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ANTINOCICEPTION AND LOCOMOTOR IMPAIRMENT INDUCTION BY METHANOLIC EXTRACT OF URTICA DIOICA

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Running title: Locomotor impairment by of *Urtica dioica*

ABSTRACT

Urtica dioica (UD), as anti-inflammatory and analgesic, has been used by people in Iran since long. The aerial parts of plant containing active materials have been utilized in the prostate proliferative disease. In the present studies Methanolic extracts of *Urtica dioica* were prepared by maceration. Antinociceptive activity of the extract was determined by hot plate and writhing tests in mice. Methanolic extract of UD at 50 and 100 mg/ kg could produce 59% and 98% writhing inhibition respectively. Morphine (5 mg/kg) with 91% writhing inhibition showed less analgesic activity than UD (100 mg/kg). Number of writhings was decreased by diclofenac (50 mg/kg) in magnitude of 78% that was less than UD at 100 mg/kg ($p < 0.05$). Methanolic extract, in all test doses (25-100 mg/kg) showed a dose dependent increase in pain threshold in hot plate thermal test which were significantly higher than control. LD_{50} of UD was obtained 300 mg/kg. UD extract at 150 mg/ kg showed significant impairment in mouse coordination in the rota rod test.

Key words: Antinociceptive activity, writhing, hot plate, thermal test, rota rod test , *Urtica dioica*

INTRODUCTION

Urtica dioica (UD) possesses many therapeutic effects such as Antiviral activity (Uncini *et al.,* 2005), analgesic (Gulcin *et al.,* 2004) and hypolipidemic activities (Daher *et al.,* 2006). Also it has been used as a cardiovascular agent (Testai *et al.* 2002). In-vitro anti-oxidant effects of the *UD* have been determined (Mavi *et al.,* 2004; Gulcin *et al.,* 2004, Exarchou *et al.,* 2006). Inhibition of nitric oxide production and adenosine deaminase also has been reported (Harput *et al.,* 2005; Durak *et al.,* 2004). Even the possibility that elevated levels of prostaglandins and leukotrienes may partially involved in maintenance of the disease, suggests that compounds with anti-inflammatory action would be effective in prostatic hyperplasia (Safarinejad, 2005). *UD* also traditionally used as antidiabetic (Rau *et al.,* 2006; Otoom *et al.,* 2006; Bnouham *et al.* 2003) and as a pivotal therapy in patients with sinusitis (Helms and Miller, 2006).

UD has been used as anti-inflammatory, analgesic, antihypertensive, hypoglycemic and diuretic, in traditional medicine in Iran, too. There is no information about UD induction locomotor impairment while it is used for their therapeutic effects in different symptoms. In this study anitinociceptive property of UD methanolic extract was determined by hot plate and writhing test which is more sensitive for non- steroidal analgesics (Tita *et al.,* 2001) and. ED_{50} of the extracts and their effect on motor coordination also were obtained in mice.

There are several isolated constituents previously reported in *UD* such as: polyphenol oxidase (Gullcin *et al.,* 2005), phenolic acids, flavonoids (Fiamegos *et al.,* 2004) and flavonoid glycosides (Akbay *et al.,* 2003), isolectins (Ganzera *et al.,* 2005), lignans (Schottner *et al.,* 1997), oxalic acid and tartaric acid (Fu *et al.,* 2006), tri- and digalactosyl diglyceride (Radunz, 1976).

Because several papers that suggest biological activity in species of plants is mostly due to presence of different polyphenols and flavonoids (Kiss et al., 2006; Rauha et al., 2000; Akbay et al., 2003) we made use of determining these components in *Urtica dioica* as a simple method for standardization of crude extract samples.

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MATERIALS AND METHODS

Chemicals:

1. Quercetin (Sigma Chemical Co., St. Louis, USA). 2. Gallic acid and Folin–Ciocalteu reagent and 3.Methanol (Merk Co., Germany).

Preparation of extract:

UD aerial parts were collected from Sari in May, 2005 and confirmed by department of Pharmacognosy. A voucher specimen has been deposited in university's Herbarium (No. 91). Materials were dried at room temperature and coarsely ground before extraction. 50 g of dried powdered sample was extracted in 400 ml of methanol by maceration (48 hrs.). The resulting extract was concentrated over a rotary vacuum evaporator until a solid extract sample was obtained. The resulting crude extract was freeze-dried. The extract was prepared in normal saline for pharmacological studies.

Determination of total phenolic compounds:

Total phenolic compounds were determined using the Folin–Ciocalteu method (McDonald *et al*., 2001). 0.5 ml of the extract in MeOH (10 mg/ml) was added to 5 ml of Folin–Ciocalteu reagent (1:10 diluted with distilled water) and 4 mL sodium carbonate (1 M). The mixture was allowed to stand at room temperature for 15 min. Absorbance of resulting blue complex was measured at 765 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Methanol was used as a control in place of the sample. The contents of phenolic compounds were expressed as gallic acid equivalents (mg/g of dry mass) from a standard concentration curve.

Determination of total flavonoid content:

The $AlCl_3$ method (Chang *et al*,, 2002) was used for determination of the total flavonoid content of the sample extracts. 0.5 ml of extract in MeOH (10 mg/ml) was mixed with 1.5 ml of MeOH, 0.1 ml of 10% $AlCl_3$.$6H_2O$, 0.1 ml of potassium acetate (1M) and 2.8 ml of distilled water. The mixture was vigorously shaken. The mixture was allowed to stand at room temperature for 30 min. Absorbance of the reaction mixture was measured at 415 nm. Flavonoid contents were expressed as mg quercetin equivalent (mg/g of dry mass) from a standard concentration curve.

Animals:

Male Swiss albino mice weighing 25-30 g were used for all experiments. They were housed in groups of five under standard light (7.00 to 19.00) and temperature (22 ± 1^{o}C) with food and water *ad libitum*. The animals were transferred to the laboratory at least 1h before the start of the experiment. The experiments were performed during day (08:00-16:00 h). Each animal was used once only. Six mice were used in each experiment

Writhing test:

The abdominal constriction was induced by i.p. injection of 0.3% acetic acid (10 ml/kg) following the method of Koster *et al*. (1959). Animals were pretreated with vehicle (3 ml/kg, i.p.), *UD (25, 50, 100 mg/kg, i.p.)* 30 min before the acetic acid injection. Diclofenac (50 mg/kg i.p.) and morphine (5 mg/kg, i.p.) were used as the reference drugs. After challenges, pairs of mice were placed in separate plexiglas cages and the number of abdominal constrictions and stretches were cumulatively counted 8 min after acetic acid injection in each mouse over a period of 20 min.

Hot plate test:

This is done according to the method of Eddy and Leimback (1953) in mice. The extract of UD was given at 25, 50 and 100 mg/kg, i.p. to the animals as a single dose. Mice were placed on a thermostatically controlled hot plate apparatus (Harvard, UK) maintained at 52 ± 0.5 0C and the reaction time (time elapsed between placing the mouse on the hot plate and appearance of signs of acute discomfort) for licking or kicking of the fore or hind paws through was recorded with a stop watch. Controlled reaction time in Gr. I was recorded before start of the experiment. Mice, which did not show any reaction after 15 sec, were discarded. Reaction time before and at 15, 30, 45 and 60 min after administration of the extracts was recorded (sec). A cut-off time of 45 s was imposed to avoid tissue damage.

Motor coordination by Rota rod test:

Skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a rotating rod. Effect on motor coordination was assessed using Rota rod apparatus (Harvard, UK) at a

rotating speed of 16 rpm (Dunham and Miya, 1957). In order to evaluate the motor coordination in the animals a pretest was carried out and only those animals that demonstrated the ability to remain twice on the revolving rod for at least 45 s were selected. In fact the animals were trained to be able to remain on the rod and those animals that could not remain for 45 s periods were rejected from the experiments. Rotarod test was carried out in groups of 6 animals after intraperitoneal administration of *UD* extract at doses of 25 - 200 mg/kg. The number of falls from the rod was counted for 45 s. The animals were observed before and 15, 30, 45 and 60 min after administration of each extract.

Toxicity studies:

Toxicity studies were carried out in mice according to the method of Reddy and Byahatti (1996) and our recently published paper (Ebrahimzadeh et al., 2006). The methanol extract was tested at 100, 200 and 300 mg/kg, in groups of 6 animals, injected intraperitoneally and observed for a week.

Statistical Analysis:

One-way analysis of variance (ANOVA) for the writhing test or repeated- measures ANOVA (for the hot plate and rotarod tests) followed by Newman-Keuls multiple comparisons test, was used for statistical analysis. Differences with $p< 0.05$ were considered significant.

RESULTS

UD extract, in doses of 25-100 mg /kg, reduced the writhing count. The effect was dose dependent and showed a significant effect when compared to the control (Fig. 1). Methanol extract of *UD* at the dose of 100 mg/kg showed higher activity than diclofenac 50 mg/ kg i.p. ($p < 0.001$). *UD* extract, in all tested doses (25-100 mg /kg) significantly increased the pain threshold in hot plate thermal test (Table 1). LD_{50} of intraperitoneal injection of *UD* extracts in mice was obtained 300 mg/kg. The animals treated with *UD* extract remained on the rotating rod for 1 minute in doses of 25 - 100 mg /kg. In these doses *UD* extract did not induce any motor in coordination but *UD* extract showed statistically significant effect on the motor coordination at higher doses (150 and 200 mg/kg). The animals treated with this dose remained on the rotating rod only for 30 seconds.

DISCUSSION

UD extract inhibited the stretching episodes in a dose dependent manner in mice ($p < 0.05$). UD extract in 50 mg/kg showed 66.7% inhibition of writhings that is 26.6% and 11.4% less than morphine and diclofenac respectively. In doses higher than 100 mg/kg, UD extract had a significant effect on motor coordination in rotarod test. So it cannot be pronounced as analgesic in higher amounts than 100 mg/kg. It was shown that aqueous extract of *UD* in 100mg/kg decreases 89.2% of writhings (Gulcin et al., 2004).

66.7% inhibition of writhings was obtained by 100 mg/kg of *UD* methanolic extract in this study. Comparing methanolic extract's activity with aqueous one determines the possibility of responsibility of both methanol and water soluble compound for the analgesic activity of *UD* and it is valuable to elucidate the active substances' structures. In hot plate test *UD* extract showed a significant analgesic activity to control group ($p < 0.05$) and its effect was comparable to morphine (Table 1). In regard to LD_{50} of *UD* extract, 300 mg/kg caused 50% mortality in the animals.

The antinociceptive property of *Urtica dioica* methanolic extract was confirmed by writhing and hot plate tests in this research. This study supports *Urtica dioica* usage in folk medicine in Iran. Further studies in this respect should be useful.

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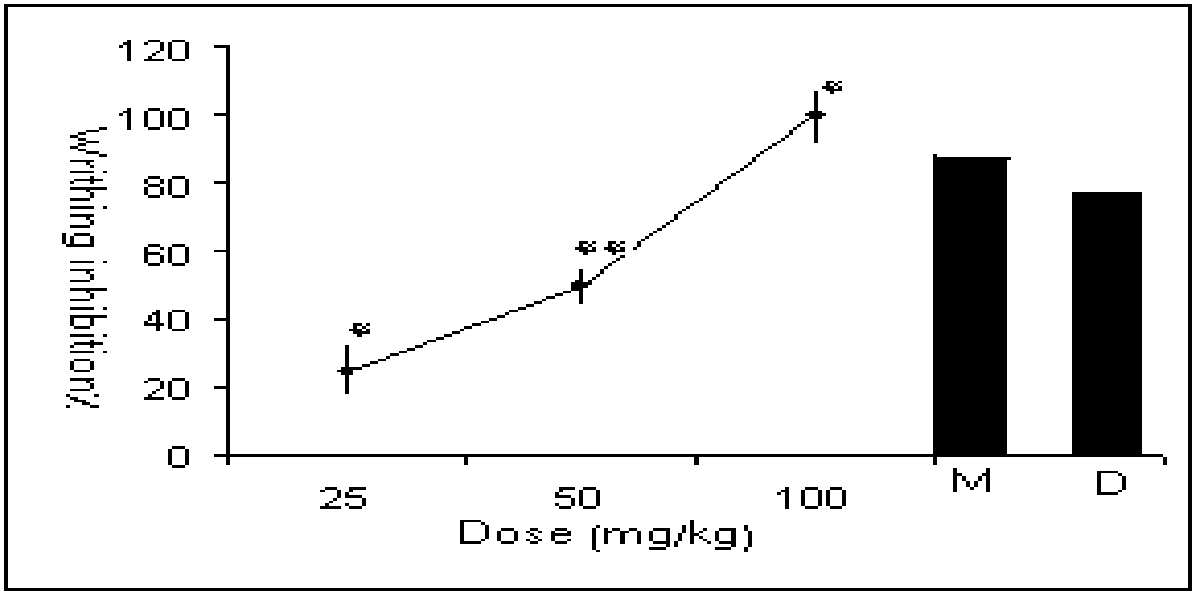

Fig. 1. Writing test. The animals (n = 6) were treated with M (morphine), D (diclofenac) or UD extracts. * p< 0.05; ** p < 0.01

Table 1. Analgesic activity of extracts of *Urticaria dioica* in mice (hot plate method).

Treatment	Dose (mg/ kg) i.p.	Response time (sec)				
		0	15	30	45	60
Control	10	6.5 ± 0.4	7.8 ± 1.2	7.2 ± 0.9	9.6 ± 1.5	8 ± 1.4
Extract	25	6.6 ± 0.3	6.9 ± 0.3 *	11.0 ± 1.2 *	12.7 ± 1.2 *	8.6 ± 0.4
	50	6.4 ± 0.8 *	8.5 ± 1.2 *	11.6 ± 1.1 *	13.6 ± 2.1 *	9.7 ± 0.7 *
	100	7.3 ± 0.6 *	12.2 ± 2.1 *	12.9 ± 2.5 *	15.15 ± 2.0*	10.0 ± 1.4 *
Morphine	5	7 ± 0.6 *	11.5 ± 1.2 *	13.1 ± 1.5 *	13 ± 0.8 *	12.2 ± 1.3*

Values are mean ± SD. (n = 6), Significantly different at * p < 0.05 with respect to control.

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