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Research Article

Phytochemical Screening and Diuretic Activity of the Aqueous and Ethanolic Extract of *Clitoria ternatea* Flowers

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Abstract

Clitoria ternatea, commonly known as butterfly pea, is a perennial herbaceous plant from the Fabaceae family. It has recently attracted a lot of interest as it has potential applications both in modern medicine and agriculture, and as a source of natural food colorants and antioxidants.Additionally, *Clitoria ternatea*has been widely used in traditional medicine, particularly as a supplement to enhance cognitive functions and alleviate symptoms of numerous ailments including fever, inflammation, pain, and diabetes.The present study was undertaken to investigate diuretic effect of aqueous and ethanolextracts of the dried flowers of *Clitoria ternatea* in normal rats.Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature.Aqueous and ethanol extracts of *Clitoria ternatea*flowers were administered to experimental ratsorally at doses of 500 mg/kg p.o. Furosemide (5 mg/kg) was used as positive control instudy. The diuretic effect of the extracts was evaluated by measuring urine volume and sodium content.Phytochemical screening of the extract showed the presence some common compounds like alkaloids, resins, steroids, tannins, saponins, and glycoside.Urine volume was significantly increased by aqueous and ethanol extracts incomparison to control group. While the excretion of sodium was also increased by both extracts.We can conclude that aqueous and ethanol extracts of *Clitoria ternatea*produced notablediuretic effect which appeared to be comparable to that produced by the reference diuretic Furosemide. Thepresent study provides a quantitative basis for explaining the folkloric use of *Clitoria ternatea*as a diuretic agent.

Keywords: *Clitoria ternatea,* Fabaceae, Phytochemical constituents, Diuretic effect, Furosemide

INTRODUCTION

Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects. Besides, the World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health-care needs[1]. Medicinal plants are used worldwide in the traditional management of some renal diseases and have a wide application as diuretic agents[2,3]. The diuretic activity of a number of plants used in ethnomedicine has been confirmed in experimental animal models[2]. The safety and efficacy of these plants for their claimed medicinal use, however, have not been extensively studied and remain to be in light of further investigation. The techniques of preparation employed by traditional healers are generally not standardized and in most cases do not comply with the requirement of good manufacturing practice[4].*Clitoria ternatea* is well known tropical perennial climber herb from family Fabaceae with slender downy stem, found throughout the tropical region of India, growing wild and also in gardens, bearing white or blue flowers. It is commonly known as Aparajita and Koyal in Hindi and Butterfly pea in English. The extracts of *Clitoria ternatea* have been used as an ingredient in MedhyaRasayana a rejuvenating herbal formulation for treatment of various neurological disorders and to strengthen intellectual ability. The root part of *Clitoria ternatea* has been used for its laxative, purgative, diuretic, inflammation, indigestion, constipation, fever, arthritis, eye ailments, sore throat and anthelmintic. Kirtikar and Basu[5] also reported the usefulness of *Clitoria ternatea* for treatment of severe bronchitis, asthma and fever. *Clitoria ternatea* is also being used by the local tribes to cause abortion, to cure abdominal swelling, sore throat, mucous disorder and fever[6]. The root juice of *Clitoria ternatea* is given with cold milk to reduce phlegm in chronic bronchitis. Taraxerol and taraxerone, pentacyclictriterpenoids and flavonol glycoside, 3,5,4′-trihydroxy-7-methoxyflavonol-3-O-β-d-xylopyranosyl- (1,3)-O-β-d-galactopyranosyl (1,6)-O-β-d-glucopyranoside are present in the root of *Clitoria ternatea*[7-9]. Besides protein and fatty acid content, *Clitoria ternatea* seeds also contain p-hydroxycinnamic acid, β-sitosterol, γ-sitosterol adenosine, flavonol-3-glycoside, ethyl-α-d-galactopyranoside,3,5,7,4′-tetrahydroxyflavone, 3-rhamnoglucoside, hexacosanol, and an anthoxanthinglucoside[10-14]. The flowers of *Clitoriaternatea* contain ternatins A1-3, B1-4, C1-5, D1-3. The flowers of *Clitoria ternatea* also contain kaempferol, kaempferol 3-neohesperidoside, kaempferol 3-2G-rhamnosylrutinoside, kaempferol 3-rutinoside,kaempferol 3-glucoside, quercetin, quercetin 3-2G-rhamnosylrutinoside, quercetin 3-neohesperidoside, quercetin 3-rutinoside, quercetin 3-glucoside, myricetin 3-neohesperidoside, myricetin 3-rutinoside and myricetin 3-glucoside[15,16]. The leaves of *Clitoria ternatea* contain β-sitosterol, flavonoids, lactones aparajitin and clitorin, essential oil, colouring-matter and mucilage[17-20], but no previous pharmacologicalor clinical study was carried

out to test thediuretic activity of this plant. Since the diureticeffect of *Clitoria ternatea*has never been experimentally confirmed, the main aim of thepresent investigation was to evaluate theclaimed diuretic activity of *Clitoria ternatea*in rats.Furosemide was selected as thereference drug, since it is used clinically insome pathologies.

MATERIALS AND METHODS

Plant material

The flowers of *Clitoria ternatea*werecollected from local area of Khargone, MP. The identification and authentication of plant was done by Dr. S.K. Mahajan, Botanist, from the Department of Botany, Govt. P.G. Collage, Khargone, MP India. A voucher specimen number 211/KRG./Sci. /Clg/Knw. was kept in Department of Botany, Govt. P.G. Collage, Khargone, MP Indiafor future reference.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction

Maceration method

In present study, plant materials were extracted by using maceration method; the flowers of *Clitoria ternatea* were collected, washed and rinsed properly. About 1kg of the powder was extracted with different organic solvents petroleum ether, water and ethanol and allows standing for 4-5days each. The extract was filtered using Whattman no.1 filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated & excessive moisture was removed and extract was collected in air tight container for further use[21].

Qualitative analysis of phytochemicals

The extracts prepared for the study were subjected to preliminary phytochemical screening by using different reagents for identifying the presence or absence of various phytoconstituents viz., carbohydrates, proteins, alkaloids, tannins, steroid, flavonoids and terpenoids in ethanolic and aqueous extracts of *Clitoria ternatea*. The above phytoconstituents were tested as per the standard method[22, 23].

Animals

Swiss albino rats (either sex, 120-125 gm±10gm) were selected randomly and further divided into various treatment groups randomly and kept in propylene cage with sterile husk as bedding. Relative humidity of 30.7% at 22±2°C and 12:12 light and dark cycle was maintained in the animal house and fed with standard pellets (Golden Feeds, New Delhi, India) and water was available *ad libitum*. Institutional Animal Ethics Committee (IAEC) has approved all animal experiments with CPCSEA.

Acute toxicity study

Organization for Economic Co-operation and Development (OECD) guideline # 423 was followed for carrying out acute oral toxicity studies. The animals of both sexes were selected by sampling technique andwere divided into 5 groups of 5 animals each. A single oral dose of the extract starting at 100 mg/kg and progressively moving from 300, 500, 700, 1000 mg/kg up to 1200 mg/kg was administered. All the animals were observed for appearance of toxic symptoms including muscle spasm, loss of righting reflex, tremors, behavioral changes, locomotion, convulsions and mortality for 24 h. Long term supervision was continued for a period of 14 days to observe any occurrence of toxic symptoms and mortality[24].

Experimental protocol

Diuretic activity was determined by the following methods of Kau et al.[25], with minor modifications. The rats were randomly divided into four groups of 6 animals each as follows: (1) Control-given 25 ml/kg body weight of normal saline; (2) Furosemide (5 mg/kg) + normal saline (25 ml/kg) of body weight; (3) Aqueous extract (500mg/kg) of body weight; (4) ethanolic extract (500mg/kg) of body weight. In all cases, the volume of the dose was administered 5 ml/kg body weight. The animals were fasted overnight (18 h) prior to the test but with free access to tap water only and then were given an oral loading of normal saline of 25ml per kg body weight. Immediately after administration, the rats were paired and placed in metabolism cages. Urine was collected in a graduated cylinder and its volume was recorded at 30 min intervals for 6 h. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 g b.w.

Measurement of urine output andanalysis of electrolytes

Na+ concentrations were measuredusing a Toshniwal group model TCM-35flame photometer. The instrument wascalibrated with standard solutions containingdifferent concentrations of Na+.

RESULT AND DISCUSSION

The crude extracts so obtained after each of the successive maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from the flowers of the plants using petroleum ether, aqueous and ethanol as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder of flowers of *Clitoria ternatea*are shown in Table 2. Ethanolic extracts of sample of *Clitoria ternatea* showed the presence of alkaloids, resins, steroids, tannins, saponins, phenols and glycoside; aqueous extracts show the presence of alkaloids, resins, steroids, tannins, saponins, and glycoside but in petroleum ether extracts alkaloids, steroids, tannins, and saponins phytoconstituents are only present.The acute oral toxicity study was done according to the OECD 425 guidelines. No adverse changes and mortality were observed in animals, which orally received extract (1200 mg/kg) of *Clitoria ternatea*flowers. This indicates that 1200mg/kg is maximum safe dose. So 1/2.4th*i.e.* 500amg/kg of body weight, of the maximum safe dose were selected for studying diuretic activity. Table 3 show that the reference diuretic, furosemide increased urine volume. Theextracts also caused an increase in urinevolume. For the aqueous extract, thedoses of 500 mg/kg body weightwas 2.5ml, compared to the control group while for theethanol extract, is the corresponding valuesare 3.5ml. Table 4 show estimation of sodium by flame photometryby dose dependent manner.

Table 1: Yield of crude extracts of *Clitoria ternatea*flowers

S.No.	Solvent	% Yield
1.	Pet. Ether	2.10
2.	Aqueous	5.17
3.	Ethanol	8.16

Table 2: Qualitative phytochemical evaluation of *Clitoria ternatea*flowers

S. no.	Phytochemical	Pet. Ether	Aqueous Extract	Ethanol Extract
1	Alkaloids	+	+	+
2	Resins	-	+	+
3	Steroids	+	+	+
4	Tannins	+	+	+
5	Saponins	+	+	+
6	Glycoside	_	+	+
7	Phenols	_	-	+

Table 3: Effect of oral administration of aqueous and ethanol extracts of *Clitoria ternatea*and Furosemide on urine volume

S. no.	Time interval (in min.) & collection of urine sample (in ml)									
	30	60	90	120	150	180	210	240	270	300
Control (saline) 25ml/kg	0 ml	0 ml	0ml	0.5ml	0.5ml	1 ml	1 ml	1.5 ml	2ml	2ml
Standard (Saline+Furosemide) 5mg/kg	0 ml	0.25 ml	0.5ml	0.75ml	1ml	1.5ml	2 ml	3 ml	3.5ml	4ml
Ethanol extract (Saline + extract) 500mg/kg	0 ml	0ml	0.25ml	0.75ml	1ml	1.5ml	2 ml	2.5 ml	3ml	3.5ml
Aqueous extract (Saline + extract) 500mg/kg	0 ml	0ml	0.25ml	0.25ml	0.75ml	1ml	1.5ml	2 ml	2.5ml	2.5 ml

Table 4: Estimation of sodium by flame photometry

Conc. (in ml)	Abs. (nm)
1	5
2	10
3	15
4	20
5	25
Unknown	17

CONCLUSION

Hence, the significant diuretic activity exerted by aqueous and ethanol extract of *Clitoria ternatea* flowerin our study may be attributed by the presence of tannins, terpenoids and phenolic substances. Studies are in progress to elucidate the molecular and cellular mechanism of the extract. Longer duration studies on chronic models may contribute towards the development of a potent diuretic drug.

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