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Research Article

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Pharmacological Activities of 'Chandrakhya' Leaves

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ABSTRACT

The herbal drug 'Chandrakhya' is commonly used in Indian traditional medicine for thousands of years. Various activities of its rhizome have been reported by several researchers but the studies on leaves are not yet reported. The aim of this study is to explore the analgesic, antibacterial and an antifungal activity of *Zingiber officinale* (ZO) [Chandrakhya] leaves on albino rats. Aqueous and alcoholic extracts of *Zingiber officinale* leaves were selected at different doses and analgesic activity was evaluated by acetic acid induced writhing test and tail clip method. The anti-bacterial and anti-fungal activity of the ZO leaves was evaluated by employing well diffusion method against *S. aureus*, *E.coli*, *P.vulgaris* and *C. albicans*. It was found that the aqueous and alcoholic extracts at the dose of 400mg/kg showed significant analgesic activity as well as shown significant anti-bacterial and anti-fungal activity at the dose of 40 mg/ml. The present study shows the usefulness of the leaves of *Zingiber officinale* and indicates that its alcoholic extract is more active than the aqueous extract in analgesic, antibacterial and antifungal activities.

Keywords: *Zingiber officinale,* analgesic, anti-bacterial, anti-fungal.

INTRODUCTION

The *Zingiber officinale* (*Zingiberaceae*) is a slender, perennial rhizhomatous herb; leaves linear, sessile, glabrous; flowers yellowish green in oblong, cylindrical spikes; rhizomes white to yellowish brown, irregularly branched. [1-2] Commonly it is known as Chandrakhya, zanjabil, adraka, sunthi. [3] It is widely cultivated in tropical Asia, Africa. [4] The rhizomes contain volatile oil, resinous matter, starch and mucilage. Oil of ginger consists of monoterpenes, sesquiterpene hydrocarbons and the sesquiterpene alcohol. The pungency of ginger is due to gingerol. [5] *Zingiber officinale* rhizomes are used as carminative, stimulant, anti-inflammatory [6], anti-platelet, anti-bacterial, anti-emetic, anti-hyperglycaemic agent [7], immuno-stimulant [8], analgesic [9] and in atherosclerosis. [10] But studies on leaves are not reported. Therefore it was decided to study the antibacterial, antifungal and analgesic activities of alcoholic and aqueous leaf extracts of *Zingiber officinale*.

MATERIAL AND METHODS

Plant Material and Extraction

The leaves of *Zingiber officinale* [ZO] (Zingiberaceae) were collected from Bhopal and was deposited as a voucher specimen [no: VNSIPCG0928] in the herbarium of Department of Pharmacognosy, VNS Institute of Pharmacy and Research, Bhopal. The samples were cut into small pieces and dried in shade for 5 days. The dried leaves were then grinded. Sixty grams of the pulverized material was macerated separately in ethanol and distilled water for 72 hours with occasional shaking in dark. Macerate was decanted and filtered. The marc was pressed and filtration was done 2-3 times. The macerates were concentrated to give alcoholic extract [8.41 % w/w] and aqueous extract [11.13 % w/w].

Phytochemical studies

Freshly prepared extracts were subjected to phytochemical screening tests for the detection of various constituents using conventional protocol. [11]

Animals

Albino rats of Wistar strain (150-200 g) of either sex were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2°C; relative humidity 60-70 %) in a 12 h light-dark cycle. The rats were given a standard laboratory diet and water *ad libitum*. Food was withdrawn 12 h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee (reg. no.778/03/C CPCSEA).

Drugs

The following chemicals and drugs were used ibuprofen,

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paracetamol doxycycline griseofulvin (Signa Pharma, Kanpur) and acetic acid.

Acute toxicity study

No adverse effect or mortality was detected in albino rats up to 3 gm/kg, *p.o.* of ZO leaves during the 24 h observation period.

Analgesic activity

The alcoholic and aqueous extracts were suspended in normal saline and employed for analgesic activity. The analgesic activity was studied in rats of either sex by acetic acid induced writhing test and tail- clip method. [12]

By acetic acid induced writhing test

Animals were divided into six groups of six rats each. Group wise the animals received various doses of alcoholic and aqueous extract of test drug intraperitonealy [200, 400 mg/kg]. Control group received normal saline and the reference group received intraperitonealy 40 mg/kg of Ibuprofen. Drug pre-treatment was given one hour before administration of 0.6 % v/v acetic acid [10 ml/kg, i.p.]. The severity of pain response was assessed by counting number of wriths [constriction of abdomen, turning of trunk and extension of hind legs] in rats. Numbers of wriths produced in 15 min. were counted in each animal after the injection of acetic acid [Table-2]. Analgesic activity was calculated as % maximum possible effect [MPE] using the following relation:

% MPE = 100 × [mean of wriths in control group – mean of wriths in treated groups] / Mean of wriths in control group

By tail-clip method

Animals were fasted over night with water given *ad libitum*, maintained at room temperature and irresponsive rats were separated by testing all rats with tail-clip. Rats that did not commence continuous efforts to remove the clip within 15 seconds were rejected. Responsive rats were tested again before the administration of aqueous suspension of standard drug or drug extract. After 15, 30, 45 and 60 minutes, the clip was again applied and the analgesic response was recorded.

The rats were divided into six groups, each containing six rats. The first group of rats served as control [10 ml/kg of 1 % v/v aqueous Tween 80 solution]. Second group of rats were administered with standard drug paracetamol at a dose of 50 mg/kg body weight, orally. And the remaining groups were treated with the different doses [200, 400 mg/kg body weight, orally] of alcoholic and aqueous extracts of test drug. [Table-3]

Anti-microbial and Anti-fungal activity

The alcoholic and aqueous extracts were tested for anti-bacterial and anti-fungal activity against *S.aureas* [NCIM 2079], *E.coli* [NCIM 2931], *P.vulgaris* [NCIM 2027] and *C.albicans* [ATCC 10231] by agar-well diffusion method. [13-14] The zone of inhibition of the extract solutions of concentrations 20 mg/ml and 40 mg/ml was determined. In each bore 50µgm/ml of extracts were introduced. The petri dishes were then incubated at 37°C for 24 h [bacterial] and 24°C for 72 h. [fungal]. Doxycycline [20 and 40 mg/ml] and Griseofulvin [12.5 and 25mg/ml] were used as the standard drug for comparison. The zones of inhibitions were noted. [Table-1]

Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A *P value <0.05* was considered significant.

Table 1: Analgesic activity of aqueous and alcoholic extracts of *Zingiber officinale* (leaves) on acetic acid induced writhings in rats

S. No.	Treatment	Dose [mg/kg body wt.]	Mean No. of wriths ±SEM	% Inhibition of wriths (MPE)
1.	Control	-	30.1±2.10	-
2.	Standard drug [Ibuprofen]	40	5.9±0.98**	80.39**
3.	Aqueous extract	200	22.5±1.94	25.32
		400	15.3±1.25	49.27
4.	Alcoholic extract	200	18.7±1.07*	37.81*
		400	8.4±1.12**	72.23**

***p<0.01, **p<0.001; n= 6 in each group**

RESULTS

The results of experiments are shown in [Table-1] [Table-2] and [Table-3]. In the acetic acid induced writhing test the extracts suppressed the acetic acid-induced writhing response significantly in a dose-dependent manner. The results were found to be highly significant (*P<0.001*) in comparison to the control. The percentage inhibition of wriths in acetic acid induced writhing test for assessment of analgesic activity shows that the alcoholic extract of the leaves was more effective than the aqueous extract at the dose of 400 mg/kg [Table-1]

In tail clip method the analgesic response mean time in seconds increased significantly for the test and standard groups after fifteen min drug administration [Table-2]. The test drug produced a dose-dependent increase in the reaction time at various time intervals of observation. Preliminary phytochemical analysis of the extracts of ZO leaves revealed the presence of terpens, resins, phenolics and flavonoid compounds.

The zone of inhibition obtained with different concentration of the extracts and the standard drugs are presented in [Table-3]. The aqueous and the alcoholic extract shown significant anti-bacterial and anti-fungal activity at the dose of 40 mg/ml. And alcoholic extract was more effective than the aqueous extract.

DISCUSSION

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. The number of writhing movements of the alcoholic extract of the leaves was more significant at the dose of 400 mg/kg. In the tail flick model, the test drug in different doses increased the pain threshold significantly during the period of observation and this indicates the involvement of a higher center. The results also suggest that the aqueous and alcoholic extracts of ZO leaves in doses of 200 and 400 mg/kg significantly shown the analgesic activity in acetic acid-induced writhing and tail flick models. However, the analgesic activity of ZO leaves was found to be more significant on the acetic acid-induced model *(P<0.001)* than the tail flick model *(P<0.01)* and thus it appears that the test drug inhibits predominantly the peripheral pain mechanism.

On preliminary phytochemical screening the aqueous extract of ZO leaves was found to contain terpens, resins, phenolics and flavonoid compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception. [15] Hence, the presence of flavonoids and gingerols may be contributory to the analgesic activities of ZO leaves. Gingerol and its analogs found in

Table 2: Analgesic activity of aqueous and alcoholic extracts of *Zingiber officinale* (leaves) by tail-clip method

S. No.	Treatment	Dose [mg/kg body wt. p.o.]	Analgesic Response Mean time in seconds ± SEM			
			15 min.	30 min	45 min	60 min
1.	Control	-	2.4±0.4	2.6±0.29	2.8±0.24	2.9±0.21
2.	Standard drug [paracetamol]	50	5.1±0.45	7.2±0.33**	8.1±0.41**	8.8±0.40**
3.	Aqueous extract	200	1.9±0.72	2.7±0.56	2.9±0.26	3.3±0.81
		400	4.6±0.56	5.8±0.67	5.9±0.49	6.8±0.77
4.	Alcoholic extract	200	2.3±0.41*	3.1±0.28*	3.2±0.72*	3.7±0.69*
		400	5.7±0.64**	6.3±0.37**	6.9±0.38**	7.7±0.82**

***p<0.01, **p<0.001; n= 6 in each group**

Table 3: Anti-bacterial and anti-fungal activity of the *Zingiber officinale* (leaves)

S. No.	Microorganisms	Zone Of Inhibition [In mm]							
		Aqueous Extract		Alcoholic Extract		Standard Doxycycline		Standard Griseofulvin	
		a	b	a	b	a	b	a_1	b_1
1.	***S. AUREUS***	10	14	11	15	18	20	-	-
2.	***E. COLI***	13	18	13	18	12	17	-	-
3.	***P. VULGARIS***	12	14	13	15	14	16	-	-
4.	*C. albicans*	08	10	09	12	-	-	11	12

Where a= 20mg/ml, b=40mg/ml, a_1= 12.5mg/ml, b_1= 25mg/ml

rhizome extracts are responsible for many pharmacological activities. [16-17]

Several types of terpene compounds are known to present antiinflammatory and antinoceptive activities. [17-18] Further studies may reveal the exact mechanisms of action responsible for the analgesic and antimicrobial activities of ZO leaves.

Hence from the above results it was evident that the leaves have shown significant antibacterial, antifungal and analgesic activity and can be used with ginger rhizomes proving the folklore claims.

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