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Evaluation of Analgesic activity of methanolic seed extract of *Clitoria ternatea*

Prathibha B Mare^{a,*}, Muralidhara R Dowlathabad^b, Venu G Khothakota^c, Pavan K Balagani^d.^{a,*} Department of Pharmaceutical Biotechnology Nirmala College of Pharmacy, Kadapa-516002, Kadapa, Andhra Pradesh.^b Department of Biotechnology Sri Krishnadevaraya University, Anantapur-515003, Anantapur, Andhra Pradesh.^c Department of Pharmaceutics, Nirmala College of Pharmacy, Kadapa-516002, Kadapa, Andhra Pradesh.^d Department of Pharmaceutics Gokula Krishna College of Pharmacy, Sullurpet-524121, Nellore Dist, Andhra Pradesh.

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*Corresponding author:

Prathibha B Mare

E-mail:

mprprathibha@gmail.com

Tel.: +91-7893753239

Abstract

Aim The objective of the present investigation was to study the analgesic activity of flavanol glycosides isolated from seeds of *Clitoria ternatea*.

Materials and methods The methanolic extract isolated from the seeds of *Clitoria ternatea* showed analgesic activity. The methanolic extract of *Clitoria ternatea* was subjected to phytochemical tests to identify the nature of chemical constituents present in plant material. The analgesic activity was studied in mice using acetic acid induced writhing and hot tail flick methods.

Results The methanolic extract and the isolated phytochemicals exhibited significant activity in the above methods.

Conclusion The methanolic extract of seeds of *Clitoria ternatea* possesses significant analgesic activity.

KEYWORDS: Methanolic seed extract, Hot tail flick method, Acetic acid induced writhing, Aspirin, Paracetamol.

INTRODUCTION

Clitoria ternatea is commonly known as Butterfly pea, Blue pea (English), Aparajita (Hindi), Dintena (Telugu), is a tropical herb belonging to the family Fabaceae. The plant is used as a brain tonic to promote memory and intelligence. The plant extract is used in a rejuvenating recipe to treat neurological disorders. Tribes use the root to induce abortion and to reduce abdominal swellings, sore throats and mucous disorders [1]. The juice of the root is mixed with cold milk and is drunk to remove phlegm and for chronic bronchitis [1]. *Clitoria ternatea* possesses the following pharmacological actions as monoamine Oxidase inhibiting activity, antioxidant activity, hypolipidemic, immunomodulatory, blood platelet aggregation inhibiting activity, vascular smooth

muscle relaxant, antiulcer, enhancing the memory [2], diuretic activity, local anesthetic effect, spermicidal activity, hepatoprotective and gastroprotective, Cardiovascular activity, antituberculosis, antidiabetic [3].

The roots are bitter, refrigerant, laxative, diuretic, antibacterial [4], anthelmintic and toxic and are useful in dementia, hemicranias, burning sensation, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites and fever while the leaves are useful in otalgia and hepatopathy and the leaves cathartic [5]. The plant is considered useful for eye infections, skin diseases, urinary troubles, ulcers, and has antidotal properties [6].

MATERIALS AND METHODS

Plant Material

Clitorea ternatea plant was collected from the local area of Kadapa, and the plant material was identified and authenticated by Dr. K. Madhava Chetty, S V University, Tirupathi. The collected seeds were shade dried under normal environmental conditions, powdered, stored at 4-6°C in refrigerator, in a closed container for further use.

Drugs and Chemicals

Aspirin, Paracetmol (Gift obtained from Aurobindo pharmaceuticals, AP), Methanol (Merek Pvt, Mumbai) and other chemicals were procured from supplier.

Preparation of extract

The powdered seeds were passed through a sieve (No. 60) and then those seeds (40gm) of *Clitorea ternatea* were extracted by sox let extraction with methanol as a solvent for 14 hrs at ambient temperature and the extract was dried under vacuum at a temperature not exceeding 50°C. The extract was subjected to various phytochemical screening tests for active constituents.

Phytochemical analysis

The methanolic extract of *Clitorea ternatea* was subjected to phytochemical tests to identify the nature of chemical constituents present in plant material.

Animals

Swiss albino mice of either sex weighing between (20 – 25 gm) were used for the present study. They were taken and grouped into 6 each consisting of 6 mice. They were housed in polypropylene cages maintained under standard conditions (12 hrs light/12 hour dark cycle) 25±3°C and 30–60 % humidity. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting.

Evaluation of analgesic activity

The suspension of extracts was prepared in sterile 0.9% NaCl solution. In all cases control received the same quantity of sterile 0.9% NaCl solution as vehicle. Analgesic activity was evaluated by two methods namely acetic acid induced writhing test and Hot tail flick methods.

Writhing tests:

The abdominal constriction test described by collier et al., (1968) [7] was followed to measure the analgesic actions of methanolic extract of *Clitorea ternatea* and the standard drug viz Aspirin. Swiss albino mice weighing between 20 – 25 gms were fasted for 24 hours with water given ad libitum. The animals were pretreated with 4% w/v acacia gum solution (0.2

ml/10gms b.w., i.p), methanolic extract of *Clitorea ternatea* CT II (400mg/kg b.w., i.p), *Clitorea ternatea* (CT III, 600mg/kg b.w., i.p) and aspirin (50mg/kg b.w., i.p) 30 minutes prior to administration of 0.6% v/v acetic acid (i.p) to cause typical stretching response. Writhing or Stretching was counted for a period of 15 minutes. The analgesic actions of drug were measured by calculating the mean reduction in the abdominal constructions for each drug as compared to the control group treated with vehicle. The % inhibition in writings was calculated by the following formula:

$$\% \text{ Inhibition of writhings} = \frac{\text{Avg writhings in control} - \text{Avg writhings in test}}{\text{Avg writhings in control}} \times 100$$

Hot tail flick method:

Swiss albino mice of either sex weighing between 20–25 gms were fasted for 24 hrs with water given ad libitum maintained at room temperature and was divided into 4 groups. Mice were treated with 4% w/v acacia gum (0.2ml/10gm b.w., i.p), with methanolic extract of CT II (400mg/kg b.w., i.p), CT III (600mg/kg b.w., i.p) and Paracetmol (100mg/kg b.w., i.p). Analgesic action of the test substance was measured using the method described by Sewell and Spencer (1976) [8]. One to two cm of the tail of mice was immersed in warm water maintained at 50°C. The response for the activity was measured by recording time required to deflect their tails. The latent period of the tail flick response was taken as analgesic and was determined before and at 15, 30, 45, and 60 minutes later the administration of drugs. The maximum reaction time was fixed at 15 seconds. The maximum possible analgesia (MPA) was calculated as

$$\text{MPA} = \frac{\text{Test reaction time} - \text{saline reaction time}}{15 \text{ saline reaction time}} \times 100$$

Statistical analysis: The experimental data was analyzed by two tail student's t test. Data were presented as mean $\pm$ SEM ANOVA of 6 observations.

RESULTS

Phytochemical screening:

Preliminary phytochemical screening of methanolic extract of *Clitoria ternatea* showed that it possess alkaloids, carbohydrates, flavonoids, phenolic compounds, gum mucilage, glycosides, triterpinoids, volatile oils etc.

Acetic acid induced writhing:

Administration of acetic acid through intraperitoneal route produced abdominal constrictions which are characterized as stretching response. Mean writhing observed in control treated with the vehicle over a period of 15 minutes was 31.67 ± 0.537 counts. Methanolic extract of *Clitoria ternatea* (CT II) and (CT

III) significantly reduced writhing ($p < 0.001$) to 14.5 ± 0.174 , 7.67 ± 0.333 counts. This writhings test also indicated that 50 mg/kg of Aspirin is equipotent to 600 mg/kg of CT III as shown in Table 1.

Hot tail flick response:

The % MPA was found to be 13.603 ± 0.715 , 22.1 ± 0.577 and 27.419 ± 0.857 for methanolic extract of CT II, CT III and paracetamol respectively. Group treated with paracetamol also exhibited analgesic activity which began at 15 minutes following the administration of the drug and the effect tested significantly throughout the 60 minutes of observation ($p < 0.001$). Similarly analgesic effect of test extract was also observed at 15 minutes following the administration of the test substance however the analgesic effect decreased with increase in time as shown in Table 2 & 3.

Table. 1: Analgesic activity of Methanolic extract of CT II and CT III using acetic acid induced writhings.

Groups	Number of writhings(mean $\pm$ SEM)	% inhibition of writhings
Control (I)	$31.67 \pm 0.537^{***}$	----
C.T II	$7.67 \pm 0.33^{**}$	75.78
C.T III	$5.16 \pm 0.125^*$	83.7
Aspirin (IV)	$5.5 \pm 0.428^*$	82.8

n = 6, $p < 0.001$, compared II, III, IV with and analgesic activity of methanolic extract of CT II and CT III using acetic acid induced writhing method.

Table. 2: Analgesic activity of methanolic extract of CT II and CT III using Hot tail flick method

S.No	Time in mins	% mean possible analgesia $\pm$ SEM	
		CT II	CT III
1	0	$0.429 \pm 0.387^*$	$2.803 \pm 0.494^*$
2	15	$13.603 \pm 0.715^{***}$	$22.131 \pm 0.577^{***}$
3	30	$15.963 \pm 0.948^{***}$	$27.684 \pm 1.37^{***}$
4	45	$11.396 \pm 0.577^{**}$	$16.764 \pm 0.703^{***}$
5	60	$2.051 \pm 0.428^*$	$5.841 \pm 0.365^*$

n=6, $p < 0.001$ compared II, III & IV and the analgesic activity of methanolic extract of CTII and CTIII using Hot tail flick method.

Table. 3: Analgesic activity of Paracetamol using Hot tail flick method

S.No	Time in mins	% mean possible analgesia $\pm$ SEM
1.	0	$3.225 \pm 0.0008^*$
2.	15	$27.419 \pm 0.857^{***}$
3.	30	$27.419 \pm 0.857^{***}$
4.	45	$27.419 \pm 0.857^{***}$
5.	60	$27.419 \pm 0.857^{***}$

DISCUSSION

Phytochemically the Ethanolic extract of *Clitoria ternatea* showed the maximum phytochemical like alkaloids, carbohydrates, steroids, proteins, phenol compounds, flavonoids, gum mucilage, glycosides, triterpenoids, volatile oils. The methanolic seed extract showed effective analgesic activity compared with other parts of the plant. NSAID such as aspirin used in the study are known to inhibit cyclooxygenase enzymes I & II which implicated in the production of inflammation mediated agent prostaglandin (PGE₂) from arachidonic acid [9]. The pattern of analgesic activity exhibited by the extract is similar to that of aspirin which suggest that the plant activity may be mediated by cyclooxygenase I & II inhibition. The analgesic activity of the methanolic extract of *Clitoria ternatea* was may be due to inhibition of phospholipase A₂ or even blocking cyclooxygenase (Cox-1 & Cox-2).

Conclusion:

The methanolic extract of seeds of *Clitoria ternatea* is having effective analgesic activity. The result produced by these methods are dose dependent, significant when

compared with control groups. The analgesic activity was found to be more significant on the acetic acid induced writhing method and the dose used was non-toxic.

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