



E-ISSN: 2278-4136

P-ISSN: 2349-8234

JPP 2019; 8(3): 1846-1848

Received: 09-03-2019

Accepted: 13-04-2019

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## Phytochemical screening and pharmacological activity with respect to muscle relaxant property of methanolic extract of *Clitoria ternatea* (flower)

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### Abstract

**Aim:** The aim of this study is to investigate the phytochemical screening and muscle relaxant property of methanolic extract of *Clitoria ternatea* flower (MECT) belonging to the family Papilionaceae using Swiss albino mice in comparison with that of diazepam.

**Method:** The phytochemical evaluation of the extract was done as per the standard methods. Acute toxicity studies were done by using Swiss albino mice of either sex weighing 25-35 gm as per OECD 425 guidelines. The extract was evaluated for its muscle relaxant action compared with control (using normal saline 0.9% NaCl solution) and standard drug diazepam at the dose of 10mg/kg/ po using Rotarod apparatus. Fifteen mice of either sex were taken and divided in to five groups of 3 each. First group was considered as control, second as standard (Diazepam), third, fourth and fifth as test group (with three different doses of MECT like 50 mg/kg/p.o, 100 mg/kg/p o and 200 mg/kg/p o). The extracts were administered orally.

**Result:** The phytochemical screening exhibits that the extract contains various phytoconstituents like Alkaloids, Tannins, Glycosides, Carbohydrates, Resins, Steroids, Saponins, Flavonoids and Phenols. The extract was found to be non-toxic upto the dose of 2000 mg/kg after the acute toxicity test. All the three doses of MECT i.e. 50 mg/kg ( $11 \pm 0.023$  sec), 100 mg/kg ( $7.67 \pm 0.014$  sec) and 200 mg/kg ( $5 \pm 0.032$ ) significantly reduced the fall off time in the Rotarod apparatus as compared to control ( $49.33 \pm 0.028$ ) (p value  $< 0.0001$ ). The result is also quite satisfying when compared with the standard drug at 10 mg/kg/p o ( $6 \pm 0.019$ ).

**Conclusion:** The result of the given study demonstrated that the methanolic extract of flower of *Clitoria ternatea* is possessing dose dependent muscle relaxant property.

**Keywords:** *Clitoria ternatea*, MECT, Rota rod apparatus, Diazepam, albino mice

### Introduction

*Clitoria ternatea* is a climbing vine which is commonly known as butterfly pea. It grows in tropical temperate climate. The most striking feature about this plant is the colour of its flowers, a vivid deep blue or pure white and this study was done on the blue flower. The Leaves are alternate imparipinnate, 2-3 pairs; leaflets opposite, ovate, 2.5-4-5x2-3. 5cm obtuse at base, entire obtuse at apex, strigose. Flowers white or blue, solitary or in pairs (Plate-1). Calyx lobes lanceolate. Petals: Standard orbicular; wings oblong 9-10 cm long. Seeds 5-9, subglobose compressed <sup>[1]</sup>. The whole plant and seed extracts are used for stomatitis, piles, sterility in females, hematemeses, insomnia, epilepsy, psychosis, leucorrhea and polyurea <sup>[2]</sup>. The roots are bitter, refrigerant, laxative, intellect-promoting, diuretic, anthelmintic, tonic and are useful in dementia, hemicrania, burning sensations, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites, fever, otalgia, hepatopathy and as a cathartic <sup>[3]</sup>. The root, stem and flower are also used for the treatment of snake bite and scorpion sting. It has also been shown to have number of pharmacological activities such as possessing anxiolytic, antidepressant, anti-stress, sedative, antipyretic, analgesic, and antimicrobial activities <sup>[4]</sup>. In previous study the ethanolic extract of the root of *Clitoria ternatea* showed skeletal muscle relaxant activity <sup>[5]</sup>. The fruit of the plant is used therapeutically as a tonic, purgative, emetic and expectorant. It is also used as a spermicidal, in treatment of piles, hysteria, epilepsy and anti-implantation, etc. This study was done to evaluate the muscle relaxant activity of the methanolic extract of the flower of *Clitoria ternatea*.

### Material and Methods

**Materials:** The flowers of plant *Clitoria ternatea* Linn were collected from Assam, India in October. The taxonomical identification and authentication of the plant was confirmed by Dr. Partha Pratim Baruah, HOD, Department Of Botany, Guwahati University, Assam.

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The Phenylalanine, threonine, cystine, valine, aspartic acid and Specimen was deposited, with plant Acc.No.18208 and identified as *Clitoria ternatea* Linn. Family. Papilionaceae.

**Preparation of extract:** The flowers of *Clitoria ternatea* Linn were separated from the stem and sun dried for 2 weeks. Then it was powdered in a mechanical grinder and the plant powders (about 50 gram) placed inside a thimble made from thick filter paper, which was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a flask containing the extraction solvent. The Soxhlet was then equipped with a condenser and heated to reflux. The chamber containing the solid material was slowly filled with warm solvent and thus by using Methanol, Propylene glycol and Petroleum Ether the extraction of the flower of *Clitoria ternatea* Linn was done. Then the extract was evaporated using rotatory evaporator and dried at 55°C. Dried extract was then stored at 20°C in labeled, sterile screw-capped bottles for further phytochemical analysis and activities.

**Drugs and chemicals:** Diazepam (as standard drug) 10mg/kg and normal saline (0.9% NaCl solution) were administered in the volume of 10 ml/kg. The extracts were suspended in distilled water and CMC and subjected for muscle relaxant activity using Rotarod apparatus. The extracts were administered orally (p.o.) in the volume of 10 ml/kg of body weight in the doses of 50mg, 100mg and 200mg/kg.

**Animals:** Swiss albino mice (weighing 20-25gm) of either sex were used for toxicity study. The animals were acclimatized to laboratory conditions for one week prior to the experiment. They were housed in polypropylene cages maintained under standard condition. The temperature in the animal house was maintained at 25±2°C. Permission from Institutional Animal Ethics Committee constituted for the purpose of CPCSEA Government of India was taken. The guidelines for the investigation of experiments in conscious animals were followed in all tests.

**Phytochemical screening:** The freshly prepared methanolic extract of flower of *Clitoria ternatea* (MECT) was subjected to various preliminary phytochemical tests to detect its phytoconstituents [6].

**Acute toxicity study:** The Acute oral toxicity study was performed as per OECD guideline 425 [7]. The animals were fasted overnight prior to the experimental procedure. The animals were divided into six groups and given different doses of plant extract (MECT) via oral route (150, 300, 500, 1000, 2000, 3000mg/kg body weight) for four consecutive

days and their mortality, loss of body weight and general behavior was recorded from the first dose upto 72 hours after the last administration of plant extract [8]. The procedure described in detail earlier in OECD 425 was followed for the determination of the acute toxicity of the extract.

#### Muscle relaxant activity Using Rotarod apparatus

The rotarod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by metallic discs, allowing the simultaneous testing of 6 mice. The rod is in a height of about 50 cm above the tabletop in order to discourage the animals from jumping off the roller. Cages below the section serve to restrict the movements of the animals when they fall from the roller. In this method, fifteen mice were divided into five groups having three in each group. Here the groups are-

**Group I:** Control mice (Normal saline 10 ml/kg)

**Group II:** Standard (Diazepam 10 mg/kg)

**Group III:** MECT 50mg/kg

**Group IV:** MECT 100mg/kg

**Group V:** MECT 200mg/kg

Here Group I served as control which received Normal saline 10ml/kg, Group II received standard drug Diazepam at a dose of 10mg/kg, p.o. Group III, IV and V received the methanolic extract of *Clitoria ternatea* orally at a dose of 50, 100 and 200mg/kg. Animals remain on Rota-Rod (25 rpm) 5 min or more after low successive trials are included in the study. After the administration of control, standard and test material the fall off time from the rotating rod was noted after 2 hrs. The difference in the fall off time from the rotating rod between the control and the treated mice was taken as an index of muscle relaxation [9].

**Statistical analysis:** The data obtained in present investigation was subjected to statistical analysis. All results are expressed as Mean ± SEM (standard error of mean); three animals in each group. All statistical comparisons were made by using Graphpad prism one-way ANOVA software in which *P* values < 0.05 were considered significant.

#### Results

All the three doses of MECT i.e. 50 mg/kg (11 ± 0.023 sec), 100 mg/kg (7.67 ± 0.014 sec) and 200 mg/kg (5 ± 0.032) significantly reduced the fall time in the Rotarod apparatus as compared to control (49.33 ± 0.028) (p value < 0.0001). The result is also quite satisfying when compared with the standard drug at 10 mg/kg/po (6 ± 0.019)

**Table 1:** Effect of MECT on muscle relaxant activity in rotarod apparatus

GROUPS	DOSE	fall off time (In Second)		
		Initial	After 30 minutes	After 60 minutes
Group I (Control)	10 ml/kg (Normal Saline)	54	48	46
Group II (Standard)	10 mg/kg (Diazepam)	7	5	6
Group III (Extract)	50 mg/kg (MECT)	12	11	10
Group IV (Extract)	100 mg/kg (MECT)	8	7	8
Group V (Extract)	200 Mg/kg (MECT)	6	4	5

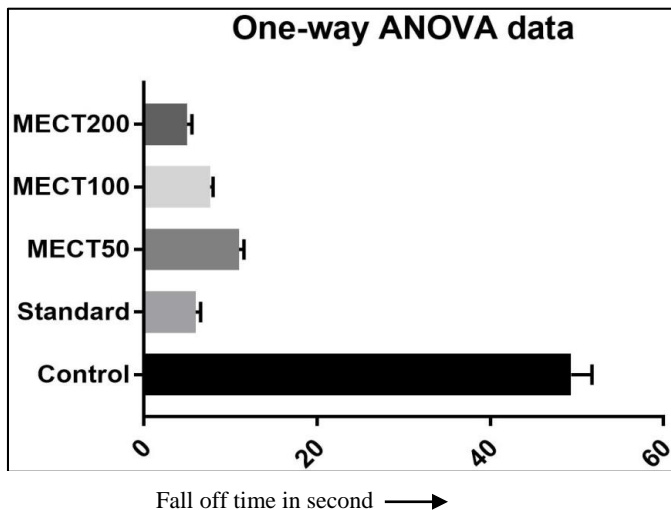


Fig 1: Muscle relaxant activity of MECT using Rotarod apparatus

### Discussion

This study was designed to evaluate phytochemical screening and the skeletal muscle relaxant properties of methanolic extract of *Clitoria ternatea* flower. The Phytochemical tests indicated the presence of Alkaloids, Tannins, Glycosides, Carbohydrates, Resins, Steroids, Saponins and Flavonoids. In previous study the ethanolic extract of the root of *Clitoria ternatea* showed skeletal muscle relaxant activity which is due to the presence of anthraquinone, steroids, saponins, reducing sugars and tannins in the plant extract. As the flower part of *Clitoria ternatea* also contains some of these chemicals so we can expect skeletal muscle relaxant activity with its extract also i.e MECT. It was also found in the previous studies that diazepam appeared to be more effective and safe so it was used as standard drug. Skeletal muscle relaxants are used to treat two different types of conditions:

1. Spasticity from upper motor neuron syndromes
2. Muscular pains or spasms from peripheral musculoskeletal conditions<sup>[10]</sup>.

Here, the experimental model is rotarod test in Albino mice. The study was carried out in albino mice weighing 25-30gms. Three mice were included in each group (total five groups) to evaluate the muscle relaxants property in different concentrations such as 50,100 and 200 mg/kg *p.o* MECT, For the studying of the drug as well extract mouse is one of the best animals as it is easy to handle can be used repeatedly since the animal is not sacrificed by rotarod method. In the present study it was found that the extract Demonstrated muscle relaxant action.

### Conclusion

In the present work attempts were made to study detail phytochemical investigation and pharmacological action, particularly muscle relaxant activity of methanolic extract of *C. ternatea*. The phytochemical analysis of the dry residue showed the presence of saponins, flavonoids, alkaloids, glycosides, tannins and carbohydrates. The most widely used animal models for muscle relaxant screening is Rotarod test and here it was used. The test is used to evaluate the activity of drugs interfering with motor coordination. In 1956, Dunham and Miya suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. This forced motor activity has subsequently been used by many investigators. The dose which impairs the ability of

50% of the mice to remain on the revolving rod is considered the end point<sup>[11]</sup>. Results showed that the administration of the MECT produced a significant decrease in fall off time and its efficacy was found to be comparable with Diazepam (10 mg/ kg).

### Acknowledgements

The author is thankful to the Management and Principa I Dr. Subhakanta Dash, Girijananda Chowdhury Institute of Pharmaceutical Science, Guwahati, Assam for providing necessary facilities and also to the guide Dr. Debaprotim Dasgupta, Assistant Professor, Department of Pharmaceutical Chemistry to carry out this work acknowledged. Also Dr.Partha Pratim Baruah, H.O.D of Dept. of Botany, Guwahati University, Assam for the authentication of the plant.

### Reference

1. Subramanian MS, Prathyusha P. Pharmacological and Phytochemical Characterization of *Clitoria ternatea* Linn. International Journal of Pharm Tech Research. 2011; 3(1):606-612.
2. Yoganarasimhan SN. Medicinal plants of India. Interline Publishing Co. 2000; 2:146-147.
3. Nadkarni KM. Indian Materia Medica, Popular Publications. 1976; 92(3):354-355.
4. Manjula P *et al.* Phytochemical analysis of *Clitoria ternatea* Linn. A valuable medicinal plant. J Indian bot. Soc. 2013; 92(3-4):173-178.
5. Gupta GK *et al.* *Clitoria ternatea* (L.) Old and new aspects. Journal of Pharmacy Research. 2010; 3(11):2610-2614.
6. Khandelwal KR, Sethi VK. Practical Pharmacognosy. Nirali Prakashan, Pune, 2013.
7. Al-Snafi AE. Pharmacological importance of *Clitoria ternatea* – A review. IOSR Journal of Pharmacy. 2016; 6(3):68-83.
8. Deka M, Kalita JC. Preliminary phytochemical analysis and acute oral toxicity study of *Clitoria ternatea* Linn. Roots in albino mice. International research journal of pharmacy. 2011; 2(12):139-140.
9. Kishan PV *et al.* Evaluation of muscle relaxant activity of aqueous extract of *Sapindus trifoliatus* (pericarp) in swiss albino mice. Journal of Chemical and Pharmaceutical Research. 2012; 4(4):1960-1964.
10. Veena NS *et al.* Skeletal muscle relaxant property of diazepam by using rotarod on albino mice. Indian Journal of Basic and Applied Medical Research. 2015; 4(4):714-721.
11. Kaur D *et al.* Antidepressant and Skeletal Muscle Relaxant Activity of Methanolic Extracts of *Basella alba* L. Asian. Journal of Biomedical and Pharmaceutical Sciences. 2016; 6(55):07-10.