British Journal of Pharmaceutical Research
6(4): 255-260, 2015, Article no.BJPR.2015.066
ISSN: 2231-2919

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of Analgesic Activity and Phytochemical Screening of *Clitoria ternatea* Linn

Shital S. Chavan[1*], Ravindra S. Jadhav[2], Deepak Kharat[2], Someshwar D. Mankar[2] and R. K. Godge[2]

[1]Department of Pharmacognosy, University of Mumbai, Government College of Pharmacy, Ratnagiri, Maharashtra, India.
[2]Department of Pharmacognosy, University of Pune, Pravara Rural College of Pharmacy, Loni, Maharashtra, India.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2015/13593
Editor(s):
(1) Syed A. A. Rizvi, Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, USA.
(2) Alyautdin Renad N, The Department of Pharmacology (Pharmaceutical Faculty), I. M. Sechenov MSMU, Moscow, Russia.
Reviewers:
(1) Anonymous, India.
(2) P. G. Latha, Ethnomedicine and Ethnopharmacology Division, Jawaharlal Nehru tropical Botanic Garden and Research Institute (JNTBGRI), India.
(3) Halima Sadiya Abdullahi Gero, Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=985&id=14&aid=8505

Short Research Article

Received 25th August 2014
Accepted 21st November 2014
Published 17th March 2015

ABSTRACT

Clitoria ternatea L. is a member of the family Fabaceae, commonly known as 'Aparajita' or Girikarnika. It is a perennial climber widely used in the traditional Ayurvedic system of Indian medicine for treating a wide variety of ailments. Nowadays available drugs for the management of pains, fever, and inflammation like conditions are having many adverse effects; hence there is need for the drugs from other safer sources which will be highly safer having little or no side effects. For this purpose, *Clitoria ternatea* Linn leaves (Fabaceae) were screened for its phyto and pharmacological especially analgesic properties using hotplate and tail immersion method with mice. The analgesic study of *Clitoria ternatea* Linn leaves showed that the petroleum ether extract of the leaves have significant activity compared to pentazocin which is used as a standard. Generally Tannins, flavonoids, alkaloids and saponins are responsible for the analgesic and anti-inflammatory activities in many medicinal plants; hence phytoconstituents from plant leaves are also

**Corresponding author: Email: shitalchavan28@ymail.com;*

studied. These results may explain the use of the plant for the management of pains and its related ailments in the region where it is commonly found.

Keywords: Analgesic activity; Clitoria ternatea; hot plate method; tail immersion test.

1. INTRODUCTION

In the traditional system of medicine 'Aparajita' is considered as a 'Medhya' drug to improve intelligence and enhance memory function [1]. It is also used in the treatment of chronic bronchitis, dropsy, goiter, leprosy, mucous disorders, sight weakness, skin diseases, sore throat and tumors. It was used traditionally to cure sexual ailments, like infertility and gonorrhea, to control menstrual discharge, and also as an aphrodisiac. Aparajita grows throughout India. It is a beautiful-looking plant, hence cultivated in gardens. It is a perennial twining herb having seven leaflets, which are elliptic and obtuse; there are few varieties with white, violet and blue flowers [2, 3,4]. The plant is used in Analgesic, Anti-inflammatory, Antipyretic, Antioxidant, blood purifier, it is an antiemetic. Antidypsetic Mild-laxative and cholagogue Therefore it is used in emesis, dyspepsia, constipation jaundice and piles. *C.ternatea* was showing the most promising mosquito larvicidal activity [5,6]. It contains glycosides, flavonal glycosides and resin glycosides. Three flavonol glycosides are 3- O- (2″- O – α – rhamnosyl - 6″ - O - malonyl) – β - glucoside, 3 – O -(2″-O-α-rhamnosyl-6″-O-malonyl)-β-glucoside, and n 3-O-(2″,6″-di-O-α-rhamnosyl)-β-glucoside together with eleven known flavonol glycosides were present in the petals of *Clitoria ternatea* [1,7]. Crude protein and crude fibre in the leaves were 21.5% & 21.5-29% respectively. Total plant protein ranges from 14-20%., sigmast-4-ene-3, 6, diene, roots contain taxaxerol and resin. The seeds contain a fixed oil, a bitter acid resin (the active principle), tannic acid, glucose (a light brown resin) and ashand taxaxerone. The leaves contains 3 – o – rhamnosyl-galactoside of kaemferol,delphinidin-3,5-diglucoside, delphinidin - 3β - glucoside, and its 3 methyl derivative, , kaemferol and cynodin chloride seeds contains myricetin 3 – 2 G - rhamnosylrutinoside; kaempferol 3-2 Ghamnosylrutinoside; 8, quercetin 3 - rutinoside; quercetin 3- glucoside; myricetin 3-glucoside.The root bark contains starch, tannin [1,7].

Analgesics are the one used to cure or decrease pain. Pain as a sensation is explained as an unpleasant emotional and sensory experience which is associated with potential tissue damage according to International Association for the study of Pain (IASP) or defined in terms of such damage [8,9]. The classical analgesic drugs primarily including non steroidal anti-inflammatory drugs and opiates have their origin in natural products but many synthetic compounds which act by the same mechanism have been synthesized and are having serious adverse side effects such as gastrointestinal bleeding, ulceration, additive potential, drowsiness, respiratory distress, nausea etc [10,11]. On the other hand drugs of plant origin have been used for management of diseases for many centuries and have not been reported of any deleterious effects to their hosts. Literature survey reports that leaves of *Clitoria ternatea* L. is used traditionally as astringent and in the treatment of ulcers [10,11]. Not much scientific work is yet done on the plant leaves extract regarding its analgesic activity. So *Clitoria ternatea L*. was selected for study of analgesic activity [12,13].

2. MATERIALS AND METHODS

2.1 Plant Material

Leaves of *C. ternetea* were collected from Ahmednagar district, Loni, authenticated (voucher no. CLITTSHIS3) cleaned and dried at room temperature in shade, away from direct sunlight and ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

2.2 Preparation of Extract

Large difference in particle size of crude drug results in long extraction time as the coarse particles increases the extraction time and fine may form bed, so the powdered material was sieved through 60-120 mesh to remove fine and the remainder powder was subjected for further study. Hot continuous extraction Technique; Soxlet Extraction was used for sequential extraction with a series of solvents of increasing polarity i.e. petroleum ether and ethanol. Pet Ether extract was used for further studies. The percentage yield was calculated using the

formula below and the extract stored in a refrigerator at 15°C until time of use.

%yield =

$$\frac{weight\ of\ extracted\ material}{weight\ of\ original\ plant\ material\ used} X \frac{100}{1}$$

2.3 Animals

Young Swiss-albino mice of aged 4-5 weeks, either sex, average weight 20-25 gm were used for experiment and kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light / 12 hour dark cycle) for one week for acclimation with free access to food and water ad libitum. Animals were treated ethically, the set of rules followed for animal experiment were approved by the institutional animal ethical committee [14].

2.4 Acute Toxicity Test

Toxicity studies conducted as per internationally accepted protocol drawn under OECD guidelines in Swiss albino mice at a dose level up to 2000 mg/kg. Mice were fasted for overnight and maintained with water *ad libitum* and separated into different groups (n= 6). In fixed dose method, dose started from 2000 mg/kg body weight. The next day the product (suspended in 5% tween 80 solutions) was administered orally at a dose of 2000 mg/kg. After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 hr, and 24 hr to detect changes in the autonomic or behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep, and coma and then monitored for any mortality for the following 14 days [15,16].

2.5 Phytochemical Screening

Phytochemical screening of extracts was performed by doing various qualitative chemical tests to identify the active chemical constituents on the basis of characteristic colour changes using standard procedures. Presence of Carbohydrates was tested with Molisch's test, saponins were detected with the capability of producing suds, glycoside tested with water and sodium hydroxide solution, steroids with chloroform and sulphuric acid, presence of flavonoids was detected with Mg and HCl while that of tannins with ferric chloride solution, Alkaloids were identified with Mayer's reagent, Hager's reagent and Dagendorff's reagent and gum with Molish reagents and concentrated sulfuric acid [14,17,18].

2.6 Analgesic activity

2.6.1 Hot plate method

Central analgesic activity of petroleum ether extract was evaluated using hot plate method. The mice of either sex were divided into three groups of six animals each. The first group served as control and received only vehicle (2% DMF), second group was administered standard drug pentazocine (10 mg/kg, IP) dissolved in vehicle. The animals of third group were treated with petroleum ether extract (50 mg/kg, i.p., each). The animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5°C. A cut off period of 15 s was observed to avoid damage to the paw [19]. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90, 120,150,180 min after oral administration of the samples [20,21]. 0 min readings are the predrug reaction time.

2.6.2 Tail immersion test

The procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55°C. Mice of either sex were divided into three different groups each containing six animals. The animals were marked individually, weighed and numbered appropriately. The first group served as control and received only vehicle PSS (10 mL/kg). Second group was administered standard drug pentazocine (PTZ, 5 mg/kg p.o.) dissolved in vehicle. The animals of third group were treated with petroleum ether extract (300 mg/ kg). The lower 5 cm portion of each tail was immersed in beaker of water maintained at 55±0.5ºC. The time, in seconds, for tail withdrawal from the water was recorded as the reaction time at the time interval of 15, 30, 45 and 60 minutes, with a cut-off time for immersion set at 10s. The reaction time was measured 1 h before and after oral administration of extract (300 mg/ kg) or PSS (10 mL/kg) [22].

2.7 Statistical Analysis

The results of statistical analysis for animal experiment were expressed as mean ± SEM and were evaluated by ANOVA followed by Dunnet's

multiple comparisons. The results obtained were compared with the vehicle control group. The $p<0.05$, 0.001 were considered to be statistically significant.

3. RESULTS

3.1 Plant Extraction

The yield of the *Clitoria ternatea* L leaves extract was 3.95% w/w dry matter and was dark in colour.

3.2 Acute Toxicity Test

Acute toxicity test of the extract produced no death or signs of toxicity after 24 hours even at the dose of 2000 mg/kg which shows that the extract was well tolerated.

3.3 Phytochemical Screening

Phytochemical analysis of the pet ether extract of *Clitoria ternatea* L leaves revealed the presence of carotenoids, tannins, lipids, free sterol and triterpens.

3.4 Hot Plate Method

Pet ether extract of *Clitoria ternatea L* leaves showed significant analgesic activity at 50 mg/kg, i.p. dose as shown in Table 1 and Graph no.1. Analgesic activity was comparable with standard drug pentazocine. Petroleum ether extract contain tannins compounds so it shows significant analgesic activity From the result it may be concluded that tannins compounds plays important role in analgesia.

3.5 Tail Immersion Method

Pet ether extract of *Clitoria ternatea* L leaves showed significant analgesic activity at 50 mg/kg, i.p. dose as shown in Table 2 and Graph no. 2. Analgesic activity was comparable with standard drug pentazocine.

4. DISCUSSION

Anti-nociceptive models; hot plate and tail immersion tests were used to evaluate the analgesic activity of *Clitoria ternatea* L leaves. Since tests of analgesic drugs involves the reaction of animals to painful stimuli [23], the stimulus may be chemical (acetic acid-induced writhing or formalin tests) may be thermal (tail immersion or hot plate tests), or it may be mechanical (tail or paw pressure tests) [24]; here we used thermal stimulus to study analgesic activity. The pet ether extract of *Clitoria ternatea* L leaves produced no death or signs of toxicity even at the dose of 3000 mg/kg which suggests that the extract was well tolerated by the mice and that the doses used were safe. The pet ether extract of *Clitoria ternatea* L leaves showed a dose-dependent and significant increase in the pain threshold post-treatment with dose of extract in the tail immersion, and hot plate tests. The effects of the extract were significantly ($P < 0.001$) lower than those produced by pentazocin in same tests. The hot plate and tail immersion models have been used to study centrally acting analgesics [25]. Thus from the results, we can conclude that the analgesic activity of *Clitoria ternatea* L leaves may be fully mediated through central mechanism, as in all above tests, the nociceptors are sensitise by sensory nerves and the involvement of endogenous substances such as prostaglandins are minimized.

Table 1. Effect of pet ether extract of *Clitoria ternatea* L leaves on latency to hot plate test in mice

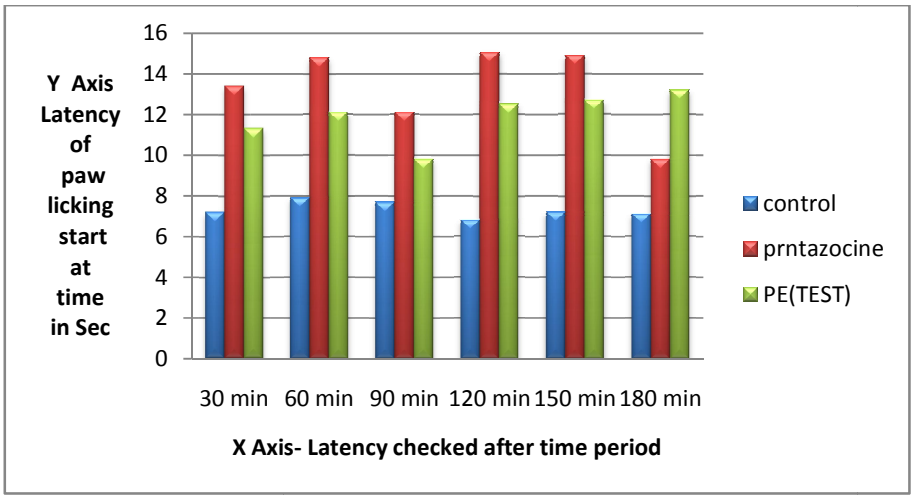
Treatment	Latency to lick the paws					
	30 min	60 min	90 min	120 min	150 min	180min
Control	7.2sec	7.9sec	7.7sec	6.8sec	7.2sec	7.1sec
Pentazocine	13.4sec	14.8sec	12.1sec	15.06sec	14.9sec	9.8sec
PE (Test)	11.3sec	12.2sec	9.8sec	12.5sec	12.7sec	13.2sec

PE: Pet ether extract of Clitoria ternatea L leaves

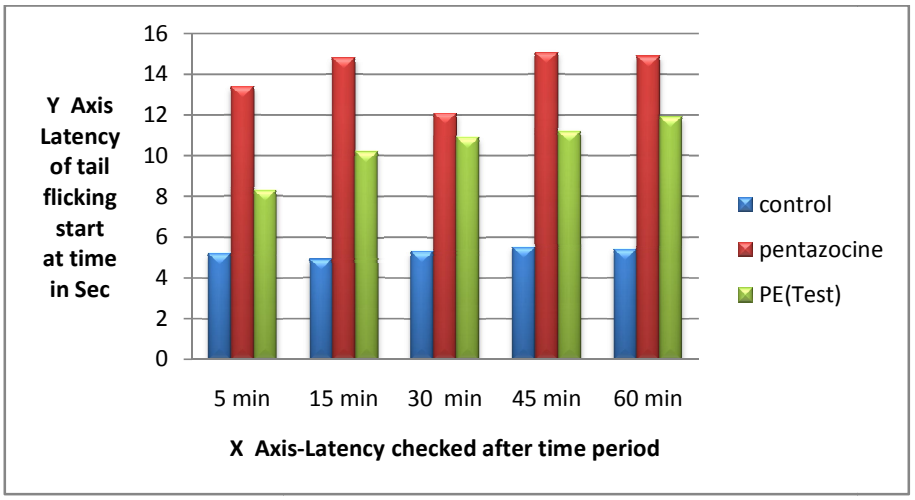
Table 2. Effect of Pet ether extract of *Clitoria ternatea* L leaves on tail immersion test in mice

Treatment	Latency to flick tail				
Time	5 min	15 min	30 min	45 min	60 min
Control	5.2 sec	4.9 sec	5.3 sec	5.5 sec	5.4 sec
Pentazocine	13.4 sec	14.8 sec	12.08 sec	15.06 sec	14.9 sec
PE	8.3 sec	10.2 sec	10.9 sec	11.2 sec	11.9 sec

PE: Pet ether extract of Clitoria ternatea L leaves



Graph 1. Effect of pet ether extract of *Clitoria ternatea* L leaves on latency to hot plate test in mice



Graph 2. Effect of pet ether extract of *Clitoria ternatea* L leaves on tail immersion test in mice

5. CONCLUSION

In conclusion, we can confirm that the pet ether extract of *Clitoria ternatea L* leaves shows central analgesic properties. However, further study is needed in order to understand the precise mechanism involved in the central analgesic effect. In future experiments, studies with purified fractions of the extract can be conducted for further phytochemical, pharmacological and toxicological characterization.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kulkarni C, Pattanshetty JR, Amruthraj G. Effect of alcoholic extract of *Clitoria ternatea* Linn. on central nervous system in rodents. Indian J. Exp. Biol. 1988;26:957-960.
2. Reid R, Sinclair DF. An evaluation of a collection of *Clitoria ternatea* for forage and grain production. Genetic Resources Communication. 1980;1:1-8.

3. Fantz Paul R. "Ethnobotany of Clitoria". Economic Botany (New York Botanical Garden Press). 1991;45(4):511–20.
4. Jain N. "Clitoriaternatea and the CNS". Pharmacology Biochemistry and Behavior. 2003;75:529.
5. Parimala DB, Boominathan R, Mandal SC. Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root. Parimala, Fitoterapia. 2003;74(4): 345-349.
6. Paul R. Fantz, omenclatural notes on the genus Clitoria.
7. Kenji O. Pain signalling pathways: from cytokines to ion channels. Int. J. B. C. B. 2007;39:490.
8. Merskey HM. Pain terms, Pain. 1986;3(5): 215-221.
9. Michael YD, Dubois CG, Allen HL. Chronic pain management in: Healy TEJ, Knight. PR eds. Wylie and Church-Davidson's: A Practice of Anaesthesis, 7th ed. London. Hodder Arnold. 2003;1235-1239.
10. Laurence DR, Benneth PN, Brown MJ. Clinical pharmacology. 8th edn. Edinburgh: ChurchHill Livingstone; 1997.
11. Mate GS, Naikwade NS, Chowki CSA, and Patil SB. Evaluation of Anti-nociceptive Activity of *Cissus quadrangularis* on albino mice. Int J Green Pharm. 2008;2:118–121.
12. Ezeja MI, Omeh YS, Ezeigbo II and Ekechukwu A. Evaluation of the Analgesic Activity of the Methanolic Stem Bark Extract of *Dialium guineense* (Wild). Annals of Medical and Health Sciences Research. 2011;1(1):55-62
13. Abdullahi MN, Ilyas N, Ibrahim H. Evaluation of Phytochemical Screening and Analgesic Activity of Aqueous Extract of the Leaves of *Microtrichia perotitii* Dc (Asteraceae) in Mice using Hotplate Method. Medicinal Plant Research. 2013; 3(5):37-43
14. Zulfiker AHM, Rahman MM, Hossain MK, Hamid K, Mazumder MEH, Rana MS. In vivo analgesic activity of ethanolic extracts of two medicinal plants- *Scoparia dulcis* L. and *Ficus racemosa* Linn. Biology and Medicine. 2010;2(2):42-48.
15. Stitzel K, Carr G. Statistical basis for estimating acute oral toxicity comparison of OECD Guidelines 401, 420, 423 and 425. Appendix O-1; 1999.
16. Banger OP, Jarald EE, Asghar S, Ahmad S. Antidiabetic activity of a polyherbal formulation (Karmin Plus). International Journal of Green Pharmacy. 2009;211-14.
17. Quibria T, Das BK, Hasan T, Uddin MA, Alam MM. Phytochemical screening and evaluation of analgesic activity of *Solanum surattense*. American Journal of Pharmaceutical Sciences and Nanotechnology. 2014;1(1):1-10.
18. Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses. 2nd edn. Asiatic Society of Bangladesh, Dhaka, Ramna. 2003;184.
19. Franzotti EM, Santos CVF, Rodrigues HMSL, Mourao RHV, Andrade MR, Antoniolli AR. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L. (Malva-branca). Journal of Ethno-pharmacology. 2000;72:273-8.
20. Eddy NB, Leimback D. Synthetic analgesic II, Dithienyl butenyl and dithienyl butylamines. Journal of Pharmacology and Experimental Therapeutics. 1953;107: 385–393.
21. Kulkarni SK. Hand book of experimental pharmacology. Vallabh Prakashan, Delhi, India. 1999;117.
22. Janssen PA, Niemegeers CJ, Dony JG. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal refl ex in rats. Arzneimittel Forschung/Drug Research. 1963;6:502-507.
23. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th edn. New Delhi India: Elsevier Science ltd; 2003.
24. George KA, Eric W, David DO, George AK. Antinociceptive Effects of *Newboulia Laveis* (P. Beauv) Stem Bark Extract in Rat Model. Pharmacog Mag. 2009;17:49–54.
25. Bachlav RS, Gulecha VS, Upasani CD. Analgesic and Anti-inflammatory Activity of *Argyreia speciosa* roots. Indian J Pharmacol. 2009;41(4):158–161.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=985&id=14&aid=8505