Assessment of anti-inflammatory, anti-ulcer and neuro-pharmacological activities of *Cyperus rotundus* Linn.

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Abstract: This article reports the assessment of anti-inflammatory, antiulcer and neuropharmacological activities of crude extract of *Cyperus rotundus*. The plant exhibited significant property to act as an anti inflammatory agent. In experimental design, inflammation was produced by carrageenan in rats and compare with saline treated and Aspirin treated group. Simultaneously the drug was also observed for its antiulcer response and found effective enough (these two activities were observed at the dosage of 300mg/kg and 500mg/kg). The anti ulcer activity was observed 41.2% as a dosage of 500mg/kg. Neuropharmacological activities (open field, head dip, rearing traction and forced swimming test) were also observed at 300 and 500mg/kg of *C. rotundus* extract. The crude extract showed mild decreased in all test and exhibited slight muscle relaxant effect. Powder drug studies and FTIR analysis were performed for the authentication of *C. rotundus*.

Keywords: *Cyperus rotundus*, antiulcer, anti-inflammatory.

INTRODUCTION

The pharmacological profile of *Cyperus rotundus* Linn. (nut grass; Gramineae) is characterized by stomachic, carminative, astringent, aphrodisiac, diuretic, anthelmintic, emmenagogue and relieve pain (Jaysweera, 1980; Gupta, 1993; Watt 1962). It is a perennial herb with long rhizomes (Stone 1970), leaves are linear, broadly grooved on the upper surface and dark green in colour. Flowers are small inflorescence with 2-4 bracts. The inflorescence consists of a few slender branches with the longest usually not more than about 7.5cm spikes. The nut is oblong, ovate, nearly half as long as the glume, strongly 3-angled, yellow or black when ripe (Ross 2003).

In the present study the potential existence of anti-inflammatory, antiulcer and neuropharmacological activities of crude ethanolic extract of *C. rotundus* have been carried out. The Anti-inflammatory activity of C. *rotundus* extract in rat paw edema induced by carrageenan. Anti-ulcerogenic effect of C. *rotundus* has been observed in Aspirin induced Ulcer. In the light of their use in folklore/traditional medicine as an antidepressant agent, the study has been carried out to observe the effects of C. *rotundus* on CNS. Microscopic and Fourier Transform Infrared spectroscopy (FTIR) studies have also been performed on powder of *C. rotundus* dried rhizome.

MATERIAL AND METHOD

Plant material and Chemical

The dried rhizome of *C. rotundus* was collected from the local market, identified and deposited in Department of Pharmacognosy (Voucher number A-2003-1), University of Karachi. The dried rhizomes were powdered and extracted with absolute ethanol. The extract was filtered and dried under reduced pressure at a temperature below 40ºC on a rotary evaporator (Rehman, 2007). All chemicals used in experiments were of analytical grade. Reference drugs (Aspirin, Cimetidin) were purchased from local market.

Animals

Male and female albino mice (24-28g) and rats (180-220) were purchased from the Agha Khan University animal house and were housed one week prior to start the experiment. The animals were maintained/conditioned in standard cage (5 mice/rats or mice per cage) and fed laboratory diet ad libitum and allowed free access to drinking water and kept on a 12/12h light-dark cycle. All animals were fasted 6-8 hours before dosing. Control animal received 0.9% NaCl solution in the same experimental conditions (Ahmad *et al.*, 2013a, b).

Anti-inflammatory activity

Determination of anti-inflammatory activity has been carried out by using plethysmometer (Plethysmometer 7150, Ugo Basile). The method was described by Winter *et al.* (1962). Each dose of drug extract and Aspirin has tested along with control group. After 40 min. of an oral administration of *C. rotundus* extract (300 and 500 mg/kg) to the rats, an injection of 0.1ml of 1% carrageen and suspension (Sigma Chemical Co., USA) was injected in to the sub plantar region of the right hind paw. The edema has been noted in volume size. The average

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volume of hind paw was also determined before starting the experiment. The reading noted at 0-5 hour. The results expressed along percentage of inhibition of inflammation in comparison with Aspirin (table 2). Percentage of inhibition (I) obtained for each group by using the following formula:

$$\% \text{ of } I = \frac{\text{Control - Treated}}{\text{Control}} \times 100$$

Anti-Ulcer activity

To support the significance of anti-inflammatory activity of *C. rotundus* and to find out the safety of drug against ulcer this test was done. The method adapted with slight modification as prescribed by Koley *et al.* (1994) and Umamaheswari *et al.* (2007). Rats of either sex divided in to five groups each containing five animals. Group I received saline solution 0.5ml, Group II received Aspirin 300 mg/kg, group III and IV received 500 and 300mg/kg of drug extract with Aspirin 300mg/kg, Group V received Cimetidine 40mg/kg with Aspirin 300mg/kg for 14 consecutive days. Drugs were administered through oral route to fasting rats. Animals sacrificed at the last day of dosing by the administration of large dose of ether. The stomach is removed and cut along the greater curvature to observe the gastric lesion or ulcer patches. According to severity an orbitrary scale has been followed as: 0=no lesions, 1=few small lesions, 2=no of ulcer patches/gastric ulcer less than 1 mm, 3= no of gastric lesions greater than 1 mm. Ulcer index calculated by the formula: Ulcer index =Number of ulcers+ Scoring of ulcers+ Area of ulcer in mm $x10^{-1}$.

General central nervous system activity

Open field test (Effect on locomotion)

Open field studies on rats (n=10) to observe the effects of drug on locomotion as described by Sakina and Dandiya (1990). The apparatus consists of a box, 60x 60x25cm with 16 squares drawn on the floor. The number of squares traveled by rats are noted for 10 min before drug and 45 min after the oral administration of drug.

Cage crossing movement

The effects of drug extract on 10mice have been carried out. Cage crossing activity recorded for 10min. after 30 min. of the oral administration of drug. The reading observed before and after the drug administration (Sakina and Dandiya, 1990).

Head dip test (Exploratory behavior test)

The head dip studies performed on 10 mice in a specifically designed square box having three small opening on each sides of the box. The count scored by both control and treated mice has been observed as they try to come out through these openings (Sakina and Dandiya, 1990).

Effect on muscle relaxant activity (Traction test)

The method of Boissier *et al.* (1961) followed for traction test and the mice with their forelimbs travel on a wire of 1 mm in diameter, which was stretched horizontally at a height of 35cm. This test is performed in control and test groups of mice, which were previously screened. The muscle activity has been observed in term of wire holding time after 30 min. of the oral administration of drug.

Fig. 1: Pakistani and Chinese dried rhizome of *C. rotundus*

This test procedure was used by Porsolt *et al.* (1977). The mice are exposed to forced swimming in to a transparent cage (30cm height, 15 cm diameter) filled with tap water and maintain at 35±2ºC temperature. The total time taken was 6 min. divided in to three periods each of 2 min. individual mice (control and treated) put in the vessel and the struggling time (mobility) noted by using stopwatch. Mice are considered mobile when it tries to escape from water or swim where as if it remains float in the water without any movement supposed to be immobile. Reading observed after 30 min. of drug administration. Each experimental group consists of 10 animals (Porsolt *et al.*, 1977).

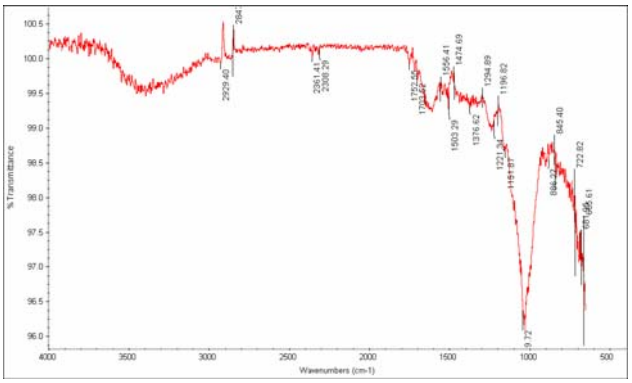

Fig. 2: FTIR analysis of *C. rotundus* powder

Powder drug study and FTIR analysis

Microscopic evaluation of powder of *C. rotundus* was carried out by the method described by Rehman (2007). FTIR analysis was carried out by using Nicolet Avatar 330 FTIR (USA) according to the method of Andrei *et al.* (2006).

STATISTICAL ANALYSIS

All data represented as mean ± standard error of mean SEM. Statistical analysis done by student's t test. Probability of 0.05 or less considered significant (Alcaraz and Jimenez 1989).

Table 1a: Anti-inflammatory effect of crude extract of *Cyperus rotundus* 300mg/kg in rats

Variation of edema with ± SEM (Time in hours)						
Group	0	1	2	3	4	5
Control	0.31±0.003	1.19±0.05	1.4±0.3	1.08±0.055	1.42±0.26	1.48±0.15
Treated 300 mg/kg (% inhibition of treated)	0.376±0.0024 (21)	0.844±0.063** (29)	1.088±0.104** (22.3)	0.914±0.09 (15.4)	0.922±0.096** (35)	1.183±0.047* (20)
Aspirin 300 mg/kg (% inhibition)	0.35±0.014 (13)	0.93±0.005* (21.8)	1.3±0.07 (7.1)	1.0±0.009 (7.4)	1.29±0.078 (9.1)	1.21±0.075 (18.2)

Table 1b: Anti-inflammatory effect of crude extract of *Cyperus rotundus* in rats

Variation of edema with ± SEM (Time in hours)						
Group	0	1	2	3	4	5
Control	2.26±0.0586	2.98±0.0601	3.79±0.082	4.18±0.0328	3.98±0.018	3.99±0.009
Treated 500mg/kg (% of inhibition)	2.01±0.257* (11)	2.26±0.184** (24)	2.59±0.034** (31)	2.66±0.121** (36)	2.95±0.052** (25.87)	2.95±0.0384* (26)
Aspirin 300 mg/kg (% of inhibition)	2.18±0.116 (3.54)	2.43±0.130* (18.5)	2.8±0.141* (25.9)	3.01±0.095* (28)	2.97±0.068* (25.56)	3.27±0.157* (17.83)

Results were expressed as mean ± SEM using student t-test at $p \leq 0.05$.

Table 2: Anti Ulcer activity of Crude Extract of *C. rotundus* in Aspirin induced Ulcer Model

Treatment	Number of Ulcer	Ulcer scoring	Ulcer area (mm^2)	Ulcer Index (% of Inhibition)
Group I Negative Control Saline	-	-	-	-
Group II Positive control (Aspirin treated)	3.8	2.8	4.8	1.14(0)
Group III CE 500mg/kg	2.7	1.5	2.5	0.67(41.2) **
Group IV CE 300mg/kg	2.5	1.6	2.9	0.7 (39) *
Group V Cimetidine 40mg/kg	2.5	1.3	2.2	0.6 (47.3) * *

Ulcer Index = No. of Ulcer + Scoring of Ulcers + Area of Ulcer in $mmx10^{-1}$

Table 3: Neuropharmacological activates of Crude extract of *C. rotundus*

Treatment Dose Orally	Mean No. of Observation ± SEM				
	Open field	Head dip	Rearing	Traction (min.)	Swimming test (min.)
Control 0.5 ml saline	120.5±7.59	44.2±5.54	110.25±6.16	3.05±0.11	4.07±0.08
CE 500 mg/kg	96.5±3.88**	30.2±1.35*	84±3.93*	3.56±0.25*	3.09±0.07*
CE 300 mg/kg	100±6.76	34.8±2.26	89.5±2.34	3.47±0.103	3.32±0.12
Daizepam 2mg/kg	85±2.12**	24.4±2.69**	68±4.77**	4.11±0.25**	2.52±0.13**

Results were expressed as mean ± SEM using student t-test at $p \leq 0.05$.

RESULTS

Anti-inflammatory

Crude extract of *C. rotundus* has been analyzed for anti-inflammatory activity and the data given in table 1a and 1b. Inhibition rate of edema as compared with Aspirin 300mg/kg. At 300 and 500mg/kg doses of crude extract the results are more effective at 2^{nd} and 4^{th} hours. The maximum percentage of inhibition (36%) observed with 500mg/kg dose. This is suggested that the protection from edema and pain stimulus are both time and dose dependent.

Anti-Ulcer activity

In this activity, positive control group received aspirin to produce gastric lesion after chronic administration. The results showed that lesions or hemorrhages observed after the chronic administration of extract of *C. rotundus* were reduced and comparable with reference drug Cimetidine (table 2).

General central nervous system activity

The results obtained are presented in table 3. These general neuropharmacological investigations showed the decrease in central nervous system activity by different assays. After an evaluation of results obtained from locomotor (cage crossing, open field) muscle relaxant (traction), exploratory behaviour (head dip test) and forced induce swimming test. It is observed an overall depressant activity of a drug thus, it may have beneficial effect in case of anxiety and aggressive behaviour.

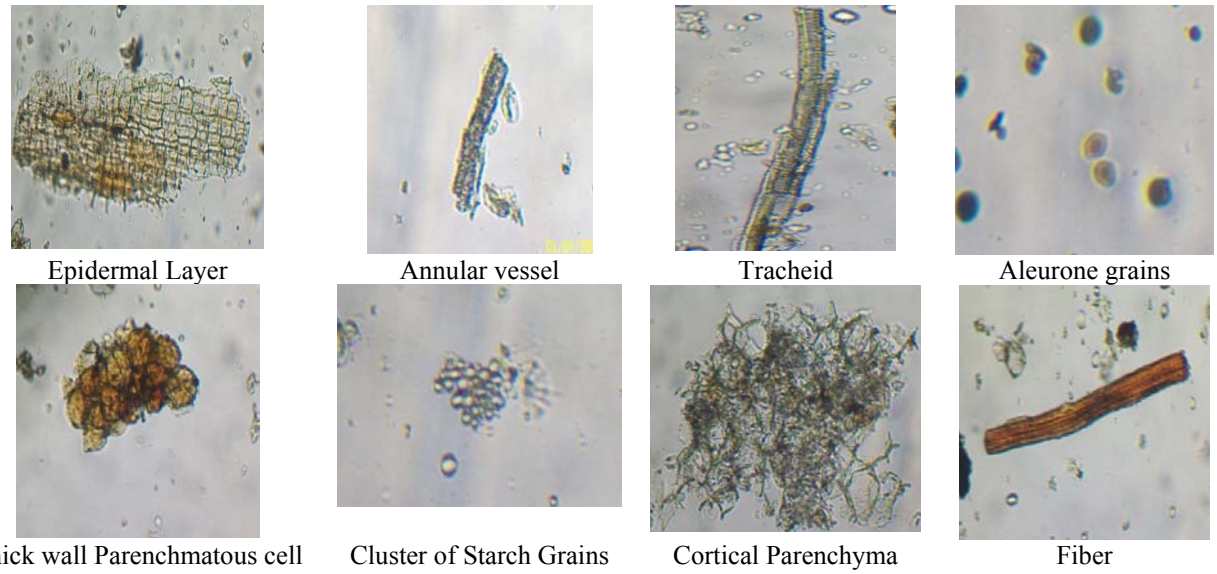

Fig. 3: represent the powder drug study. It indicates the presence of markers constituents, which help to assess the quality of drug.

Powder drug study chemical constituents and FTIR analysis

The reported chemical compounds of *C. rotundus* are α-cyperone, α-rotunol, β-cyperone, β-pinene, β-rotunol, Beta-selinene, Calcium, Camphene, Copaene, Cyperene, Cyperenone, Cyperol, Cyperolone Cyperotundone Dcopadiene, D-epoxyguaiene, D-fructose, D-glucose, Flavonoids, Gamma-cymene, Isocyperol, Isokobusone, Kobusone, Limonene, Linoleic-acid, Linolenic-acid, Magnesium, Manganese, Rotunduskone, Myristic-acid, Oleanolic-acid, Oleanolic-acid-3-oneohesperidoside, Oleic-acid, *p*-cymol, Patchoulenone, Pectin, Polyphenols, Rotundene, Rotundenol, Rotundone, Selinatriene, Sitosterol, Stearic-acid, Sugeonol, Sugetriol (Khan *et al*.,2011; Lawal *et al*., 2009; Sonwa & Konig, 2001; Jeong *et al*., 2000).

Fig. 2 represents the finger prints of *C. rotundus* powder analysis. The peaks area gives an identification mark for the presence of following important constituents in rhizome of *C. rotundus*: 3400 (OH), 2929.40(C-H), 1752.55 (C=O), 10972 (C-O-C).

DISCUSSION

In present study significant anti-inflammatory activity has been observed by the extract of *C. rotundus* without producing any toxic symptom. The extract may have such type of active principles that produces the anti-inflammatory effect more than Aspirin. Akbar *et al,* 1982 and Gupta *et al*, 1971 also reported the anti-inflammatory response in chloroform extract, which confirm our research work.

It is very common to have ulcer and gastric problems with the use of non-steroidal anti-inflammatory drugs. Beside this stress may provoke the ulceration (Onwudiwe 2012). In this study crude extract of *C. rotundus* at 500mg/kg reduced the ulcer index up to 41.2%, which is near to the inhibition of ulcer by reference drug cimetidine. From this activity the *C. rotundus* extract has exhibited the more benefit over other NSAIDs. Previously Arshad *et al.* (2012). reported the antiulcer activity on *C. rotundus* by single high dose but in this study in the continuation of our previous work (Ahmad *et al*., 2012, 2013a,b) we used low dose sub chronic study (14 days) in which *C. rotundus* extract was administered orally at 300 and 500 mg/kg. Since ulcer is present in acute and chronic conditions (Yuan *et al.*, 2006) therefore, this antiulcer study on *C. rotundus* gives an additive beneficial profile.

In present, neuropharmacological studies in mice, the crude extract of *C. rotundus* has decreased the open field and cage crossing, rearing and head activity. The results are statistically significant (table 3). Previously CNS effect on *C. rotundus* root and rhizome were carried out by Pal *et al.,* 2009. This is an expanded work, which is carried out on other neuropharmacological activites. All psychotropic drugs decrease locomotion and exploratory behaviour. The literature and the research done on *C. rotundus* indicated that it is a remedy for mood swing and depression. Many psychotropic drugs are mylorelaxant and responsible for the decreased muscle tone & motor activity, Decreased cage crossing activity (Akperbekova and Guseinov, 1966b; Mokkhasmit *et al*, 1971) and same case was observed with *C. rotundus*. Any alteration in response was not observed in animals after *C. rotundus* administration for condition avoidance response. Present

findings are in accordance with previous reports that *C. rotundus* did not alter condition avoidance response because *C. rotundus* is reported to enhance memory and learning behavior (Ross, 2003, Meguro *et al,* 1969; Gupta *et al*, 1971). *C. rotundus* contains choline which is an important constituent of brain and may be the probable cause of boosting of the memory. Memory booster chemical agents are vasodilator whereas choline is a natural chemical exist in plants, humans and animals (Meguro *et al.,* 1969; Gupta *et al*., 1971). Improvement in learning and memory enhancement may also be due to hypotensive action of *C. rotundus* (Ahmad *et al,* 2013a). Methylphenidate, which is worldwide approved as memory booster also act by providing more blood supply to cerebral vessels and brain (Kim *et al*, 2006). *C. rotundus* showed muscle relaxation in traction test and force induced swimming test and decreased exploratory behavior by producing sedation. All hypotensive are sedative to some extent. *C. rotundus* is also a hypotensive drug (Akperbekova and Guseinov, 1966b; Mokkhasmit *et al.,*1971). The drug shows slight effect on muscle relaxant activity in traction test.

The relaxant and sedative effect of *C. rotundus* did not prolong the struggling time in forced swimming test as compared to the control animal while antidepressant drug having capability to prolong the struggling time on the basis of present finding it may be suggested that *C. rotundus* may act as relaxant, sedative, and hypnotic but it may not be antidepressant. It is also correlated with other behavioral tests like exploration, cage crossing, open field etc. Although microscopic study has been carried out by Sharma and Singh (2011) and Rai *et al*. (2010) but this is expanded work to see the active constituents and markers by powder drug study (using three solvents) and FTIR spectroscopy.

CONCLUSION

This research study concluded that the crude extract of *C. rotundus* can safely be use as an anti-inflammatory drug without affecting gastrointestinal tract. Mild sedative action of *C. rotundus* gives an additive beneficial effect.

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