ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Issue 1, 2014

ISSN - 0974-2441

Research Article

EVALUATION OF BRINE SHRIMP CYTOTOXICITY OF 50% AQUEOUS ETHANOLIC LEAF EXTRACT OF *CLITORIA TERNATEA* L.

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Received: 28 October 2013, Revised and Accepted: 8 November 2013

ABSTRACT

Objectives: To study the anticancerous property of leaves of *Clitoria ternatea* L.
Methods: The leaves were soaked in 50% aqueous ethanol, extract was filtered and concentrated. Cytotoxicity of the extract was tested by brine shrimp lethality assay.
Result: The experimental results revealed that 0.28-0.38% concentration of 50 % aqueous ethanolic leaf extract possessed cytotoxic potentiality against brine shrimp.
Conclusion: Hence the plant may be identified as a source of bioactive principle having antitumour activity.

Keywords: cytotoxicity, lethal conc., mortality, LC50, brine shrimp, phytochemical products.

INTRODUCTION

Medicinal plants are natural resources yielding valuable phytochemical products, which are often used in the treatment of various diseases. A substantial part of the population in developing countries, use folk medicines for their daily healthcare [1]. Some of the traditional medicine involves the use of crude plant extracts, which may contain an extensive diversity of molecules, often with indefinite biological effects [1]. However, most of the information available to the consumer with regard to the medicinal herbs is not backed by credible scientific data. For this reason, research is carried out, to determine the cytotoxicity of medicinal plants.

Clitoria ternatea L. commonly known as Butterfly pea belonging to the family Fabaceae and sub-family Papilionaceae is a perennial leguminous twinner, which originated from tropical Asia and later was distributed widely in South and Central America, East and West Indies, China and India, where it has become naturalised. It is also commonly called as *Clitoria*, blue-pea, kordofan pea (Sudan), cunha (Brazil or pokindong (Philippines) and is a vigorous, summer growing, legume of old world origin.

The major phyto-constituents found in the plant are the pentacyclic triterpenoids such as taraxerol and taraxerone. [2,3].

The seeds contain nucleoprotein with its amino-acid sequence similar to insulin, delphinidin-3,3,5-triglucoside, essential amino-acids, pentosan, water soluble mucilage, adenosine, an anthoxanthin glucoside, greenish yellow fixed oil [4,5], a phenol glycoside, 3,5,7,4-tetrahydroxy-flavone-3-rhamoglycoside, an alkaloid , ethyl D-galactopyranoside, p-hydroxy cinnamic acid polypeptide, a highly basic protein-finotin, a bitter acid resin, tannic acid, 6% ash and a toxic alkaloid [6]. According to Yoganarasimhan seeds contain ß-sitosterol, and hexacosanol and anthocyanin glucoside [7,8,9,10]. It also contains anti-fungal proteins and has been shown to be homologous to plant defensins [11]. Naeem et al.2007 reported a lectin present in the seeds of *Clitoria ternatea* agglutinated trypsin-treated human B erythrocytes [12]. Since the purified lectin was found to be potential tool for cancer studies so an attempt was made for the alternate high yielding purification method for *Clitoria ternatea* lectin designated CTL, present in the seeds of this member of leguminosae family [13]. Phytochemical screening of the roots shows the presence of ternatins, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, resins, starch, taraxerol and taraxerone .

From ancient times "Shankhpushpi" is known as reputed drug of Ayurveda and reported as a brain tonic, nervine tonic and laxative. It is considered as a Medhya-Rasayana in Ayurvedic texts. It comprises of entire herb with following botanicals viz *Convolvulus pluricaulis* (Convolvulaceae), *Evolvulus alsinoides* (Convolvulaceae), *Clitoria ternatea* (Papilionaceae) and *Conscora decusata* (Gentianaceae). It is an Ayurvedic drug used for its action on the CNS, especially for boosting memory and improving intellect. Extracts of this plant have been used as an ingredient in Medhya-Rasayana, are rejuvenating recipe used for treatment of neurological disorders [14].

The roots are being used as diuretic and seeds as cathartic [15]. In the traditional system of medicine particularly in Ayurveda, the roots, seeds and leaves of *C. ternatea* have long been widely used as a brain tonic and is believed to promote memory and intelligence. Infusion of leaves is used for eruptions. Root juice applied in the nose for migraine. Overall it is a traditional Ayurveda medicine used as a brain tonic, memory and intelligence enhancer, anti-depressant, anxiolytic, sedative and anti-convulsant [14]. Entire plant is used as antidote for snake-bites. *C. ternatea* petals have been recognized to possess anti-oxidant activity. Two related citations from this journal were viewed in this regard.[16,17].

Thus this plants contains a wide varieties of phytochemical constituents ranging from nucleoprotein to terpenoids, from flavonoids to alkaloids. However the claim that safe usage of *C. ternatea leaf* extract in folk medicine is unsubstantiated by scientific studies. Hence, the current study has been undertaken to investigate the toxicity of 50 % aqueous ethanolic leaf extract of *C. ternatea* against brine shrimp.

MATERIALS AND METHODS

Plant materials

The leaves of the plants were collected from the adjoining areas of Kalyani, Distt: Nadia of West Bengal. The plant was identified in the Department of Botany, University of Kalyani. Leaves were cut into small pieces and dried at about 40°C for one week and were powdered using a blender.

Extract preparation

100 gm. of dried powered leaves of Clitoria ternatea L. were soaked in and 50% aqueous ethanol for seven days. The extract was

collected, evaporated under reduced pressure in a vacuum rotary evaporator. Dark brown residual solid collected from the fraction, was subjected to experimental studies. Various concentrations lying in the range of 1mg/ml – 100mg/ml of the residual solid were made.

Brine Shrimp Lethality Assay

Brine shrimp (*Artemia salina*) eggs were hatched in artificial sea water prepared from commercial sea salt 38 g/L [18]. A lamp was placed above the open side of the tank to attract the hatched shrimps close to the tank wall. After 48 hours, the shrimps matured as nauplii (*Artemia salina)* and were ready for the assay. The brine shrimp lethality bioassay was carried out with the above mentioned solution by the standard procedure [19, 20]. Each concentration was tested in triplicate. A test tube containing 5 ml of salt water was used as the negative control. A suspension of larvae (0.1 ml), containing about 15 – 25 larvae, was added into each test tube and incubated for 24 hours. The test tubes were then examined and the number of dead larvae in each test tube was counted after 3, 6, 9, 12, 15, 18, 21 and 24 hours. The total number of shrimps in each bottle was counted and recorded. The percentage of dead shrimp at each observation was calculated using the following formula

$$\text{Percentage of Death} = \frac{\text{Total naupii} - \text{Alive naupii}}{\text{Total naupii}} X100$$

The lethal concentration at which 50% of the shrimp died (LC_{50}) was determined by statistical software SPSS by probit analysis

RESULTS AND DISCUSSIONS

Number of survived shrimp was observed in 3 hour intervals and the percentage of death was calculated using the previously mentioned formula. The observed results were as follow (table:1).

Table 1: Brine shrimp lethality assay of 50% ethanolic extract of *C. ternatia*.

sets	Concentration of extract.(mg/ml)		Number of dead shrimp after 0 hrs	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21hrs	24hrs
1	1	Mean	48.00	46.33	46.00	41.67	41.00	40.33	39.67	39.67	39.33
		Std. Deviation	1.00	1.53	1.00	1.53	1.00	0.58	1.53	1.53	0.58
2	2	Mean	20.33	18.00	17.33	16.33	13.33	14.33	15.00	14.67	14.67
		Std. Deviation	2.52	2.00	2.52	2.52	2.52	2.52	1.00	1.15	1.15
3	3	Mean	14.33	14.00	11.33	11.67	13.00	11.33	11.00	9.33	**8.67**
		Std. Deviation	1.53	1.00	1.53	2.08	2.00	1.53	2.65	0.58	0.58
4	4	Mean	12.00	12.33	11.33	9.33	9.00	7.33	5.00	4.33	**4.00**
		Std. Deviation	2.00	2.52	1.53	2.52	3.00	2.52	1.00	0.58	1.00
5	5	Mean	18.17	14.33	14.67	12.67	11.17	8.50	5.17	4.83	3.67
		Std. Deviation	3.87	4.76	6.62	5.39	4.36	3.62	1.17	0.75	0.82
6	6	Mean	18.33	16.33	16.33	13.00	10.33	8.00	6.00	2.67	1.33
		Std. Deviation	2.52	1.53	2.52	1.00	1.53	1.00	1.00	0.58	0.58
7	7	Mean	18.67	17.33	10.67	8.00	6.33	6.33	4.00	1.33	0
		Std. Deviation	2.08	1.53	2.08	1.00	1.53	2.31	1.00	0.58	0
8	8	Mean	14.00	12.67	11.00	11.00	11.00	7.67	4.00	1.00	0
		Std. Deviation	1.00	2.08	2.00	1.00	2.00	1.53	1.00	0	0
9	9	Mean	11.67	9.33	9.00	7.33	5.33	6.33	4.33	0	0
		Std. Deviation	1.53	1.53	1.00	1.53	1.53	1.53	1.53	0	0
10	10	Mean	13.00	11.00	7.00	4.67	1.67	0	0	0	0
		Std. Deviation	1.00	2.00	1.00	1.53	1.53	0	0	0	0
11	20	Mean	12.00	9.33	5.67	1.67	2.00	0	0	0	0
		Std. Deviation	1.00	1.53	2.08	1.15	1.00	0	0	0	0
12	30	Mean	21.00	11.33	6.00	2.33	2.67	0	0	0	0
		Std. Deviation	2.65	1.53	1.00	1.53	1.53	0	0	0	0
13	40	Mean	34.33	18.33	16.00	4.67	0	0	0	0	0
		Std. Deviation	2.08	2.08	1.00	0.58	0	0	0	0	0
14	50	Mean	21.00	15.33	12.00	3.33	0	0	0	0	0
		Std. Deviation	1.00	0.58	6.08	2.89	0	0	0	0	0
15	60	Mean	29.00	21.00	10.67	1.67	0	0	0	0	0
		Std. Deviation	1.00	1.00	1.15	0.58	0	0	0	0	0
16	70	Mean	14.67	5.67	2.67	0	0	0	0	0	0
		Std. Deviation	0.58	0.58	0.58	0	0	0	0	0	0
17	80	Mean	20.67	7.67	0	0	0	0	0	0	0
		Std. Deviation	0.58	0.58	0	0	0	0	0	0	0
18	90	Mean	33.67	11.00	0	0	0	0	0	0	0

		Std. Deviation	3.21	1.00	0	0	0	0	0	0	0
19	100	Mean	30.67	6.67	0	0	0	0	0	0	0
		Std. Deviation	1.15	1.15	0	0	0	0	0	0	0

From the above table it is very clear that with the increasing dose and time the survivality of shrimp decreases. At the lowest used concentration 1mg/ml, most of the shrimp remain survived even after 24 hours while at the highest used concentration 100mg/ml, all the shrimp lost their survivality just within 6 hours. From the table it seems that LC_{50} i.e. the concentration at which 50% of the shrimp will remain survived after 24 hour may be in between 3mg/ml and 4mg/ml. To determine the exact LC_{50}, probit analysis was done with the help of statistical software SPSS 13. Probit regression line (Probit transformed response), obtained from the analysis was as follow:

From the analysis it is also further revealed that in case of present study

LC_{50} = 3.25 mg/ml (lower concentration: 3.00 and upper concentration 3.48 mg/ml

CONCLUSION

This study presents valuable data on the toxicity level of *C. ternatea* leaf extract, which should be very useful for clinical study of this plant leaf extract.

ACKNOWLEDGMENTS

The authors express heartfelt gratitude towards the Department of Botany, University of Kalyani for providing laboratory facilities and University Grant Commission for financial assistance.

REFERENCES

1. Alkofahi A., Batshoun R., Owais W., and Najib N., Biological activity of some Jordanian, medicinal plant extracts. Fitoterapia. 1996; 68(5): 435.
2. Banerjee SK, Chakravarti RN, Taraxerol from Clitoria ternatea, Bull Calcutta School Trop Med, 1963; 11: 106-107.
3. Banerjee SK, Chakravarti RN, Taraxerone from Clitoria ternatea, Bull Calcutta School Trop Med, 1964; 12: 23.
4. Joshi SS, Shrivastava RK, Shrivastava DK, Chemical examination of *Clitoria ternatea* Seeds, Journal of American Oil Chemical Society, 1981; 58(6): 714-715.
5. Tiwari, R.D., Gupta, R.K.,. Chemical examination of the oil from the seeds of *Clitoria tematea* Linn. Journal of Oil Technology Association of India 1957;13: 9–13.
6. Potsangbam L, Ningombam S, Laitonjam WS, Natural dye yielding plants and indigenous knowledge of dyeing in Manipur, Northeast India, Indian Journal of Traditional Knowledge, 2008; 7(1): 141-147.
7. Yoganarasimhan SN, Medicinal Plant of India, Bangalore, India, Interline Publishing Co, 2000; 2: 146-147
8. Sinha A, Studies on the unsaponifiable matter of the seeds of Clitoria *ternatea* Linn. And isolation of ?-sitosterol, Proceedings of the National Academy of Sciences, 1960b; 29: 23-26.
9. Sinha, A.,. Studies on the unsaponifiable matter of the seeds of *Clitoria tematea* Linn. and isolation of _-sitosterol. Proceedings of the National Academy of Sciences of India 1960b; 29: 23–26.
10. Sinha, A.,. Mucilage from the leaves of *Clitoria ternatea*. Proceedings of the Institution of Chemists, India1960c; 32:, 228–231.
11. Osborn RW, Samblanx GW. De, Thevissen K, Goderis I, Torrekens S, Van Leuven F, *et al.* Isolation and characterisation of plant defensins from seeds of Asteraceae, Fabaceae, Hippocastanaceae and Saxifragaceae, FEBS Letters, 1995; 368: 257-262.
12. Naeem A, Ahmed E, Khan RH, An alternate high yielding purification method for *Clitoria ternatea* lectin, International Journal of Biological Macromolecules, 2007; 41: 481-486.
13. Naeem A, Haque S, Khan RH, Purification and Characterization of a Novel ß-D-Galactos I d es - Specific Lectin from *Clitoria ternatea*, The Protein Journal, 2007; 26(6): 403.
14. Girish Kumar Gupta , Jagbir Chahal , Manisha Bhatia , *Clitoria ternatea (L.)*: Old and new aspects, Journal of Pharmacy Research 2010; 3(11):2610-2614
15. Parimaladevi, B., Boominathan, R., Mandal, S.C.,. Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root. Fitoterapia 2003;74: 345–349.
16. N.K. Sathish, N.M. Raghavendra, In-vitro and in-vivo antitumor activity of 1, 3 -diacetoxy acridones against *Ehrlich ascites* Carcinoma, Asian Journal of Pharmaceutical and Clinical Research, 2010; 3(4):80-82
17. Ajoy Ghosh , Padma Chatterjee Brine shrimp cytotoxic activity of 50% alcoholic extract of *Croton bonplandianum* Baill. Asian Journal of Pharmaceutical and Clinical Research,2013; 6, Suppl 3: 40-41
18. Finney D.J., "Probit analysis". (1971), 3rd Ed. Cambridge: Cambrige University Press
19. McLaughlin J.L., Methods in plant biochemistry, K. Hostettman (ed.), London: Academic press, 1991;6: 1.