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EVALUATION OF DIURETIC ACTIVITY OF SHANKAPUSHPI ETHANOLIC EXTRACT IN WISTAR RATS

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ABSTRACT

Aim: The present investigation aimed to determine the diuretic activity of Shankapushpi ethanolic extract in Wistar rats.

INTRODUCTION

Clitoria ternatea (Fabaceae) generally known as Butterfly pea. The condiment *Clitoria ternatea L*. generally known as butterfly pea showed its anti-inflammatory action in macrophage cells by suppressing inflammatic due to presence of polyphenols. Kaempferol, B- sitosterol, Sankhapushpine, N- hexaconazole, and Hydroxy cinnamic acid are major chemical constituents. it helps in enhancing diuretics, attention, learning capabilities, internal fatigue, wakefulness, stress, anxiety, depression, etc. and has been extensively screened for different pharmacological activities.

METHODOLOGY

The ethanolic extract of *Clitoria ternatea* (leaves) were orally administered to mainly Wistar rats. Furosemide were used as diuretic reference. Urine output was recorded up to 5 hours. the urinary excretion rate and Ph and electrolyte was determined. Toxic study was carried out to determine the cure of the medicine and phytoconstituents presence was analyzed by phytochemical tests.

RESULTS

Phyto chemical evaluation revealed the presence of alkaloids, carbohydrates, tannins, phenols, and terpenoids.

CONCLUSION

This study will give a quantitative base for explaining the natural medicinal use of *Clitoria ternatea* as a diuretic agent, promoting the safe diuretic excretion process.

KEYWORDS

Clitoria ternatea, Butterfly pea, diuretic.

1. Introduction

Diuretics refers to increased urine production and excretion of kidney and it also loss the electrolytes such as Sodium chloride and Potassium [1]. The net excretory effect of diuretic agents causes changes in urine flow, pH, ionic composition of urine and blood [2]. The diuretics adequately control fluid retention and restore and maintain normal volume status in patients with congestive symptoms (dyspnea, orthopnea, oedema) or the sign of elevated filling pressures (peripheral oedema) [3]. diuretics which are meant to regulate body water balance by reducing urine production and excretion, there by opposing diuresis [1]. The currently used anti-diuretics like vasopressin (ADH), argipressin, desmopressin, lypressin, ornipressin, oxytocin, and terlipressin are usually associated with many adverse reactions such as increased urination and sodium loss, development of new-onset diabetes, metabolic alterations, impairment of sexual function and activation of neuroendocrine system [1,4]. Antidiuretic hormone (ADH) is found in most mammals. In some condition like diabetes insipidus, bed-wetting and increased rate of urination due to head surgery or trauma are treated by the use of diuretic agent/drugs. It also affects the blood potassium levels in the body [1]. Various plant extract used in traditional medicine that have shown significant diuretic activity when tested in animal model [3]. Natural herbal medicine is considered a quite important resource for drug discovery as it has less side effects with cost effectiveness & has increasingly attracted the attention of researchers [5]. In this current study an attempt has been made to evaluate the diuretic activity of the ethanolic extract of *Clitoria ternatea L* (Shankhpushpi)[3].

3.Material and Methods

3.1 Chemicals and Drugs

Ethanol was used as an extracting solvent. Furosemide was used as diuretic reference, which was a high-ceiling loop diuretic in clinical practice and at the given dose of 12mg/kg. Chemicals like Alcohol, Acetic acid, Dragendroff's reagent, Wager's reagent, Mayer's reagent, Hager's reagent, α naphthol solution, Conc sulfuric acid, Fehling solution A&B, Benedict reagent, Chloroform, Acetic anhydride, Dil HCl, ZnCl, Alcoholic ferric chloride, 2N sodium hydroxide, Acetone, 5% NaoH, Vinyl Cl were used for phytochemical tests.

3.2 Collection and Authentication of Plant materials

The fresh leaves of Clitoria ternatea L (Shankhpushpi) were collected from Medicinal Herbal Garden of RR college of Pharmacy during August September 2021 at day time. The plant materials were identified and authenticated by S. Noorunnisa begum (Voucher no 5677) at Foundation for Revitalization of Local Health Tradition (FRLHT) The university of Trans-Disciplinary Health science and technology, Karnataka.

3.3 Review of Literature

Butterfly pea, or Clitoria ternatea, is a plant in the Fabaceae family and subfamily Papilionaceae. There are at least 12 other species recognized in this species. *Clitoria Ternatea* also called clitoria, blue-pea, kordofan pea, Cunha is vigorous, summer growing, legume of world origin. It is characterized as a woody genus with showy,

papilionaceous flowers, an in-fundibular calyx with persistent bracteoles, stipules and stalked ovaries. In Indian traditional this medicine is known as Aparajit (Hindi), Kokkattan (Tamil), Aparajita (Bengali). In ayurvedic texts, it is known by a number of other names, including Aparajita, Girikarnu, Asphota, and Vishnukranta in Sanskrit. English names: Butterfly pea, Mazerion and Winged leaved clitoria. Local name: Gorani (Guj), Gokarna (Mar), Aparajit (Hin), Buzrula (Arabic) and Aparajita (Beng)[6].

The perennial leguminous herb *Clitoria ternatea*, has interest in agricultural, medical applications, ornamental and as a source of an ecofriendly insecticide [7]. Butterfly pea is 90 to 162cm tall, long-lived herb [8]. The seeds are yellowish-brown or blackish in color and sub globose or oval in shape. Root system consists of a fairly stout taproot with few branches and many slanders lateral roots. The leaflets are ovate or oblong 2-5cm long and sub coriaceous, rubiaceous stomata with wavy cell walls are present on both upper and lower epidermis of the leaflets. *Clitoria ternatea* has 2 varieties in flowers, white and blue in color. The white flowered one is found to be therapeutically more active and bluer flowered used as a substitute for the white flowered one [9].

Butterfly pea has many pharmacological effects including antioxidant, hypolipidemic, anticancer, analgesic, anti-inflammatory, antidiabetic, CNS. The leaves and roots of *Clitoria Ternatea* are used in the treatment of a number of ailments including body aches, especially infections, urinogenital disorders and as an anthelmintic [9]. The juice of flower is reported to be used in insect bites and skin diseases. The roots are used in the treatment of various diseases like indigestion, constipation, fever, arthritis and eye ointments. It also employed in cases of enlargement of the abdominal viscera, sore throat and skin diseases [6]. Various constituents are found in different parts of this plant [10].

(a) (b)

(b) **Fig.2.** *Clitoria ternatea*

3.4 Preparation of plant technique

The *Clitoria ternatea L* plant leaves were dried in the shade, powdered & extracted by using Soxhlet apparatus. The Ethanolic extract (EE) of *Clitoria ternatea* was prepared by 90% ethanol for 24 hours. The extract has been stored in a airtight container under normal temperature.

PRELIMINARY PHYTOCHEMICAL TESTS

The Ethanolic leaf extracts of *Clitoria Ternatea* were subjected to qualitative testing for alkaloids, flavonoids, phenolic compounds, carbohydrates, saponins, tannins, coumarins, triterpenoids and carboxylic acids.

Sl no	**Tests**	**Ethanolic extract of** *Clitoria ternatea* **(+/-)**
1	**Alkaloids**	
a	Dragendorff 's test	+
b	Mayer's test	+
c	Hager's test	+
d	Wagner's test	+
2	**Steroids**	
a	Libermann-buchard test	-
b	Salkowski's test	-
3	**carbohydrates**	
a	Molisch's test	+
b	Fehling's test	+
c	Benedict's test	+
4	**Tannins**	
a	With $FeCl_3$	+
5	**flavonoids**	
a	Shinoda's test	-
6	**Saponins**	
a	With $NaHCO_3$	-
7	**Terpenoids**	
a	Tin & thionyl chloride test	+
8	**Coumarins**	
a	With 2-N NaOH	-

9	**Phenols**	
a	With alcoholic ferric chloride	+
10	**Carboxylic acid**	
a	With water & $NaHCO_3$	-
11	**Resin**	
a	With aqueous acetone	-
12	**Quinone**	
a	5% NaOH	-
13	**Amino acids**	
a	Ninhydrin reagent	-

Table 1: Results of preliminary phytochemical tests

Test	**Ethanolic extract of Clitoria ternatea**
Alkaloid	+
Carbohydrate	+
Tannin	+
Terpenoid	+
Phenol	+
Steroids	-
Flavonoids	-
Coumarins	-
Carboxylic acid	-
Quinone	-
Amino acids	-

Resin	-
Saponins	-

Note:(+) present (-) absent.

3.6 Study Design

3.6.1 Experimental Animals

Male Wister *albino* Rats and Male Swiss *albino* Mice were purchased from Sri Raghavendra Enterprises (Bangalore, India). Healthy adult Wister *albino* male rats (weight 120-125g) were used in the diuretic study and Swiss *albino* male mice were used in toxicity study. The animals were kept in Sapthagiri Institute of Medical Science and Research Centre under standard animal's house condition [13]. Rats were kept for acclimatization for 5 hours. The animals were fed standard pellet diet maintained on natural light and dark cycle and had free access to water and food [5].

3.6.2 Acute Toxicity studies

The study was determined using Swiss *albino* male mice(30-g) maintained under standard husbandry conditions [4]. A/C to OECD guidelines No.423 2000mg/kg ethanolic extract was administered & observed for the mortality up to 24 hours study period. Based on toxicity 1/10th dose of the extract was determined. there were no toxic symptoms like lacrimation, writhing, stupor, squint, convulsions were observed.

3.6.3 Experimental study design

Evaluation of diuretic activity:

Twenty-four Wister *albino* male rats are selected for the diuretic activity. The rats were deprived of water for 18 hours. The rats were then orally administered with 15 ml of saline (Nacl,0.9% W/V) to impose uniform water load. After 45 minutes, urinary bladder of each rat was emptied by gentle compression of the pelvic area and by pulling of the tail.

Grouping of rats for the experiment:

Four groups of rats, each consisting of six rats, were as follows: -

Group 1-Normal rats, which received 1ml of normal saline.

Group 2-Treatment control group, which received Furosemide 12mg/kg

(Diuretic reference drugs).

Group 3- This group rats received ethanol extract at the dose of 200mg/kg respectively.

Group 4-This group rats received ethanol extract at the dose of 400mg/kg respectively [15].

After oral administration, each rat was housed in an individual metabolic cage specially designed to separate faces and urine. Urine output was measured hourly over 5 hours from the point of administration of saline to the rats [14]. During this period no food or water was made available to animals [18]. The volume of urine collected was measured at the end of 5 hours and total urine volume and concentrations of Na^+, K^+, Cl^- in the urine were determined. The concentrations of the electrolytes in urine were expressed in terms of mmol/L and urine volume was expressed in ml/5h [14].

4.Results & Discussion

STATISTICAL ANALYSIS

The data are expressed as mean ± SEM. Statistical analysis was performed using the Mann-Whitney U-test by Minitab 14.1 computer package. A significant level was set at $p<0.05$. linear regression analysis was performed to access dose dependencies.

Table 3. Effect of oral administration of Ethanolic extract of *Clitoria ternatea* on some urine parameters (up to 5h) on rats.

Sl no	Parameters	Negative control	Positive control	Standard	Test	
					2000mg/kg	4000mg/kg
1	pH	4.05±0.21	5.10±0.20	3.25±0.12	4.52±0.01	5.52±0.02
2	Na^+(ppm)	4300.65±560.18	4346.67±564.19	5012.31±401.12	6012.31±600.12	7343.33±610.12
3	K^+(ppm)	2512.00±220.25	2514.00±320.30	2526.10±100.09	4411.30±600.11	5481.50±690.13
4	Ca^{2+}(ppm)	381.10±20.40	387.20±30.50	98.35±78.20	100.55±81.31	116.66±88.32
5	Mg^{2+}(ppm)	412.00±70.90	531.00±74.95	450.25±40.00	511.66±44.00	671.67±74.80

*$p<0.05$, compared to control by non-parametric test (Mann Whitney U-test); N.D = not detected.

DISCUSSION

This study examined the effect of Ethanolic extract of *Clitoria ternatea* on the urine output of rats. The results showed that Ethanolic extract has moderate diuretic activity (in terms of diuretic action, percentage of urinary excretion, percentage change of urine output). It is reported that the alkaloid, carbohydrate, tannin, terpenoid and phenol can produce a diuretic effect. Toxicity studies showed that Ethanolic extract (10mg/kg) is well tolerated (in terms of overt signs of toxicity) and did not produce any toxicity. Additionally, there was no evidence of mortality during the toxicity studies. These observations suggest that Ethanolic extract of *Clitoria ternatea* may be safe for regular consumption.

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