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ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF LEAVES OF WHITE FLOWERED VARIETY OF *CLITORIA TERNATEA* LINN.

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ABSTRACT

In this study, we investigated the effects of methanolic extract of *Clitoria ternatea* (MECT) leaves on anti-inflammatory and analgesic activities in vivo using rat as an animal model at the doses of 250 mg/kg and 500 mg/kg body weight. The anti-inflammatory activities were investigated by utilizing carrageenan induced paw edema in rat. The analgesic activity was examined against hot plate method in rats. The results showed that CT significantly (p<0.001) reduced carrageenan induced paw edema in rats. In hot plate method, methanolic extract of *Clitoria ternatea* has shown increase in reaction time i.e. paw licking maintained at 55°C indicating analgesic activity. Preliminary phytochemical screening of the extract revealed the presence of phenolic compounds, alkaloids, tannins, glycosides and flavonoids. These results demonstrated that the methanolic extract of *Clitoria ternatea* (CT) leaves exhibited significant analgesic and anti-inflammatory activities.

INTRODUCTION

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agents as well as to remove the consequent necrosed cells and tissue and it is manifestation of the body's response to tissue damage and infection. The result of each inflammatory reaction may be beneficial (defense the body against agents deranging its homeostasis) or harmful (damage to surrounding tissues). Pain and fever are being the most common complaints associated with inflammation. The NSAIDs used in the inflammatory conditions do not cure and remove the underlying cause of the disease but they only modify the in flammatory response to the disease. Large numbers of NSAIDs are available in the market with their advantages and disadvantages. Though there are standard drugs like Aspirin, Indomethacin, Phenylbutazone, etc., these drugs limitation. Herbal medicines used in Ayurveda remain the major source of health care for the world's population.

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are not entirely free of side effects and have their own WHO has recognized herbal medicine as an essential building block for primary health care of vast countries like India [1]. *Clitoria ternatea* L. (CT) a perennial twining herb, found throughout India in tropical areas. CT is commonly known as 'Butterfly pea' belongs to Family: Fabaceae. CT has two flowered varieties one is white flowered variety and second blue flowered variety. CT has been traditionally used as a remedy for various disease like urinogenital disorder, bronchitis, purgative, diuretic, anthelmintic, rheumatism, demulcent, anticancer, antidote for animal stings [2-5].

CT has been used as an ingredient in 'Medhya Rasayana' a rejuvenating recipe used for treatment of neurological disorders [6]. CT has been scientifically studied for various pharmacological activities like antioxidant [7], local anesthetic [8], anthelmintic [9,10] antipyretic, anti-inflammatory, analgesic [11], anxiolytic, antidepressant, anticonvulsant, sedative [12], hypoglycemic[13], anticancer [14] also enhances acetylcholine content in rat hippocampus [15]. A wide range of chemical constituents are present in CT. The anti-inflammatory activity of leaves and seeds of blue flowered

variety CT was carried out. Aim of this present study to explore the anti-inflammatory and analgesic potential white flowered variety of *Clitoria ternatea* L.

MATERIAL AND METHOD

Collection, Authentication and Extraction of Plant material

The leaves of white flowered variety of *Clitoria ternatea* were collected from local habitat. The plant specimens were authenticated (Specimen No. 9493) by Botany Department Rashtrasant Tukdoji Maharaj, Nagpur University, Nagpur. The leaves of white flowered variety of *Clitoria ternatea* were dried at room temperature. The dried leaves were subjected to size reduction to get coarse powder by using grinder. This powder was packed into soxhlet apparatus and extracted with methanol [16]. The extracts were evaporated to dryness at 40^{o}C (yields 10.5% w/w). A phytochemical screening of residues was performed for the presence of phytochemical constituents [17,18].

Animals

Healthy adult albino wistar rats weighing about 180-200gm were obtained from TVES HLMC College of Pharmacy, Faizpur. These animals were housed in polypropylene cages and fed on standard pellet diet and provided with water *ad libitum* during the experiment. The animals were housed under standard conditions (24-28^{o}C, relative humidity 60-70%, 12 h light and 12 h dark cycle). Ethical clearance for the handling of experimental animals was obtained from Institutional Animal Ethics Committee (Reg. No. 652/02/a/CPCSEA).

Acute toxicity testing

Healthy Swiss albino mice of either sex, starved and divided into 5 groups. These animals orally fed the methanolic extract of CT (MECT) in increasing dose level of 250, 500, 1000, 1500, 2000 mg/kg body weight. Mice were observed for 24 hours for any lethality [19-21].

Anti-inflammatory activity

Carrageenan-induced paw edema test

Inflammation was produced by administering 0.1 ml of (1%) carrageenan into sub-plantar surface of rat hind paw. Albino rats of either sex weighing 150-250 g were fasted overnight with *ad libitum* access to water. The animals were divided in to four groups (n=6)

Group I : Normal saline solution (5 ml/kg) + Carrageenan (0.1ml of 1% in normal saline)

Group II: Diclofenac sodium (10mg/kg, *p.o.*) + Carrageenan (0.1 ml of 1% w/v in saline solution

Group III : MECT (250 mg/kg, *p.o.*) + Carrageenan (0.1 ml of 1% w/v in saline solution

Group IV : MECT (500 mg/kg, *p.o.*) + Carrageenan (0.1 ml of 1% w/v in saline solution.

In the present study, a mark was made on right the hind paws just below the tibio- tarsal junction so that every time the paw could be dipped in the column of the plethysmograph upto the mark to ensure constant paw volume. The extract at the dose level of 250 and 500 mg/kg body weight were administered orally to the treated group and Diclofenac sodium at the dose level of 10 mg/kg was administered orally to the standard group. After 30 min. an inflammatory edema was induced in the left hind paw by injecting of carrageenan (0.1 mL) in saline (1% w/v), in the planter tissue of all the animals. The paw volume was measured at 0min, 15min; 30min; 60min; 120min. and 180min. after administration of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume [22-24].

Analgesic activity

Hotplate Method

The method as described by Wolfe and Mc Donald (1944) was used. In this method Wister male albino rats (180-200 g) were used for the study. The animals were segregated into four groups of six animals each.

Group 1 - Normal saline solution (5 ml/kg),
Group 2 - Aspirin as standard (25 mg/kg),
Group 3 – Methanol extract (250 mg/kg),
Group 4 - Methanol extract (500 mg/kg),

Methanolic extracts were administered orally using intragastric tube. The animals were placed gently on Eddy's hot plate, set at 55^{0}C temperature. The pain threshold (Number of licking of paw/jumping) were measured at 0, 30, 60, 90 min after administration of standard and test solution. The animals were removed from hot plate soon after they exhibited jumping. Cut off time was 20 seconds [25].

RESULT & DISCUSSION

Acute toxicity study revealed the non-toxic nature of extracts of Clitoria ternatea. There was no lethality or any toxic reactions found at any of the doses selected. A phytochemical screening of residues revealed the presence of Phenolic compounds, tannins, flavonoids, glycosides, triterpennoid, sterol, carbohydrates and protein. The effect of methanolic extract of *Clitoria ternatea* on carrageenan-induced rat paw edema was showed in Table 1. After administration of methanolic extract of *Clitoria ternatea* (MECT-I) at 250 mg/kg orally, the paw volume was reduced by 56.86%, whereas after administration of methanolic extract of *Clitoria ternatea* (MECT-II) at 500 mg/kg orally showed 66.66 % inhibition after 3 h, which indicate that the effect of MECT 250 and 500 mg/kg showed anti-inflammatory activity in a dose-dependent manner. The standard drug showed the 70.58% inhibition of edema after 3 h. The standard drug and MECT 500 mg/kg drug treated animals showed statistically significant (*p<0.001) inhibition of paw edema.

Table 1. Anti-inflammatory activity of *Clitoria ternatea* (Carrageenan-induced paw edema test)

Treatment	Dose mg/kg	Mean paw volume in ml						Percent Inhibition
		0 min	15min	30min	60min	120 min	180 min	
Control	Saline solution 5ml/kg	0.79 ±0.03	1.04 ±0.08	1.29 ±0.06	1.57 ±0.09	1.78 ±0.08	1.53 ±0.09	--
Standard drug	10	0.77 ±0.01	0.86 ±0.06	0.69 ±0.04**	0.57 ±0.09*	0.48 ±0.05*	0.45 ±0.05*	70.58
MECT-I	250	0.73 ±0.07	1.02 ±0.07	0.91 ±0.07	0.79 ±0.06	0.71 ±0.08**	0.66 ±0.05**	56.86
MECT-II	500	0.78 ±0.09	0.87. ±0.08	0.81 ±0.006	0.70 ±0.08**	0.58 ±0.09*	0.51 ±0.05*	66.66

Figures in parenthesis indicate edema inhibition percentage, *N*=6 animals per group
**p*<0.001by Student's t-test when compared with the corresponding values of control
***p*<0.05 by Student's t-test when compared with the corresponding values of control

Table 2. Analgesic activity of *Clitoria ternatea* (Hot plate method)

Sr. No.	Drugs	Latency of paw licking (after treatment)			
		At 0 Min	At 30 Min	At 60 Min	At 90 Min
1	Normal saline solution	4.3 ± 0.6	4.5 ± 0.7	5.6 ± 0.4	5.3 ± 0.3
2	Aspirin as standard (25 mg/kg),	4.6 ± 0.8	7.2 ± 0.8	9.6 ± 0.6	8.1 ± o.6
3	MECT (250 mg/kg),	4.1 ± 0.4	5.8 ± 0.5	6.5 ± 0.6	6.1 ± 0.4
4	MECT (500 mg/kg),	4.5 ± 0.35	7.0 ± 0.2	8.3 ± 0.3	7.9 ± 0.2

The analgesic effect of methanolic extract of *Clitoria ternatea* on Eddy`s hot plate method were showed in Table 2. The increase in reaction time to thermal stimuli indicating analgesic activity. The paw licking time of MECT 250 & 500 mg /kg treated animals is extended. The reaction time for the hot plate is increases up to 6.5 & 8.3 seconds respectively. Aspirin (25 mg/kg) treated animals showed increase in reaction time up to 9.6 seconds. MECT showed the anti-inflammatory as well as analgesic activity. The carrageenan-induced hind paw oedema model in rats is known to be the acute inflammatory model sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents (NSAID), which primarily inhibit the cyclooxygenase involved in prostaglandin (PG) synthesis.

CONCLUSION

Form the results obtained in the present study, it can be concluded that methanolic extract of *Clitoria ternatea* leaves possesses significant anti-inflammatory and analgesic effect which may be due to its inhibition of inflammatory mediators like cyclooxygenase (COX), prostaglandin [26].

The methanolic extract of *Clitoria ternatea* showed presence of Phenolic compounds, tannins, flavonoids, glycosides, triterpennoid, sterol compounds which may be further supported its potential for anti-inflammatory and analgesic activity.

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