

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ACUTE ORAL TOXICITY STUDY OF *CLITORIA TERNATEA* LINN. ROOTS IN ALBINO MICE

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Article Received on: 12/10/11 Revised on: 08/11/11 Approved for publication: 19/12/11

*E mail: manalishahd@gmail.com**ABSTRACT**

Clitoria ternatea has been using since the ancient times for its medicinal values. Almost all the parts of the plant have medicinal property. The root of the plant is reported to have anti diarrheal, Anti histamic, cholinergic activity etc. Traditionally the root has been using for the treatment of many diseases like leucorrhoea, diarrhea, urinary problems, diuretic, impotency, stomach trouble etc. The present study was designed to investigate the preliminary phytochemical analysis and acute oral toxicity of the root of the plant. The shed dried materials were grinded and used in the study. The preliminary phytochemical analysis was done by following standard protocols. For acute oral toxicity study, methanolic extract of the root was used. The extract was prepared by standard protocol. The preliminary phytochemical analysis showed the presence of proteins, carbohydrates, glycosides, resins, saponin, flavonoid, alkaloids, steroids and phenol. The acute oral toxicity study showed no mortality up to a dose of 3000 mg per kg body weight. The presence of plant chemicals revealed the medicinal values and the non toxic property of the plant indicated the value of the plant as medicine. Thus we can conclude that, the root of the plant can be used as a safe drug against many diseases.

KEY WORDS: *Clitoria ternatea*, phytochemical analysis, acute oral toxicity, methanolic extract.

INTRODUCTION

Clitoria ternatea is a vigorous, strongly persistent, herbaceous perennial legume. Almost all parts of this plant are reported to have medicinal properties. Flowers of this plant has been using in a number of religious purposes since the ancient times. The plant has been using traditionally to treat infertility, worm infestation, skin disease, tonsillitis, appetizer, digestant, vermicide, cough, asthma etc. Many of the medicinal values are evaluated by many workers such as Anthelmintic¹; Anti hyperglycemic²; Anti inflammatory³; Anti diarrhoeal⁴; Anti oxidant⁵; hepatoprotective⁶; Immunomodulatory⁷; Anti histamic⁸; cholinergic activity⁹ and many more. *C. ternatea* is reported to be a good “Medhya” (toning the brain) drug mainly used in the treatment of “Masasika” roga (mental illness), but it is also said to be useful in hectic fever, severe bronchitis, asthma and remedy for snakebite and scorpion sting¹⁰. A preliminary study using fresh flowers of *C.ternatea* showed hypoglycemic and hypolipidemic effects¹¹. The present study was carried out to investigate the preliminary phytochemical analysis and acute oral toxicity of root in mice.

MATERIALS AND METHODS**Plant materials**

The whole plant of the plant was collected from the local garden and made herbarium. The herbarium was identified for authenticity and preserved in the department of Botany, Gauhati University, Assam, India. For the analysis, fresh roots of *C. ternatea* were collected and washed properly to remove all the debris and soil. The roots were then shed dried and made powder in a mechanical grinder. This material was used in the study.

Extraction

The powdered roots were soxhlet-extracted with Methanol. The extract, on removal of solvent in vacuum, gave a reddish brown semisolid residue (yield: 6.6 % w/w).

Animals

Swiss albino mice used in the present study were obtained from the Animal House of the Department of Zoology, Gauhati University, Assam, India. Animals were bred in our own laboratory facility. They were maintained under uniform conditions of natural photoperiod (12hrs light/dark cycle). Mice had free access to food and water. Experiments were conducted using healthy adult Swiss albino mice of approximately 3 months of age and weighing 20-25gm.

Preliminary phytochemical analysis

Phytochemical screening procedures were performed using standard procedures^{12, 13, 14}

- **Test for Proteins:** Few drops of nitric acid were added by the sides of the test tube very gently to 1 ml methanol extract. Formation of yellow colour indicated the presence of protein in the sample (Xanthoprotein test).
- **Test for carbohydrates:** 1 ml each of Fehling A and Fehling B were added in diluted extract and heated for 30 minutes and observed for the formation of brick red colour.
- **Test for Resins:** Five milliliter of distilled water was added to the methanol extract and observed for turbidity.
- **Test for Tannins:** 5 ml of 45% ethanol was added to 2 g of the ground sample and boiled for 5 min. The mixture was cooled and filtered. Then 3 drops of lead sub acetate solution was added to 1 ml of the filtrate. A gelatinous precipitates were observed which indicates the presence of Tannins. Another 1 ml of the filtrate was added 0.5 ml of bromine water. A pale brown precipitates were observed indicating the presence of Tannins.
- **Test for Saponins:** 0.5 g of methanol extract was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a persistent froth. The frothing was mixed with 3 drops of Olive oil and shaken vigorously after which it was observed for the formation of an emulsion.
- **Test for Flavonoids:** 0.5 g of the macerated sample of was introduced into 10 ml of ethyl acetate and heated in boiling water for 1 min. The mixture was then filtered. 4 ml of the filtrate was shaken with 1 ml of 1% aluminum chloride solution and kept. Formation of a yellow colour in the presence of 1 ml dilute Ammonia solution indicated the presence of flavonoids.
- **Test for alkaloids:** 5 gm of ground material was extracted with 10 ml Ammonical Chloroform and 5 ml chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5 M Sulphuric acid. Creamish white precipitate was observed for the presence of Alkaloids.
- **Tests for Steroids:** 2 ml of acetic anhydride was added to 0.5 g of methanol extract and 2 ml of Sulphuric acid was added by the sides of the test tube and observed for the colour change from violet or blue-green.
- **Test for Phenols:** Methanol extract was taken in a test tube and mixed with distilled water and warmed. To this 2 ml f Ferric

chloride solution was added and observed for the formation of green or blue colour.

- **Test for Glycosides:** About 0.5 ml of methanol extract was taken in a test tube and added 1 ml glacial acetic acid containing traces of ferric chloride. To this solution 1 ml conc. Sulphuric acid was added and observe for the formation of reddish brown colour at the junction of the two layers and the upper layer turned bluish green in the presence of glycosides.

Acute Oral Toxicity Study

Acute Toxicity was studied by the method followed by Handa *et al.*, 1990¹⁵. Albino mice of both sexes were taken for this experiment. Animals were divided in six groups (n=6) and were given different doses of plant extract via oral route (150, 300, 500,1000, 2000, 3000mg/kg body weight) for four consecutive days and their mortality, loss of body wt. and general behaviour was recorded from the first dose up to 72 hours after the last administration of plant extract. One group was taken as control group and was administered with normal saline through the same route.

RESULTS

Preliminary phytochemical analysis: The results of the preliminary phytochemical analysis of *C.ternatea* L. root are shown in the table 1.

Acute Oral Toxicity Study: The investigation of acute toxicity is an initial step in the characterization of the biological effects of any substance which is necessary for conducting any biological experiment. The oral acute toxicity study revealed no mortality up to the dose 3000 mg/kg.

DISCUSSION

The plant has many medicinal values and has been in use from the ancient times. The present study revealed the presence of phytochemicals like Resins, Saponin, Flavonoid, Alkaloids, Steroids and Phenol. All these phytochemicals help in preventing many diseases. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones¹⁶. The presence of protein, glycosides and carbohydrate also indicate the palatability of the material. The root has been evaluated for the medicinal values like anti diarrhea⁴, Anti histamic⁸, cholinergic activity⁹ etc. As already stated, the plant has many medicinal values, so it is an essential factor that, the plant should not have any toxicity. The acute oral toxicity study showed no toxicity up to a range of 3000 mg/kg body weight. Therefore, the oral administration of the root of the plant will not affect the animal in terms of its mortality. Hence the plant here can be seen as a potential source of useful drugs.

ACKNOWLEDGMENT

Authors are thankful to head of the department Zoology, Gauhati University, Assam, India for providing all necessary facilities to do the experiments.

REFERENCES

1. Kamrun Nahar, Muhammad Ashikur Rahman Most. Nazma Parvin and Shamy Sarwar(2010) Evaluation of Anthelmintic Activity of Aqueous Leaf Extract of *Clitoria ternatea* Linn. Stamford Journal of Pharmaceutical Sciences 3(1): 46-48.
2. P. Daisy, Kanakappan Santoshand M. Rajathi 2009 Antihyperglycemic and antihyperlipidemic effects of *Clitoria ternatea* Linn. in alloxan-induced diabetic rats. African Journal of Microbiology Research Vol. 3 (5) pp. 287-291.
3. B.Parimala Devi, R.Boominathan, Subhash C.Mandal (2003) Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root Fitoterapia 74 ,345–349.
4. Nitinkumar Upwar, Roshan Patel, Naheed Waseem, Naveen Kumar Mahobia 2010 Evaluation Of Antidiarrhoeal Activity Of The Root Of *Clitoria Ternatea* Linn. International Journal of Pharmaceutical Sciences Review and Research. Volume 5, Issue 1; Article-020.
5. K.Sarumathy, M.S.Dhana Rajan, T.Vijay,J.Jayakanthi 2011 Evaluation of phytoconstituents, nephro-protective and antioxidant activities of *Clitoria ternatea* Journal of Applied Pharmaceutical Science 01 (05): 164-172.
6. Y.B.Solanki and S.M Jain 2011 Hepato-protective effects of *Clitoria ternatea* and *Vigna mungo* against Acetaminophen and Carbon tetrachloride-induced hepatotoxicity in Rats. Journal of Pharmacology and Toxicology 6(1):30-48.
7. Yogendrasinh B. Solanki and Sunita M. Jain 2010 Immunomodulatory Activity of Ayurvedic Plant Aparajita (*Clitoria Ternatea* L.) In Male Albino Rats. Global Journal of Science Frontier Research. Vol. 10 Issue 3 (Ver 1.0), pp. 2-8.
8. Dnyaneshwar J Taur and Ravindra Y Patil 2011 Antihistaminic activity of *Clitoria ternatea* L. roots Journal of Basic and Clinical Pharmacy Vol-002 Issue-001.
9. Vyawahare, N. S., Nikam,A. P., sharma, R. G., Deshpande,M.M., Tarnalli.A. Dand bodhankar S. L. 2011 Effect of *clitoria ternatea* extract on radial arm Maze task performance and central cholinergic Activity in rats journal of cell and tissue research vol. 7 (1) 949-952.
10. Chopra RN, Chopra IC, Handa KL, Kapur LD 1982. Indigenous drugs of India. Academic Publishers, Calcutta, India pp. 476.
11. Rajathi M, Daisy P 2000. Effect of plant extracts on the blood glucose and cholesterol level of alloxan diabetic rabbits. Indian J. Comp. Ani. Physiol. 18: 14-17.
12. Trease GE, Evans WC 1989. Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillan publishers.
13. Harborne JB 1973. Phytochemical methods, London. Chapman and Hall,Ltd.
14. Sofowara A 1993 Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria.
15. S.S.Handa, A.Sharma. 1990 Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride, Indian Journal of Medical Resarch, vol.92, 276-283
16. Okwu DE 2001. Evaluation of the chemical composition of indigenous spices and flavouring Agents. Global J. Pure Appl. Sci. 7(3): 455-459.

Table1: Results of the preliminary phytochemical study:

Sl no	Phytochemicals	Result
1.	Protein	+ ve
2.	Carbohydrate	+ ve
3.	Resins	+ ve
4.	Tannins	- ve
5.	Saponin	+ ve
6.	Flavonoids	+ ve
7.	Alkaloids	+ ve
8.	Steroids	+ ve
9.	Phenols	+ ve
10.	Glycosides	+ ve

Source of support: Nil, Conflict of interest: None Declared