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Phyto-chemical analysis of *cyperus rotundus* and its effect on ethanol treated rats

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ABSTRACT

The present study was aimed to evaluate the antioxidant activity of the extract of tubers of Cyperus rotundus. It is a well known plant in Indian traditional medicine. An ethanol extract of tuber parts of Cyperus rotundus was examined for antioxidant activity. The study was carried out on equal four groups (n=3) namely as follows: group I (control group) group II (18%ethanol only), group III (18%ethanol and test drug Cyperus rotundus extract), group IV (18% ethanol and Ascorbic acid) was treated with 5ml/kg weight of body orally for 45 days. Antioxidant enzymes activity such as Lipid peroxidation was measured in the gastric tissue. Histopathological studies were carried out for ischemic injury. The serum enzyme level of AST and ALT followed a standard pattern of elevation after ethanol treatment. The extract of *C.rotundus* significantly reduced the triglyceride level in test animals. The results obtained in the present study indicated that *C.rotundus* rhizomes extract can be a potential source of natural antioxidant.

Introduction

India has a rich wealth of medicinal plants, most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for many generation. There is an increasing interest in the measurement and use of plant antioxidant for scientific research as well as industry purposes. This is mainly due to their strong biological activity excluding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis. Obviously, there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts (*MacClements and Decker, 2000*). A number of plants have been investigated for their biological activities and antioxidant principles (*Baris et al., 2006; Saleem et al., 2001*).

Various scientific studies reported the analgestic, anticancer, antiviral, antimalarial, antibacterial and antifungal, antifeedent and antifertility activity of some plants (*Vishnukanta, 2008*). Oxidation is increased by the processes like iron release, lipoxygenase activation, phagocyte stimulation, disruption of mitochondrial electron transport chain and decreases in antioxidant defenses.

The genus Cyprus includes common weeds found in upland and paddy fields in temperature to tropical regions. *C.rotundus*, is used as a traditional folk medicine for treatment of stomach and inflammatory diseases (*Gupta et al 1971; Singh et al., 1970). C.rotundus*, have been reported to contain Oils, alkaloids, glycosides, saponins, flavonoids, tannins (Vu, 1993). The rhizomes of *C.rotundus*, have been used in ancient medicine in India for fever, dysentery, purities, pain, vomiting and various blood disorders (*Kirtikar et al., 1944*). In particular, plant extracts offer a rich potential source of novel antiplatelet agents (*Tsai et al., 2000; Ballaberi et al., 2007).*

When antioxidant defense of the body is inadequate, oxidative stress can damage a variety of biomolecules, inactivate receptors and membrane bound enzymes. Some species of *Piper nigrum, Zingiber officinale* present in piperne, piperidine are known for hepato-protective and antioxidant activity (*Koul and Kapil, 1993).* Amrita bindu was found to combat free radical insults and antioxidant depletion in nitrosamine- induces tissue damage studied albino rats (*Shanmugasundram et al, 1994*).

Lipid peroxidation involves highly reactive oxygen species formed constantly in the human body and are removed by enzymes. Eg. Superoxide dismutase, catalsase, Glutathione, peroxidase etc.) and non enzymatic antioxidant defense system.

However, anti-oxidant activity of *C.rotundus*, a very important medicine plant has not been investigated. Sofar, therefore the present study was aimed to elucidate the effect of tubers of Cyperus rotundus against ethanol induced peroxidative damage and its efficacy to inhibit lipid peroxidation.

Materials and Method

Plant material

Root parts of *C.rotundus* L. were harvested from out field near Monday Market, Kanyakumari District, Tamilnadu, India. Botanical identification was carried out by Department of Botany, Annamalai University, and Chidhamparam. Fresh plant roots were rinsed several times with clean tap water to make it dust and debris free. Then the roots were spread evenly and dried in shady condition for 3 to 4 days until they become crispy while still retaining the brownish coloration. Dried roots were ground in electric chopper to get fine powder for further use.

Preparation of plant extracts

The dried and powdered *C.rotundus* plant roots were successively with double distilled with water, ethanol, petroleum ether, chloropharm and benzene for 10-12 hrs using a Soxhlet apparatus. The collected solutions were filtered through

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Whatman No.1 filter paper. The extracts were evaporated to dryness under reduced pressure at 90^0c by Rotary vaccum evaporator to obtain the respective extracts and stored in a freeze condition at -18^0c for further analysis.

Preliminary phytochemical analysis

Plant material were screened for the presence of alkaloids, glycosides , carbohydrates, phenolic compound and tannins and sterols using the methods previously described by *Tona et al (1998*).

Animal used

Albino mice of either sex weighing 25-35g were used to determine the LD50 of the extract. The effect of *C.rotundus* plant root extract on the motility of gastro-intestinal tract was evaluated by Ethanol test in mice. Albino mice of either sex weighing 25-35 gms were selected and fasted for 24hrs, prior to the experiment; of course water was given *ad libitum*. They were divided into 4 groups of 3 mice in each group. The groups were treated as follows,

Group I – Normal control

Group II – Rats were given 18% ethanol (5 ml/100 gm body weight for 45 days)\

Group III-Rats were given 18% Ethanol (5 ml/100 gm body weight for 45 days) Test drug Cyperus rotundus for 45 days (n=4)

Group IV-Rats were given 18% Ethanol (5 ml/100 gm body weight for 45 days) Standard drug Ascorbic acid for 45 days (n=4)

After the experimental period, the overnight fasted rats were anaesthetized with diethyl ether; blood was collected by carotid artery bleeding. Blood samples were kept for 30 minutes without any disturbance in dry test tubes, and then centrifuged for 10 minutes at 2000 rpm to separate the serum.

Assay

AST, ALT, Triglycerides and total cholesterol levels were estimated in the serum samples using auto analyzer. Results of Biochemical analysis are given in Table 3-6.

Histopathological studies

Liver from one animal of each group was isolated to study the histopathological changes. Results of the histopathological studies are shown in fig 1-4.

Statistical analysis

The results were expressed as Mean± Standard error of mean (S.E.M). Student's T-test used for determining significance. Statistical analysis was carried out with student's T-test was analyzed.

Results and Discussion:

Studies carried out using the extract of Cyperus rotundus revealed that the LD50 of this plant is 1250mg/kg as per the acute toxicity studied. (Table 2). The phytochemical evaluation showed the presence of carbohydrates, glycosides, alkaloids, phenolic compounds and tannins in the alcoholic extract of this plant. (Table- 1)

Lipid peroxidation

In addition to free radical scavenging activity, the rhizome extract was evaluated for its ability to protect biomembrane from oxidative damage. Initiation of the lipid peroxidation by ferrous sulphate takes place either through ferryl-perferryl complex or through OH radical by Fenton's reaction. CRRE inhibited FeSo4 ascobate induced lipid peroxidation is albino mice brain mitochondria in a dose dependent manner as given in Table 2 -6.

The marked elevation in the concentration of total cholesterol and triglycerides was observed in the ethanol treated rats (G2). After treatment with the test drug (extract of *C.rotundus*), level was increased a significant reduction in triglyceride level was noticed whereas total cholesterol in G3 (Ethanol and drug treated). The standard drug (Ascorbic acid) used was effective in reducing both triglyceride as well as total cholesterol.

The serum enzyme level of AST and ALT followed a standard pattern of elevation after ethanol treatment as depicted by the result of G2. The plant drug treatment was not able to reduce these enzyme to a significant level. Even the standard drug did not reduce AST level below the level of G2 whereas it brought down the elevated ALT to that of G1 (Control group).

Histopathological studies

Evaluation based on macroscopic features showed significantly lower score values for drug treated and ethanol treated group compared to the control group. Score values of the drug treated group were comparable with the scores obtained in ethanol treated group (Fig 1-4).

Lipid peroxidation assay showed significant increase in ethanol, ethanol & test drug and ascorbic acid treated group when compared to control group.

Sections of liver tissue obtained after ethanol administration showed liver parenchyma with sheet of hepatocytes showing mild fatty acid changes. Sinusoidal dilatation along with portal tract infiltration with inflammatory cells was also seen.

Sections of liver tissue obtained from control animals showed liver parenchyma with sheets of hepatocytes and sinusoidal spaces whereas in the test group section showed parenchyma with central vein and cords of hepatocytes radiating from the central vein.

The inflammatory response initiated by acetic acid includes activation of cyclooxygenase and lipozygenase pathways (*Shron and Stenson, 1985).* Thus, the postulated mechanism is that altered mucosal prostaglandin synthesis compromises intestinal integrity, resulting in mucosal response to the bacterial products (*Banerjee and Peters, 1989*).

Otherwise, flavonoids are known to inhibit lipid peroxidation and exert these effects as antioxidants, free radical scavengers, and chelators of divalent cations (*Shon et al.,2004*) the results of our experiments confirm known antioxidant activities of flavonoids (*Vaya et al., 2003; Park, et al.,2004).* In summary, C.rotundus extracts could give rise to anticarcinogenic agents and could be promising candidates for further studies designed to obtain more evidence on their components with potential of disease preventive activity.

***Conclusion*:**

The results obtained in the present study indicated that ethanol induced lipid- peroxidation and *C.rotundus* rhizomes extract exhibits free radical scavenging, reducing power and antioxidant activity. The tubers could therefore serve as a cheap source of raw material for chemical industries. The plant tubers will also serve as source of non-protein nitrogenous phytocompounds whose allelopathic properties will be of great economic value in general agriculture.

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FIGURES

Fig.1. Liver of rat treated with 18% ethanol and test drug

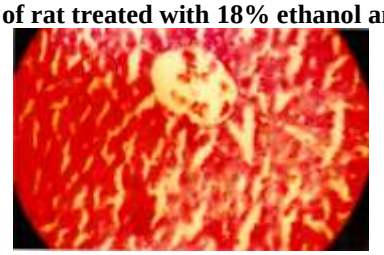

Fig.2. Photomicrograph showing normal liver architecture

Fig.3. Liver of rat treated with 18% ethanol and standard drug

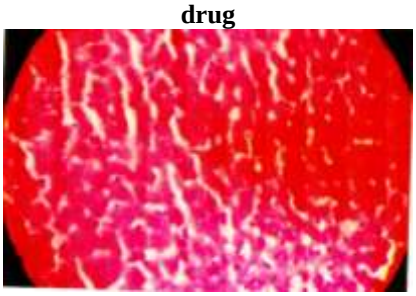

Fig.4. Liver of rat received 18% ethanol

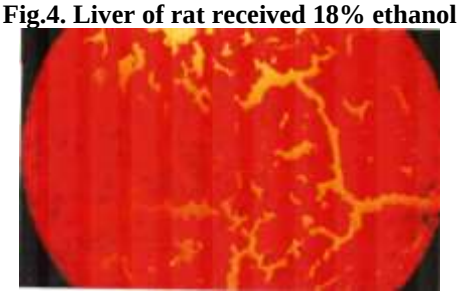

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Table: 1 Phytochemical evaluation of *Cyperus rotundus* Linn.

Phytochemical parameters	Petroleum ether extracts	Benzene extracts	Chloroform extracts	Alcohol extracts	Aqueous extracts
Carbohydrates	-	-	-	+	+
Glycosides	-	-	-	+	+
Fixed oils and fats	+	+	+	-	-
Proteins and amino acids	-	-	-	-	-
Phenolic compound and tannins	-	-	-	+	+
Phytosterols	+	+	+	+	-
Alkaloids	-	-	-	+	-
Flavonoids	-	-	-	-	-
Saponins	-	-	-	-	-
Gums and Mucilages	-	-	-	-	+

(+) - Presence of Constituents (-) - Absence of Constituents

Table: 2 LD50 Determination of alcoholic extract of Cyperus rotundus

Group	Dose (mg/kg)	No. of Animals	Dose difference (a)	Mortality	Mean mortality (b)	Product (a x b)
1	500	4	0	0	0	0
2	750	4	250	0	0	0
3	1000	4	250	1	0.5	125
4	1250	4	250	2	1.5	375
5	1500	4	250	3	2.5	625
6	1750	4	250	4	3.5	875

Table: 3 Serum levels of total cholesterol

Parameter	Group n=4	Total Cholesterol level ±SEM (mg/dl)	Groups compared	t-value	Significance
Total Cholesterol	G1	54.85±5.25			
	G2	71.7±0.237	G2 Vs G1	3.206	*
	G3	76.92±1.106	G3 Vs G2	4.614	**
	G4	66.55±0.480	G4 Vs G2	4.537	**

Table: 4 Serum levels of total triglycerides

Parameter	Group n=4	Total Cholesterol level ±SEM (mg/dl)	Groups compared	t-value	Significance
Total triglycerides	G1	79.57±9.02			
	G2	120.3±6.177	G2 Vs G1	3.726	**
	G3	77.75±2.01	G3 Vs G2	6.550	***
	G4	98.02±1.564	G4 Vs G2	3.497	*

Table: 5 Serum level of total Aspartate amino transferase (AST)

Parameter	Group n=4	Total Cholesterol level ±SEM (mg/dl)	Groups compared	t-value	Significance
Total Aspartate amino transferase (AST)	G1	227±1.080			
	G2	252.3±6.75	G2 Vs G1	3.71	**
	G3	292.3±7.553	G3 Vs G2	4.00	**
	G4	262.4±18.83	G4 Vs G2	0.4515	NS

Table: 6 Serum levels of total Alanine amino transferase (ALT)

Parameter	Group n=4	Total Cholesterol level ±SEM (mg/dl)	Groups compared	t-value	Significance
Total Alanine amino transferase (ALT)	G1	53±4.65			
	G2	81.5±2.66	G2 Vs G1	5.327	**
	G3	107.8±0.270	G3 Vs G2	9.850	***
	G4	68.17±3.988	G4 Vs G2	2.78	*