid	Protein
	----- (mg dL <sup>-1</sup> ) -----			(g dL <sup>-1</sup> )
<b>Serum</b>				
Control	0.45±0.06	15.1±1.2	3.6±0.36	10.5±0.2
<i>P. spinosa</i> extract (1 mg kg <sup>-1</sup> )	0.38±0.05	15.0±1.1	3.2±0.33	10.8±0.2
<i>P. spinosa</i> extract (10 mg kg <sup>-1</sup> )	0.44±0.06	15.0±0.7	3.8±0.38	10.2±0.5
<b>Urine</b>				
Control	53.80±9.50	6.5±0.9	49.2±0.56	13.3±0.7
<i>P. spinosa</i> extract (1 mg kg <sup>-1</sup> )	56.90±8.20	6.5±0.7	48.6±0.62	13.3±0.6
<i>P. spinosa</i> extract (10 mg kg <sup>-1</sup> )	41.90±7.60	7.2±0.5	49.9±0.59	15.9±0.7*

Rats were orally treated for 14 successive dosing days with *P. spinosa* extract. Urine and serum biochemical analysis was performed on the last day of experiment. Each data is expressed as mean±SEM (n = 6). \*: p<0.05 in compare with the control group (Student's t-test)

Hydroalcoholic extract of *Pycnocycla spinosa* in doses of 0.25, 0.5 and 1 mg kg<sup>-1</sup> inhibits castor oil induced diarrhoea (Sadraei *et al.*, 2003a). Antidiarrhoeal activity of *P. spinosa* extract is seen with doses that are close to oral dose of loperamide in animal studies (Sadraei *et al.*, 2006a). Thus, *P. spinosa* extract looks like an alternative herbal remedy for treatment of diarrhoea, provided it has no toxic effect on biological function. There had been a number of reports on effect of *P. spinosa* extract on bladder, uterine and cardiovascular function (Sadraei *et al.*, 2006a; Samuelsson, 1999; Klaassen, 1996), all of which indicate that extract of *P. spinosa* is not toxic. Study of nervous system function also shows that *P. spinosa* extract at antidiarrhoeal dose and with dose of 10 mg mL<sup>-1</sup> has no effect on animal behavior treated with extract over 3 week (Sadraei *et al.*, 2006b, c). Acute LD<sub>50</sub> of the extract also indicate a large margin of safety (Sadraei *et al.*, 2006c). In this research, we have studied possible effect of *P. spinosa* extract on a number of blood

serum and urine chemical parameters including creatinine, uric acid, urea nitrogen and total protein. Any changes in these parameters may indicate a change in renal function. Animals were treated orally with either 1 or 10 mg kg<sup>-1</sup> of the extract. Dose of 1 mg kg<sup>-1</sup> is an antidiarrhoeal dose of the extract in animal model, while dose of 10 mg kg<sup>-1</sup> is 10 times greater than the dose of the extract that has maximum inhibitory action on castor oil induced diarrhoea (Hajhashemi *et al.*, 2000; Sadraei *et al.*, 2003a). In this study there was no significant increase in the level of serum creatinine, urea nitrogen, uric acid and total protein in single dose study or repeated dose study in comparison with the control groups. Total protein was increased likely in the urine of the rats, which were treated with high dose of *P. spinosa* extract (10 mg kg<sup>-1</sup>) while there were not significant changes in creatinine, uric acid and urea nitrogen in the urine. Generally, there are four ways in which proteinuria can occur: increased glomerular permeability, in which the urinary protein is mainly albumin; defective tubular reabsorption, in which the urinary protein is mainly normal, low-molecular weight plasma protein; over flow from plasma to urine of an abnormal low-molecular weight protein and production protein by the urinary tract. As total protein was only increased in urine but no in the serum it may indicate that high dose of *P. spinosa* extract, which was 10 times more than that of therapeutic dose, may affect the renal function which demands further investigation. There was no significant change in the urine total protein and other measured parameters with antidiarrhoeal dose of *P. spinosa* extract (1 mg kg<sup>-1</sup>). Therefore it may be concluded when *P. spinosa* extract is used orally for treatment of diarrhoea it won't affect serum and urine concentration of creatinine, uric acid, urea nitrogen and total protein, therefore, no toxic effects on renal function. Our results show that there is no significant change in creatinine clearance when rats treated with of *P. spinosa* extract. Renal clearance of creatinine is usually used to assess kidney function; therefore, the doses of 1 and 10 mg kg<sup>-1</sup> have likely no effect on renal function.

The *P. spinosa* extract at the dose of less than 1 mg kg<sup>-1</sup> could be used as drug for prevention and cure of diarrhea. However, the *P. spinosa* extract could be considered for clinical use, if the risks of adverse effects on other organs such as liver will be under investigations.

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