



Pharmacological and Phytochemical Evaluation of *Clitoria ternata* flower and *Tribulus terrestris* seed

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ABSTRACT

To evaluate the anti-inflammatory and anti-diabetic potential of extracts *Clitoria ternata* flower (CTF) AND *Tribulus terrestris* seed (TTS) by in-vivo pharmacological models using rats. Ternate flower (CTF) AND *Tribulus terrestris* seed (TTS). Extraction by cold maceration techniques using hydro-alcoholic solvent. Evaluation of chemical constituents by various chemical tests. Acute toxicity studies as per OECD guidelines and calculation of ED₅₀ Anti-inflammatory activity by Carragenan induced rat paw models. Anti-diabetic activity by Alloxan induced diabetes in rats. Statistical analysis by Students t Test. Extraction by Cold Maceration method. Estimation of Phytochemicals by various chemical tests.(3) Acute Toxicity studies by OECD guidelines and dose selected were 200mg and 400mg/kg(4) Phytoconstituents are mostly presents in the *Clitoria ternata* flower and photosterols and flavonoids are not present in the *Tribulus terrestris* seed which are highly present in *Clitoria ternata* flower. Anti-inflammatory Activity of *Clitoria ternata* flower and *Tribulus terrestris* seed highly seen in group III and V of CTF AND TFS. Least amount present in group II of 39% in CTF and 45% of group IV in TTS..Anti-diabetic Activity of *Clitoria ternata* flower and *Tribulus terrestris* seed values are Mean \pm SEM of Six Animals. Statistical Significance: a = p < 0.001 and b = p < 0.05 as compared to control From the acute toxicity studies the ED₅₀ of the extract were fixes as 200 and 400 mg/kg. The anti-inflammatory and anti-diabetic activity of both CTF & TTS were calculated by *in-vivo* methods using rat models. The 400mg/kg of both *Clitoria ternata* flower (CTF) and *Tribulus terrestris* seed (TTS) extracts showed potential anti-inflammatory and anti-diabetic activity. Further studies are required for structural elucidation of the active component(s) involved in the anti-hyperglycaemic and anti-inflammatory activity of *Tribulus terrestris* L. and *Clitoria ternata*

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INTRODUCTION

An ancient Indian Sanskrit literature that described the diabetes mellitus as ‘madhumeha’ which deals the health care systems and duly acknowledged in modern medical texts. Current studies were estimated, that diabetes mellitus is most popular and commonly appearing disorder in human population all over the world. (1)

Inflammation is the body's attempt at self-protection to remove harmful stimuli and begin the healing process. Inflammation is part of the body's immune response. Infections, wounds, and any damage to tissue would not be able to heal without an inflammatory response(2)

Aim

To evaluate the anti-inflammatory and anti-diabetic potential of extracts Clitoria ternate flower (CTF) AND Tribulus terrestris seed (TTS) by in-vivo pharmacological models using rats.

Objectives

1. Collection of powders of Clitoria ternate flower (CTF) AND Tribulus terrestris seed (TTS).
2. Extraction by cold maceration techniques using hydro-alcoholic solvent.
3. Evaluation of chemical constituents by various chemical tests.
4. Acute toxicity studies as per OECD guidelines and calculation of ED₅₀
5. -inflammatory activity by Carragenan induced rat paw models.
6. Anti-diabetic activity by Alloxan induced diabetes in rats.
7. Statistical analysis by Students t Test.

MATERIAL AND METHOD

Extraction of the powdered plant material

All the two plants powders were procured from ayurvedic shop. The powdered plant materials were then passed through a sieve No 22 and stored in air tight container for further use. Extraction was carried out using ethanol by a simple maceration technique. Seven hundred and fifty milliliter (750 ml) of ethanol was added to 75 g of powder and kept on mechanical shaker for 4 h and filtered through Whatman No.1 filter paper. The filtrate was evaporated under reduced temperature and pressure to constant weight. The percentage yield of ethanolic extract was calculated for each plant.

Animals

Wistar albino rats of either sex (150-200 g) were used for the *ex vivo* study. They were housed in standard polypropylene cages and kept under controlled room temperature ($24 \pm 20^{\circ}\text{C}$, relative

humidity 45-55%) in a 12 h light-dark cycle. The rats were given a standard laboratory diet and water *ad libitum*

Phytochemical screening

Preliminary phytochemical screening of *Clitoria ternata* flower & *Tribulus terrestris* seed extracts were done to test the presence of the active chemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, fixed oils and fats (Khandelwal, 2008)

Acute toxic studies

Acute toxic studies were performed for both CTF and TTS extracts at a dose of 2000 mg/kg body weight in 2 groups of 6 rats respectively. The CNS activity of both group of rats were observed for its behavioral change. No death is reported for this selected dose and 1/10th of the dose selected for the anti urolithiastic activity (200mg and 400mg).

Anti inflammatory activity

Swiss albino rats were divided into five groups of six animals each. The test groups received orally 200 & 400 mg/kg of sample. The reference group received diclofenac sodium (10 mg/kg, p.o) while the control group received vehicle (1 % tween 80). After 1h, 0.1 mL, 1 % w/v carrageenan suspension in normal saline was injected into the subplanatar tissue of the right hind paw. The paw volume was measured at 30 min, 1, 2, 3 and 4 h after carageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula: % inhibition = $(V_c - V_t / V_c) \times 100$

Whereas V_c was the average inflammation (hind paw edema) of the control group of mice at a given time, V_t was the average inflammation of the drug treated (i.e sample or reference diclofenac sodium) mice at the same time.

Antidiabetic activity

Antidiabetic screening For experiment overnight fasted Wistar rats was induced by a single intraperitoneal administration of Alloxan monohydrate (150 mg/kg.b.wt) in 0.1 M citrate buffer, pH 4.5. Those animals with fasting blood glucose level more than 300 mg/dl after alloxan administration were selected for the study and they were divided into four groups of six animals each.

Group I served as diabetic control and received 0.3% CMC, Group II served as positive control and received glibenclamide (10 mg/kg.b.wt), Groups III and IV received the ethanolic extract of *Clitoria ternate* flower (200 mg/ kg.b.wt and 400 mg/kg.b.wt respectively). Groups V and VI received the ethanolic extract of *Tribulus terrestris* sedd (200 mg/ kg.b.wt and 400 mg/kg.b.wt respectively).

Statistical analysis

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Table-1 Phytoconstituents of *Clitoria ternata* flower & *Tribulus terrestris* seed Extracts

S.NO	Phytoconstituents	<i>Clitoria ternata</i> flower	<i>Tribulus terrestris</i> seed
1	Saponin	++	++
2	Tanins	++	++
3	Alkaloids	++	++
4	Glycosides	++	++
5	Phytosterols	++	-
6	Carbohydrates	+	+
7.	Flavonoids	++	-
8.	Terpenoids	++	+

Note:

++ - Presence of Chemical constituents, - Absence of Chemical constituents.

Table-2 Results of *In vivo* anti inflammatory activity of *Clitoria ternata* flower (CTF) & *Tribulus terrestris* seed (TTS)

S.No	Group	Dose	Paw volume (ml)				Difference (b-a)	Men value	%anti-inflammatory activity (VcVt/Vc)X100
			0 hour	1 hour	2 hour	3 hour			
Group-I	Control	1% CMC	0.2	0.6	0.5	0.5	0.4	0.33	-
			0.2	0.6	0.6	0.7	0.4		
			0.2	0.5	0.6	0.6	0.3		
			0.2	0.5	0.6	0.7	0.3		
			0.2	0.5	0.5	0.8	0.3		
Group-II	CTF	(400 mg/kg b.wt)	0.2	0.5	0.7	0.8	0.3	0.05	84%
			0.3	0.4	0.1	0.1	0.1		
			0.3	0.4	0.1	0.1	0.1		
			0.3	0.3	0.2	0.1	0		
			0.3	0.3	0.1	0.1	0		
Group-III	CTF	(200mg/kg b.wt)	0.3	0.4	0.2	0.2	0.1	0.2	39%
			0.3	0.6	0.2	0.2	0.3		
			0.3	0.3	0.2	0.3	0		
			0.3	0.6	0.2	0.3	0.3		
			0.3	0.6	0.4	0.5	0.3		
Group-IV	TTS	(200mg/kg b.wt)	0.3	0.5	0.3	0.4	0.2	0.18	45%
			0.3	0.5	0.1	0.1	0.2		
			0.3	0.6	0.4	0.3	0.3		
			0.3	0.5	0.2	0.1	0.2		
			0.3	0.5	0.1	0.2	0.2		

Group V	TTS	(400 mg/kg b.wt)	0.3	0.4	0.2	0.2	0.1	0.05	84%
			0.3	0.4	0.1	0.1	0.1		
			0.2	0.3	0.2	0.1	0.1		
			0.2	0.3	0.2	0.1	0.1		
			0.1	0.1	0.2	0.2	0		
			0.2	0.2	0.1	0.2	0		
Group- VI	Standard Diclofenac sodium	(10mg/k)	0.2	0.3	0.1	0.1	0.1	0.08	75%
			0.2	0.2	0.2	0.1	0		
			0.2	0.3	0.1	0.1	0.1		
			0.2	0.3	0.1	0.1	0.1		
			0.3	0.4	0.1	0.1	0.1		
			0.2	0.3	0.2	0.1	0.1		
			0.3	0.3	0.1	0.1	0.1		
0.2	0.4	0.2	0.1	0.1					
0.3	0.3	0.2	0.1	0					

Table-3 Antidiabetic activity of *Clitoria ternata* flower (CTF) & *Tribulus terrestris* seed (TTS)

Group	Dose	Normal level	Glucose level after alloxan	Glucose level after drug treatment
control	0.3%cmc	93.64±4.25	312.16±12.04	338.10±10.12
glimeclamide	10mg/kg	98.34±6.08	302.05±8.56	102.34±8.34 ^a
CTF	200mg/kg	102.12±10.12	308.46±20.03	198.16±15.08 ^b
CTF	400mg/kg	88.63±1.02	304.24±5.08	103.64±3.14 ^a
TTS	200mg/kg	112.12±10.12	328.46±10.03	188.16±15.08 ^a
TTS	400mg/kg	83.63±1.02	314.24±5.08	113.64±3.14 ^b

Values are Mean ± SEM of Six Animals. Statistical Significance: a = p < 0.001 and b = p < 0.05 as compared to control.

RESULTS AND DISCUSSION

In our study the ethanolic extracts of *Clitoria ternatea* flower showed potential anti-inflammatory activity at a dose of 400mg/kg level. From this study it was predicted that saponins and flavonoids after absorption from the GIT they cross the blood brain barrier (BBB) and then enter into the brain and amplify the signaling in the basal hypothalamus energy sensing function. Previous studies showed, the anti inflammatory, analgesic studies of petroleum ether extract (60-80c) from the flowers of *Clitoria ternatea* showed that it exhibited significant anti inflammatory activity at both the dose level (200 and 400 mg/kg body weight) (P<0.01).(11). *Clitoria ternatea* roots methanol extract when given by oral route to rats was found to inhibit both the rat paw oedema caused by carrageenin and vascular permeability induced by acetic acid in rats (12)

In our study the ethanolic extracts of *Clitoria ternatea* flower showed potential anti-diabetic activity at a dose of 400mg/kg level. Previous studies showed, Antihyperglycemic *Clitoria*

ternatea showed antihyperglycemic activity (13). Flavonoids, tannins, alkaloids, saponins, tri terpinoids, glycosides are highly soluble in ethanol than the ethyl acetate and hexane(14,15) Flavonoids, tannins(16), alkaloids(18), tri terpinoids (17), glycosides having potential anti-diabetic activity.

In our study the ethanoloic extracts of *Tribulus terrestris seed* showed anti-inflammatory activity at a dose of 400mg/kg level. From the previous literature it was revealed that, *T. terrestris* has been used in traditional medicine for relieving rheumatic pain and as an analgesic plant for a long time. In this investigation the analgesic effect of methanolic extract of this plant on male albino mice was evaluated by formalin and tail flick test. *T. terrestris* extract has a suitable analgesic effect and further studies are required to produce a more effective product of this plant to substitute for conventional analgesic drugs (19). Hence the extract having some considerable anti-inflammatory activity.

In our study the ethanoloic extracts of *Tribulus terrestris seed* showed potential anti-diabetic activity at a dose of 400mg/kg level. This observed potential anti-hyperglycaemic activity might be due to the large presence of saponins in *T. terrestris* [20]. Saponin from *T. terrestris* reported to possess hypoglycemic properties and produced protective effect in streptozotocin-induced diabetic rats by inhibiting oxidative stress [21].

SUMMARY AND CONCLUSION

The two plants powders were procured from ayurvedic shop. Extraction was carried out using ethanol by a simple maceration technique. *Wistar* albino rats of either sex (150-200 g) were used for the *ex vivo* study. Preliminary phytochemical screening of *Clitoria ternata* flower & *Tribulus terrestris* seed extracts were done to test the presence of the active chemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, fixed oils and fats. Acute toxic studies were performed for both CTF and TTS extracts at a dose of 2000 mg/kg body weight in 2 groups of 6 rats respectively. From the acute toxicity studies the ED 50 of the extract were fixed as 200 and 400 mg/kg. The anti-inflammatory and anti-diabetic activity of both CTF & TTS were calculated by *in-vivo* methods using rat models. The 400mg/kg of both *Clitoria ternata* flower (CTF) and *Tribulus terrestris* seed (TTS) extracts showed potential anti-inflammatory and anti-diabetic activity. The pharmacological activity of extracts may be due to., Anti-inflammatory of CTF - The presence of saponins and flavonoids. Anti-diabetic activity of CTF – The presence of Flavonoids, Tannins and Terpinoids Anti-inflammatory of TTS - Previous Research work on analgesic activity. Anti-diabetic activity of TTS - Large presence of Saponins

Further studies are required for structural elucidation of the active component(s) involved in the anti-hyperglycaemic and anti-inflammatory activity of *Tribulus terrestris* L. and *Clitoria ternate*.

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