



ANTI-INFLAMMATORY, ANTI-ARTHRITIC, ANALGESIC AND ANTICONVULSANT ACTIVITY OF CYPERUS ESSENTIAL OILS

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ABSTRACT

The aim of present study was to evaluate the Anti-inflammatory, anti-arthritis, analgesic and anticonvulsant activity of *Cyperus esculentus* Linn. and *Cyperus rotundus* Linn. Essential oils. The oils were subjected to phytochemical tests and the flavonoids, triterpenoids, carbohydrate, proteins were found. In India it has been popularly used for the treatment of wound healing, antimicrobial, antidote, anti mutagenic and anti diarrhoeal. In this study we evaluate the effects of oils in anti-inflammatory (carrageenan induced), antiarthritic (formaldehyde induced), analgesic (formalin induced writhing) and anticonvulsant (MES produced convulsion). The results showed dose dependent activity, indicated by reduction in paw edema in anti-inflammatory and antiarthritic activity, and significant reduction ($p < 0.01$) in the MES induced convulsion in comparison to control. From literature survey as well as experimental performed, it can be said that essential oil possess a good Anti-inflammatory, anti-arthritis, analgesic and anticonvulsant activities.

Keywords: Epilepsy, Cyperus, Arthritis, Essential oil

INTRODUCTION

Epilepsy is a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness. Epilepsy is the second most common chronic neurological condition seen by neurologists. It is estimated that there are 55, 00,000 persons with epilepsy in India, 20, 00,000 in USA and 3, 00,000 in UK. Three to five per cent of the populations have a seizure sometime in their life and half to one percent of the population have 'active epilepsy'. It is said that 60 out of 1, 00,000 children in the US and the UK are affected with juvenile rheumatoid arthritis. With as many as 12,000 children in Mumbai estimated with pediatric rheumatoid arthritis, the formation of Juvenile Arthritis Support group at Mumbai's Jaslok Hospital signifies an important milestone². Essential oils are used extensively in aromatherapy and various traditional medicinal systems. Anti-inflammatory are the agents pain-relieving drugs reduce swelling and inflammation. Anticonvulsant Drugs are medicines used to prevent or treat convulsions (seizures). Since the essential oils are noncorrosive and safe to take internally, the oil has been chosen. *S C rotundus* (*Cr*) showed multiple pharmacological activities like antidiarrhoeal³, hepatoprotective⁴, antimutagenic and radical scavengers⁵. Traditionally these plants are also reported for its analgesic and anticonvulsant activity⁶. Hence the present study is to investigate the Anti-inflammatory, anti-arthritis activities of *Ce* and *Cr* essential oils.

MATERIALS AND METHODS

Essential oils of *Cyperus rotundus* (*Cr*) and *Cyperus esculentus* (*Ce*) were obtained from M/S Shanbag Ayur Products, Yallapur, Karnataka.

Animals

Swiss albino rats of either sex (200-250 g) were used for present study. Animal were kept under a 12 h light/ 12 h dark cycle, with free access to food and water. All experimental protocols were prepared and performed based on ethical guidance of Institutional Animal Ethical Committee.

Phytochemical studies

The essential oils were subjected for qualitative chemical estimation to assess major phytochemical entity and Flavonoids, sesquiterpenes, glycosides were found to be presents.

Acute toxicity studies

The acute toxicity studies for extracts and fractions of leaves were performed as per OECD guideline 423 by using albino rats. Overnight fasted rats of either sex (200-250 g) were administered with different fractions of essential oils at 5000 mg/kg, p.o. After 24 hrs no mortality was found. 250, 500, and 750 mg/kg, p.o. doses were selected for the further study.

Evaluation of anti-inflammatory activity

Carrageenan induced paw edema in rats⁷

The previously described method of carrageenan-induced paw edema assay in rats was used in this work. Paw edema was induced in the hind paw of mice by the sub plantar injection of 50 μ l of carrageenan, (1 % w/v). The contralateral paw was injected with the same volume of the vehicle and used as control. The course of the edema was monitored by measuring the thickness of footpad swelling at 1, 2, 3 and 4 h after carrageenan injection by using a vernier caliper. Animals received dose of oils (250 mg/kg, 500mg/kg of *Cr* and 200mg/kg, 400mg/kg of *Ce* p.o.), and distilled water (control animals), 1hrs before the carrageenan administration. Indomethacin (10 mg/kg) was used for animals of standard group.

Formaldehyde induced arthritis⁸

Male Wistar rats weighing between 150-200 g. will be randomly selected. They will be grouped in a group of 6 animals each into 4 groups. On the 0th day, the basal paw volume of left hind paw of each animal will measured using Plethysmometer. On day 1 and day 3, they will be injected into the sub-plantar region of the left hind paw with 0.1 ml of 2 % v/v formaldehyde in normal saline. Dosing with standard drug, Diclofenac sodium and extracts will be started on same day and continued for 10 days. Group I served as - Arthritis control, Group II - Diclofenac Sodium (standard drug) treated. Group III & IV - *Ce*-250, 500mg respectively, group V & VI received *Cr*-250, 500mg/kg respectively. Paw volume of injected paw will be measured daily.

Evaluation of analgesic activity

Formalin induced pain⁹

Pain was induced by injecting 0.05 ml of 2.5% formalin in distilled water in the sub-plantar of the right hind paw of rats. Animals received dose of essential oils (250 mg/kg, 500mg/kg p.o.), indomethacin (10mg/kg), and 1% CMC (p.o.) 30 min prior to injecting formalin. The amount of time spent licking the injected paw

was indicative of pain. The number of lickings from 0 to 5 min (first phase) and 15–30 min (second phase) were counted after injection of formalin. These phases represented neurogenic and inflammatory pain responses, respectively.

Evaluation of anticonvulsant activity

Maximal electroshock (MES) induced convulsion in rats¹⁰

The anticonvulsant property of the drug in this model was assessed by its ability to protect against MES induced convulsions. The animals were first weighed and were selected for the experiment depending on weight. Rats of either sex were used. The rats were then divided into four groups of 6 rats each Group -1 received saline; Group- 2 received 25 mg/kg of Phenytoin. Group- 3 received 250 mg/kg of oil. Groups-4, received 500 mg/kg of oil. Maximal electroshock (Inco Electroconvulsimeter model# 100-3) of 150 mA current for 0.2 seconds administered through ear electrodes to induce convulsions in the control and drug treated animals. The drugs and chemicals were prepared fresh; the concentration, dose and duration before induction of convulsion.

Statistical analysis

The results are presented as Mean and \pm S.E.M. The statistical significance of differences between the groups was obtained using analysis of variance (ANOVA) complemented by Dennett's test. $P < 0.05$ and $P < 0.01$ considered to be significant. IC50 values were determined to be the effective concentration at which free radicals were scavenged by 50%. The IC50 value was obtained by interpolation from linear regression analysis.

RESULTS

Results of Phytochemical constituents

The both essential oil contains Triterpenoids, Flavonoids, Proteins and Saponins as a major active constituents

Acute oral toxicity studies:

The LD50 cut off dose of oil was 5000mg/kg. Therefore, ED50 was selected as 1/10th and 1/20th of LD50 i.e. 500mg/kg and 250mg/kg

Carrageen induced paw edema

When compared with the control, treatment with *Cr* and *Ce*, significantly ($P < 0.01$) reduced the paw edema from 2nd hr after Carrageenan injection. Pretreatment with *Cr* and *Ce* doses (250,500 and 250,500 for both mg/kg) showed a dose dependent effect. There was significant activity showed by *Cr*-(500 mg/kg) and *Ce*-(500 mg/kg) than other *Cr*-(250 mg/kg) and *Ce*-(250 mg/kg). However, 10 mg/kg indomethacin significantly suppressed paw edema from 2nd hr and remains significant upto the 4th hr. The determination of inhibition percentage showed that administration of *Cr*-(500 mg/kg) produced a comparable effect with indomethacin (69.7% and 72.1% respectively) 4 hr after carrageenan injection. (Table.1, Fig.1)

Formaldehyde induced arthritis

The anti-arthritis activity was also evaluated by using formaldehyde induced arthritis model in Wistar albino rats. The assessment made on the 10th day showed that, treatment with *Cr*(500 mg/kg) and *Ce* (500 mg/kg) more significantly reduced ($P < 0.01$) the swelling in the injected (left) hind paw as compared to Diclofenac sodium treated group. On the 10th day the % inhibition of paw edema exhibited by *Cr*(500 mg/kg) and *Ce* (500 mg/kg) were 75.54%, 76.58%, respectively; while Diclofenac sodium treated animals showed maximum % of inhibition of paw edema 81.37 on 21st day. The results are shown in (Table 2).

Formalin induced writhing

Analgesic effects on both first (0–5 min) and second phases (15–30 min) of formalin induced pain. These phases corresponded to neurogenic and inflammatory pains, respectively. Essential oil was inhibited both, neurogenic and inflammatory pain at $P < 0.01$ at dose of 500mg/kg level whereas lower doses of essential oil significantly $P < 0.05$ blocked the inflammatory pain. Indomethacin showed highest activity in blocking inflammatory pain and did not show significant activity in neurogenic pain. *Ce* (500 mg/kg) was found to inhibit the pain resulting from inflammation better than the neurogenic induced pain.

Table 1: Percentage inhibitions of carrageen-induced paw edema by essential oils and standard drug in injected (left) hind paw

Extracts	Change in paw edema					Inhibition
	0 th day	4 th day	8 th day	14 th day	21 st day	
Normal control	0.831 \pm 0.023	0.833 \pm 0.056	0.835 \pm 0.203	0.837 \pm 0.013	0.840 \pm 0.023	
Arthritic control	0.836 \pm 0.0133	1.585 \pm 0.099	1.672 \pm 0.0195	1.838 \pm 0.0186	2.067 \pm 0.056	
Diclofenac sod. (13.5mg/kg BW)	0.888 \pm 0.0268	1.485 \pm 0.017**	1.202 \pm 0.017**	1.137 \pm 0.0194**	1.116 \pm 0.015**	79.36
(<i>Cyperus sculentus</i> 250mg/kg)	0.890 \pm 0.0278	1.670 \pm 0.010**	1.352 \pm 0.013**	1.263 \pm 0.0311**	1.127 \pm 0.014**	71.90
(<i>Cyperus sculentus</i> 500mg/kg)	0.8850 \pm 0.0168	1.633 \pm 0.008*	1.464 \pm 0.010**	1.380 \pm 0.0673**	1.187 \pm 0.033**	68.21
(<i>Cyperus rotundus</i> 250mg/kg)	0.891 \pm 0.0401	1.598 \pm 0.010*	1.527 \pm 0.008**	1.278 \pm 0.0633**	1.150 \pm 0.033**	68.48
(<i>Cyperus rotundus</i> 500mg/kg)	0.835 \pm 0.0321	1.648 \pm 0.011 ^{NS}	1.595 \pm 0.0078*	1.467 \pm 0.0388*	1.542 \pm 0.044**	58.12

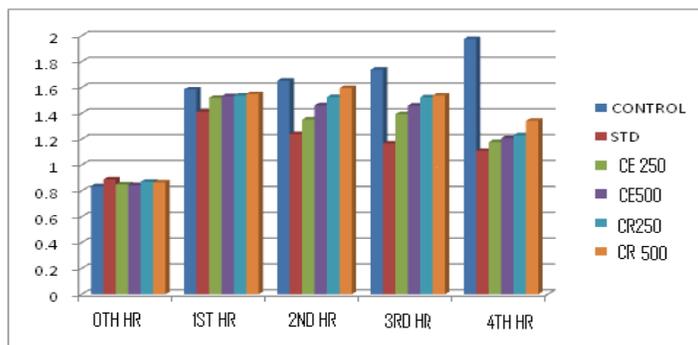


Fig. 1: Column statistic of various extracts on 0th to 21st day in injected (left) hind paw

Table 2: Percentage inhibition of paw volume produced by *Ce* and *Cr*

Group	Paw volume(mean ± SEM)		% Inhibition (10 th day)
	0 th day	10 th day	
Control	0.9433±0.0284	2.002±0.0104	
Standard	0.9317±0.0265	1.116±0.0224**	80.65
Ce250	0.9400±0.0713	1.256±0.0388**	65.11
Ce500	0.8917±0.0392	1.200±0.0535**	76.58
Cr250	0.905±0.0453	1.317±0.0170**	65.84
Cr500	0.8923±0.0543	1.195±0.046*	75.54

Table 3: Effect of *Ce* and *Cr* on Formalin induced writhing

Groups	0-5 min	15-30 min
Control	66.34 ±2.87	103.32±1.75
Standard	56.48 ±1.98**	57.23±2.43**
Ce250	61.45 ±1.73	85.67±2.56*
Ce500	54.52 ±2.54**	71.43±1.45**
Cr250	60.43 ±2.72	82.54±3.56*
Cr500	52.45± 1.54**	74.67±2.56**

MES induced convulsant activity

The essential oil of *Ce*-500 and *Cr* 500mg/kg decreases the duration significantly ($P < 0.01$), of clonus (12.00 ± 0.7303 sec) and stupor (74.20 ± 0.6325 sec) phase of MES induced convulsion as compared to control, clonus (15.67 ± 0.6667 sec) and stupor (96.00 ± 1.949 sec). In other words the essential oil of *Ce* 500 and *Cr* 500mg/kg are

able to decrease the duration of hind limb extension (extensor phase), clonus and also the duration of stupor phase, which indicate the essential oil of *Ce* ce-500 and *Cr* 500mg/kg does possess potent anticonvulsant activity against generalized tonic-clonic seizure (grand mal) while other doses essential at dose of *Ce* 250 and *Cr* 250mg/kg did not show statistically any significant effect in extensor phase as compared to control (Table 4).

Table 4: Effect of *Ce* and *Cr* on MES induced convulsions

Drug	Dose mg/Kg b.w.	Time (Sec) in various phases of convulsions (Mean±SEM)				
		Flexion	Extension	Clonus	Stupor	Recovery
Control (Saline 1ml/rat)	-	3.255±0.2828	7.980 ± 0.320	4.080 ± 0.256	188.3 ± 2.782	Recovery
Standard Phenytoin	25	0.0833± 0.0251	0.0316± 0.020	1.207 ± 0.382	1.700 ± 1.057	Recovery
Ce	250	3.245 ± 0.278**	7.800± 0.345	2.447± 0.303	174.8 ± 4.49	Recovery
Ce	500	1.953 ± 0.211**	7.050 ± 0.345 **	3.505± 0.214**	144.6 ± 4.931**	Recovery
Cr	500	0.933±0.122 ***	2.523 ± 0.189***	1.638 ± 0.253***	95.67± 8.003***	Recovery
Cr	250	2.603 ± 0.215*	7.160 ± 0.488	4.057 ± 0.162**	171.6 ± 3.094	Recovery

DISCUSSION

Carrageenan is the sulphated polysaccharide obtained from the seaweed, which is widely used phlogistic agent which shows signs and symptoms of inflammation, which can be assessed as increase in paw thickness in mouse as a result of increased inflammation, edema and increased vascular permeation. Inflammation produced by carrageenan is a triphasic response. In the first phase of inflammation, histamine and serotonin like inflammatory mediators are involved which cause the edema and redness. In the second phase, different cytokines and kinins get released in response to the inflammation produced and the mediators already secreted at the localized site. In the third phase, the COX enzyme plays pivotal role and there is production of prostaglandins which induces pain. In the present study, CE showed inhibition of paw thickness at 3rd and 4th hour after carrageenan injection which probably suggested that CE inhibit the prostaglandin formation in the third phase of inflammation¹¹.

All the essential oils under evaluation of anti-inflammatory action were subjected to phytochemical study and showed to contain triterpenoids, flavonoids and proteins. Triterpenoids are one of the most numerous and widespread groups of phenolic in plants, exhibiting a range of biological and pharmacological effects such as anti-inflammatory. In this study, essential oils were subjected for phytochemical screening and biological activity. The study indicated that essential oil has both peripheral and central analgesic properties. Its peripheral analgesic activity was deduced from its inhibitory effects on chemical induced nociceptive stimuli¹².

Formalin test investigated both. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase¹¹. The formalin test is a very useful method for not only assessing antinociceptive drugs but also helping in the elucidation of the action mechanism. The neurogenic phase is probably a direct result of stimulation in the paw and reflects centrally mediated pain with release of substance. while the late phase is due to the release of histamine, serotonin, bradykinin and prostaglandins. Essential oil was able to block both phases of the formalin response but the effect was more prominent in the second phase. In the present study, *Ce*, *Cr* showed maximum protection against formalin induced writhing followed by other models probably explained the peripheral analgesic potential of *Ce*, *Cr* prostaglandin inhibitory activity.

MES induced tonic seizures can be prevented either by drugs that inhibit voltage dependant Na⁺ channels such as Phenytoin, Valproate, Felbamate and Lamotrigine or by drugs that block glutamatergic excitation mediated by the n-methyl-D-aspartate (NMDA) receptor, such as Felbamate. *Essential oil* follows any one of the above mechanism¹³.

CONCLUSION

The *Ce* and *Cr* were found to be more active in both anticonvulsant and anti-inflammatory activities. The study confirms the anticonvulsant and anti-inflammatory activities of *Ce* and *Cr* in dose dependent manner.

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