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STUDY ON *ZINGIBER CHRYSANTHUM* ROSC. RHIZOME ESSENTIAL OIL FOR ITS ANTHELMINTIC ANTI-DIARRHOEAL AND ANTIBACTERIAL ACTIVITIES.

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ABSTRACT

Zingiber chrysanthum Rosc.(Zingiberaceae) rhizome essential oil (ZCREO) was studied for its effect on isometric contractions of *Ascaridia galli*. The amplitude and frequency of spontaneous contractions of *A. galli* were recorded on physiograph through force transducer. The oil exhibited significant effect and percent response inhibition on the contractility of *A galli* in terms of amplitude frequency and base line tension in dose dependent manner with an IC_{50} value of 6.25 ± 0.23 mg/ml. Antidarrhoeal activity of ZCREO was also evaluated on castor oil-induced diarrhea in mice. The oil showed dose dependent increase in time of onset of first wet faeces and reduces total number of faeces and number of wet faeces. This indicates that ZCREO reduces peristaltic movements of gastro-intestinal tract, and acts as potent anti-diarrheal drug. The oil exhibited moderate antibacterial activity against pathogenic bacteria viz; *E.coli, S.enterica enterica, P.multocida and S. aureus* with MIC values ranging 3.90-31.25 µl/ml.

KEYWORDS: *Ziniber chrysanthum*, anthelmintic, castor oil, antidarrhoeal activity, antibacterial activity.

INTRODUCTION

The family Zingiberaceae is well known for its uses in food & flavour, spices, ornamentals and in indigenous system of medicine. [1, 2] Several species of ginger like *Zingiber officinale* Rosc. *Z. capitatum* Roxb, *Z. roseum* and *Z. chrysanthum* Rosc. are known to grow in Kumaun and Garhwal region of Uttarakhand in India. [3] Z. *chrysanthum* is a half-hardy, perennial herb. It is found in sub-tropical forest margins in the foothills of the Himalayas, It's leafy steams grows 1.2 m to 2 m with fresh green leaves about 25 cm long and 7 cm wide with a downy pubescence in the under surface, flower appears in autumn on a very short inflorescence borne direct on the rhizomes at the base of the leafy shoots. [4] The species, *Zingiber* occupy an important place in Indian traditional texts; It has attracted wide attention due to several medicinal properties associated with them such as antispasmodic, [5] anti-inflammatory, [6] antioxidant, [7] anthelmentic, [8] antidarrhoeal activity. [9] In continuation to our studies on family Zingiberaceae we already have reported β-humulene (20.3%), terpinen-4-ol (10.0%), β-phellandrene (7.4%), sabinene (7.0%) and β-caryophyllene (6.8%) as major compounds in the essential oil of *Z. chrysanthum*. The oil was also reported to exhibit *in vitro* antioxidant activity, carbachol- and KCl-induced myorelaxant activity on isolated rat duodenal smooth muscle. [10] We also have reported antioxidant and hepatoprotective activity of acetone and methanolic extracts of *Z, chrysanthum***.** [11] Antimicrobial properties of essential oil obtained from leaf and rhizome of *Z. chrysanthum* against gram positive and gram negative bacteria has been reported. The essential oils have also been reported to posses, antifungal activity against 20 species of plant pathogenic fungi**.** [12] Very few reports exists on the biological activity of this zingiberaceous herb, in view of its medicinal properties, present study reveals the first time reported anthelmentic and antidarrhoeal activity of *Z. chrysanthum* rhizome oil essential oil (ZCREO).

MATERIALS AND METHODS

2.1 Plant Material

Fresh rhizomes were collected from Tarai region of Uttarakhand in India. The plant was identified by Dr. D. S. Rawat plant taxonomist Department of Biological Science. G. B. Pant University of Agriculture and Technology Pantnagar and a voucher specimen were submitted to the Department.

2.2Isolation of Essential oil

Sliced and crushed rhizomes were subjected to hydro-distillation using Clevenger's apparatus. The distilled oil was extracted with diethyl ether and was dried over anhydrous Na_2SO_4. Removal of solvent yielded 0.02% oil (v/w).

2.3 Anthelmintic Activity

2.3.1 Tissue mounting

2.3.1.1 Isometric mounting of A. galli and mechanical recording of the spontaneous muscular activity.

The worm *A. galli* was mounted isometrically on a tissue bath of 4 ml capacity in Tyrode solution (Composition in mM: NaCl 136, KCl 5, $CaCl_2$ 2, $MgCl_2.6H_2O$ 0.098, $NaH_2PO_4.2H_2O$ 0.36, Glucose 5.5, $NaHCO_3$ 11.9 and pH adjusted to 7.4) maintained at 40 $\pm 1^0$C and allowed to equilibrate for 30 min without any tension. During the equilibration period the bath fluid was changed once every 10 min. Isometric contraction were made after giving a tension of 0.5g using force transducer (T-301, Pt-1979) and spontaneous muscular activity was recorded using ink writing physiograph (Biodevice, India) at 0.25 mm/sec chart speed. Control recordings were made for 15 min before the addition of a drug. Three parameters namely frequency (total number of contractions in 10min), amplitude (average of all peaks per 10min or average tension) of spontaneous muscular contractions and baseline tension (average of all minimum levels of contractions used for measuring amplitude) of the isometrically mounted *A.galli* were measured. [13]

2.3.2 Effect of different extracts and albendazole on autorythmicity of A. galli

Cumulative doses of the ZCREO at a dose rate of 3.5-28 mg/ml dissolved in Tyrode solution and of albendazole at a dose rate of 0.32-2.56 mg/ml were added in the tissue bath containing the isometrically mounted worm. Each dose was allowed to act for 15 min with continuous recording of rhythmic movements of *A. galli.* The effects of various concentrations of plant extracts on frequency and amplitude of spontaneous muscular contractions and on base line tension of the mounted worm or recorded and compared with the control.

2.4 Antidiarrhoel activity

To check antidiarrhoel activity of ZCREO the mice were placed in six groups. Each group contained six mice. All the mice of each group were fasted for 18 hours before starting the experiment. The animals were placed separately in cages with white chart paper during

experiment. Group I served as negative control in which distilled water was given @ of 1 ml/kg body wt., orally. Group II served as positive control in which cathartic agent (castor oil) 0.5ml/mice was orally administered; Group III received atropine sulphate (standard drug) @ 3.0 mg/kg body weight 60 min prior to castor oil administration. [14)] The cathartic agent (castor oil) was orally administered to the animals @ 0.5 mL/kg body weight after 60 min of tragacanth administration. The time elapsed between administration of castor oil and excretion of first wet faeces was evauluated for each animal. The total number of faeces as wel as the number of diarrhoeic faece (wet faeces) excreted in four hours was determined. Different doses of the ZCREO were administered orally to the animals of groups IV, V and VI, 60 min before the administration of cathartic agent. Distribution of different doses of ZCREO in animals of groups IV, V and VI are summarize in table 1.

Table: 1.Effect of rhizome oil of Z. chrysanthum (ZCRO) on castor oil induced diarrhoea in mice (n=6, mean± S.E.)

Group	Treatment	Dose (ml/kgbodywt.)	Dose of castor oil (ml/mice)	Onset of First wet faeces (min)	No. of wet faeces in 4 hrs.	Total no. of foeces in 4 hrs.	% inhibition diarrhoea
I	Negative control (DW)	1	-	-	-	7.5±0.62^{b}	-
II	Positive control(DW)	1	0.5	71.83± 3.4^{n}	15.17± 1.83 ^{a}	18.11±1.74^{n}	
III	Atropine Sulphate	3 mg/kg,(i.p)	0.5	138.67± 0.8^{b}	5.17± 0.48^{b}	7.5±0.84^{b}	58.72
IV	ZCRO	0.3	0.5	66.4± 1.75^{n}	9± 1.57^{c}	10.6±1.71^{b}	41.66
V	ZCRO	1	0.5	161± 5.12^{b}	4.67± 0.56^{b}	6.5±0.56^{b}	64.22
VI	ZCRO	3	0.5	68.5± 3.49^{n}	7.83± 0.87^{b}	8.67±0.62^{b}	52.28

(p>0.050 values bearing common super script does not vary significantly

2.5 Antibacterial activity

The ZCREO was individually tested against four gram- negative bacteria, *Pasteurella multocida* (MTCC-1148), *Escherichia coli* (MTCC-443), *Salmonella enterica enterica* (MTCC-3223) and *Shigella flexneri* (MTCC-1457) and against the gram-positive *Staphylococcus aureus* (MTCC- 737). All the bacterial strains were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India as Microbial Type Culture Collection (MTCC) and maintained in the laboratory by regular sub culturing on to nutrient agar. Antibacterial screenings were performed, as reported before**,** [15] by the disc-diffusion method, [16] with slight modifications. The test was performed in sterile petri dishes containing solid and sterile nutrient agar plate. The respective essential oil (50ml) was absorbed on sterile paper discs (Whatman; 5mm in diameter), and placed on the surface of

media previously inoculated with a sterile microbial suspension (single type of organism per dish). All dishes were sealed with sterile laboratory films to avoid eventual evaporation of the test samples, and than incubated at 378 for 20 h. The diameter (in mm) of the inhibition zone was measured. Teracycline, amikacin, ciprofloxacin, ampicillin, and zentmicin were used as reference antibiotics (pos control). Minimum- inhibitory- concentration (MIC) values were determined by the tube- dilution method**.** [17]

2.6 Statistical analysis

The results are presented as mean ± standard error of the mean. To measuer the significance one way T test was applied.

RESULTS

Spontaneous muscular activity of *Ascaridia galli* was recorded by mounting the worm on tissue bath containing Tyrode solution, using force transducer connected to physiograph. Freshly prepared solution of ZCREO was added at cumulative doses on suspended worms and rhythmic motility was recorded. The spontaneous contractions of *A. galli* were recorded in the form of their frequency, amplitude and baseline tension (The cumulative doses (3.5, 7, 14, 28 mg/ml) of ZCREO significantly decreased the amplitude, frequency and baseline tension of *A. galli* as compared to control (table.2).

Table: 2 Effect and percent response control of ZCREO on contractility of *A. galli* at

SN	Response of ZCREO on contractility of *A. galli* (n=5)				% control of ZCREO on contractility of *A. galli* (n=5)		
	Dose (mg/ml)	Amplitude (mg)	Frequency (min^{-1})	Baseline tension (mg)	% amplitude control	% Frequency control	% base line tension control
1	0(Control)	199.4± 42.31	5.25± 0.22	511.54± 5.49	100± 0	100± 0	100± 0
2	3.5	155.1± 38.30	4.26± 0.16**	467.43± 18.11*	76.56± 4.23*	82.08± 6.34**	91.54±3.92*
3	7.0	112.9± 41.12	3.80± 0.09**	501.28± 12.72	50.78±9.20**	72.62± 2.39**	98.022±2.54
4	14	85.05± 38.04*	3.10± 0.09**	487.25± 9.41	35.38± 10.39**	59.16± 3.72**	95.32± 2.38*
5	28	26.70± 8.87**	1.74± 0.18**	465.8± 18.33**	11.39± 2.67**	32.83±2.45**	91.23 ± 4.43*

different dose levels

* P < 0.05% and ** P< 0.01 % as compared to control in the same column

Albendozole (0.32-2.56 mg/ml), a well proven anti-helmentic drug of benzimidazole group [18] was used as a reference drug in the present study. It showed better anthelmentic response by inhibiting amplitude, frequency and baseline tension of *A. galli* in a dose-dependent manner (table 3). The maximum decrease in frequency, amplitude and baseline tension was

observed at the dose of 2.56 mg/mL. IC_{50} values of ZCREO and Albendozole, calculated on the basis of their effect in amplitude, are presented in fig.1

Table:3 Effect and percent response control of albendazole on contractility of *A. galli* at different dose levels

SN	Response of albendazole on contractility of *A. galli* (n=5)				% control of albendazole on contractility of *A. galli* (n=5)		
	Dose (mg/ml)	Amplitude (mg)	Frequency (min^{-1})	Baseline tension (mg)	% amplitude control	% Frequency control	% base line tension control
1	0 (Control)	455± 46.94	248.33± 23.1	504.42± 21.32	100±00	100±00	100±00
2	0.32	312.5±27.95**	139.66± 24**	324.30± 12.81**	69.65±1.76**	54.08±4.63**	64.36±0.18**
3	0.64	240.0± 19.0**	61.66± 20.12**	166.25± 46.39**	54.46±3.77**	22.01±6.05**	35.22±10.68**
4	1.28	106.66± 2.78**	68.33± 21.61**	274.30± 5.90**	24.94±2.74**	24.52±6.42**	55.12±3.50**
5	2.56	11.33± 0.40**	18.33± 0.74**	335.41± 23.29**	2.64±0.27**	7.57±0.40**	66.10± 1.82**

P < 0.05 and **P<0.01% as compared to control in the same column.

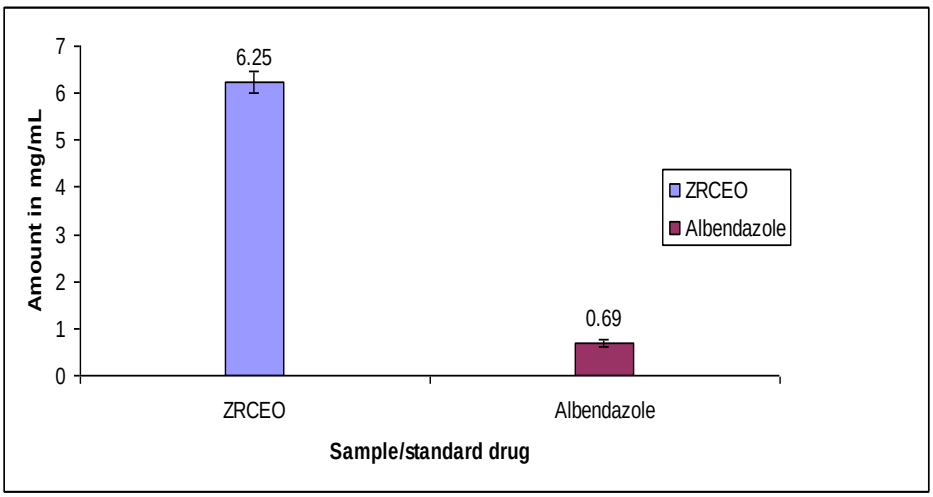

Fig: 1 IC_{50} values of ZCREO and albendazole against *A. galli* in tissue bath experiment

The antidarrheal effect ZCREO was evaluated on castor oil-induced diarrhea in mice and is presented in Table.3. Under normal conditions, in group I mice (Negative control), the number of faeces in 4 h was 7.5±0.62. In group II (Positive total control), in which mice received 1 ml distilled water 60 min prior to castor oil, cathartic effect of castor oil was found (appearance of first wet faces) at 71.8±3.4 min after oral administration of castor oil. Number of wet faeces and total number of faeces in 4 h were 15.2±1.8 and 18.2±1.7, respectively. There was a significant (P<0.05) increase in the time of onset of first wet faeces by atropine sulphate at 138.67±0.8 min after administration of csator oil in group III mice in comparison to group II mice. Significant (P<0.05) reduction in the number of diarrhoeal faeces and total number of faeces (5.17±0.84 respectively) was observed in group III mice. When compared

to the values of group II animals ZCREO, at the rate of 0.3 ml/kg body weight orally, did not alter the time of onset of first wet faeces (66.1±1.75 min) in mice of group IV as compared to group II. This dose of oil significantly (P<0.05) decreased the number of wet faeces and total number of faces in 4 h to 9±1.57 and 10.6±1.71 respectively when compared to the values of group II mice. In group IV animals, the time of onset of first wet faeces was increased significantly (P<0.05) in comparison to group II mice. This value of group V mice was also significantly higher then that of group III animals. The number of wet faeces and total number of faeces and total number of faeces was decreased significantly in group V animals in comparison to group II but not in group III mice. ZCREO, at the dose rate of 3 ml/kg body weight the time of onset of first wet faeces remained unaffected (68.8 ±3.49 min) when compared to group II animals. The number of wet faeces and total number faeces were recorded to be 7.83 ± 0.87 and 8.67 ± 1.02, respectively. These values were significantly less than those corresponding values in group II. ZCREO was found to be effective against all tested bacterial strains. The activity order in term of zone of inhibition was observed as *S. enterica enterica* >*E. coli* > *S. aureus* >*P. multocida* respectively. The results obtained in comparison to the standard drug gentamicin to exhibit maximum zone of inhibition (mm) and minimum inhibitory concentrations (MICs) in parenthesis is recorded in table 4.

Table 4: Anti bacterial assay of ZCREO

.N.	Essential oils	Zone of inhibition[mm](MIC[µl/ml])			
		E.coli	***S.enterica enterica***	***P.multocida***	***S. aureus***
3.	ZCREO	15(3.90)	16(3.90)	10(31.25)	12(31.25)
10.	Gentamicin	46(0.019)	39(0.019)	42(0.039)	41(0.0048)

DISCUSSION

A. galli was selected as a model for evaluating anthelmentic activity ofZCREO. These worms are easily available from freshly slaughtered birds and can survive in vitro for 48 h at 40±1^{o}C in Tyrode solution. The whole worm could be mounted easily in the organ bath to conduct in vitro spontaneous motility studies. *A. galli* is also recommended as a suitable model for screening of anthelmintic drug. [19] Many herbs, for example, the juice of *Clerodendron infortunatum* leaves, leaves of pine apple, powdered fruits of *Embelia ribes*, juice of *Erytrima indica* leaves, *Costus speciosus*, *Achyranthas traindra*; Juice of *Butea frundosa* seeds, *Hyoscyamus niger* and decoction of the roots of *Punica gramatum*, are used to destroy intestinal worms in indigenous system of medicine.[20,21] ZCREO at increasing cumulative concentrations caused dose-dependent suppression of frequency, amplitude and baseline

tension of contractility. The maximum decrease in frequency, amplitude and base line tension was observed at the cumulative dose of 28 mg/ml. The in vitro anthelmentic activity could be possibly due to the medicinally activity constituents, β- humulene or germacrene-D-4-ol, which are 20.3% and 2.4 %, respectively in ZCREO**.** [10] It has been reported that zingiberene and bisabolene, the aromatic principles while gingerols and shoals, the pungent principles present in oil of zingiberaceous plants are responsible for their medicinal activity**.** [22] In conclusion the rhythmic and gross motility in the present study suggested that ZCREO possessed anthelmentic activity against *Ascaridia galli* but it is 9.056 times less potent than albendazloe as far as anthelmentic efficacy is concerned.

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, resulting in an excess loss of fluid in the faeces. In some diarrhoeas, the secretory component predominates, while other diarrhoeas are characterized by hypermotility**.** [23] The use of castor oil induced diarrhoea model in our study is logical this have been implicated in the causation of diarrhoea in man. [24]

The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins which stimulate motility and secretion. [25] The results in the present study showed that there was dose- dependent increase in the time of onset of first wet faeces by ZCREO (up to 3mg/ml) which indicates that the peristaltic movement of the gastrointestinal tract of animals was reduced by the oil. This antidarrhoeal activity of the oil in the present study was further confirmed by its significant and dose-dependent decrease in number of wet fasces and total number of fasces. Among the there dose of ZCREO, the dose at the rate of 1 ml/kg body weight was found to be most effective as there was maximum increase in onset of first wet faeces as compared to other in number of wet faeces and number of total faeces. In this study atropine sulphate was taken as a reference drug and produced significant reduction in number observed of stools and increased intestinal transit time but ZCREO at the dose rate of 1 ml/kg body weight was found to be more effective than atropine sulphate (3 mg /kg).

According to the present investigation it can be concluded that the essential oil from rhizome of *Zingeber crysanthum* exhibited good anti-helmentic activity, antidiarrhoal activity and antibacterial activity. Hence the oil can be a natural antihelmentic, antidarrhoeal and antibacterial agent after proper clinical trials.

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