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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *CLITORIA TERNATEA* LINN. LEAVES EXTRACT ON RAT MODEL

Manisha Bhatia [1], Jagbir Chahal and Sumeet Gupta*[2]

Department of Pharmacognosy [1], Department of Pharmacology [2], M. M. College of Pharmacy, M. M. University, Mullana, Ambala-133203, Haryana, India

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Correspondence to Author:

Dr. Sumeet Gupta

Professor, Department of Pharmacology, M. M. College of Pharmacy, M. M. University, Mullana, Ambala, Haryana-133203, India

E-mail: sumeetgupta25@gmail.com

ABSTRACT: *Clitoria ternatea Linn* is used both in traditional system and folk medicine to treat various inflammatory diseases such as arthritis and burns. The aim of the present study was to evaluate the analgesic and anti-inflammatory activity on scientific basis of different extracts of *Clitoria ternatea* Linn using carrageenan-induced paw edema and tail flick method in rats respectively at various dose levels. From the results obtained among different extracts, only ethanol extract (400 mg/kg) showed maximum inhibition (23.80%) at 6th h and the early onset of action was found at 3rd h with only petroleum ether extract (400 mg/kg) showed statistical significant effect ($p<0.001$) compared to positive control group. Petroleum ether extracts (100 mg/kg, 200 mg/kg & 400 mg/kg) showed statistical significant inhibition from 4th to 6th h. In analgesic activity, both ethanol & petroleum ether extract showed the same type of effect but ethanol treated extract exhibited long lasting effect up to 2 h. The findings of the study proved justification of the use of the plant in the treatment of inflammatory conditions.

INTRODUCTION: Inflammations and pain are the initiator signs of inflammatory conditions. Drugs which are used now days in treatment of pain and inflammatory conditions belong to the category of either narcotics or non-narcotics.

All of these drugs are very expensive and show remarkable side effects. Nature has offered a complete store-house of remedies for all ailments of mankind by providing drugs from plants and algae.

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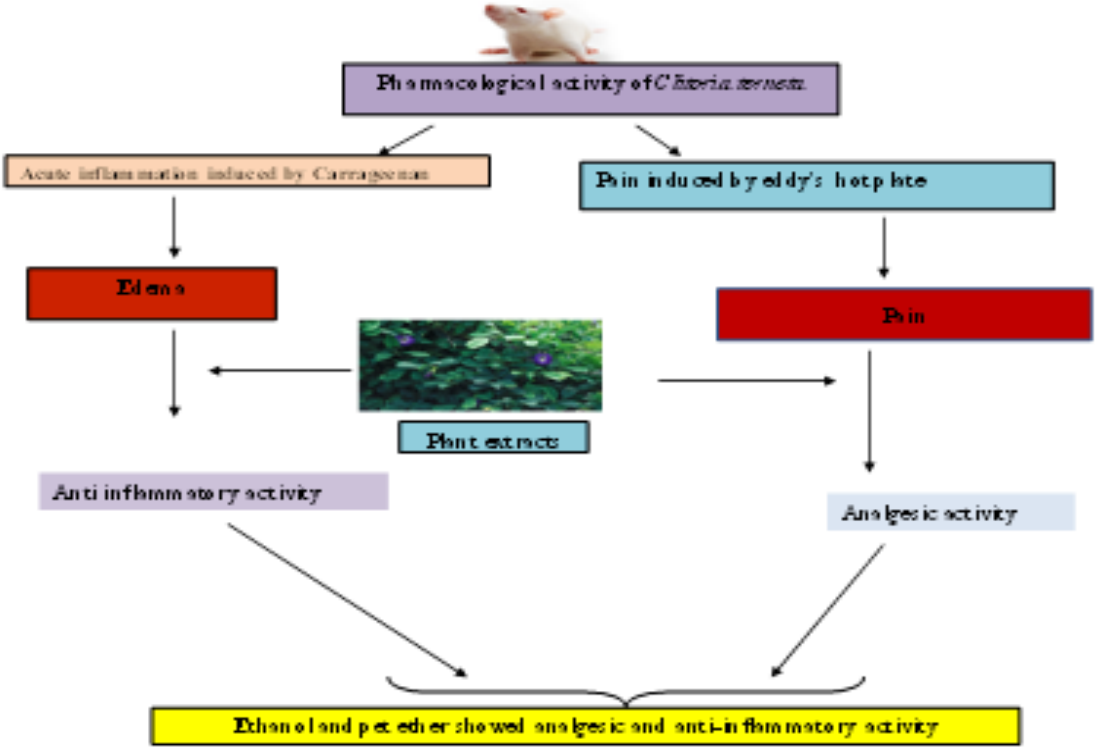
Medicinal plants are used by 80 % of the world population and it has become imperative to investigate the acclaimed ones for their possible therapeutic benefits, especially nowadays, when treatment of many serious diseases still faces diverse challenges.

The family Fabaceae (Leguminosae), commonly known as the legume family, is the second largest dicotyledeon which comprises three subfamilies Minosoideae, Caesalpinioideae and Fabaideae [1]. It belongs to largest families of plants with 650 genera and over 18000 species all over the world [2]. The genus *Clitoria* comprises 60 species distributed mostly within the tropical belt and few species found in temperate areas [3]. *Clitoria ternatea* Linn., commonly known as Butterfly pea or Shankpushapi, belonging to the family Fabaceae, previously it was known in the family of

papilionaceae [4]. It is also called as Asian pigeon wings [5]. *C. ternatea* is a perennial climbing or trailing herb growing from a woody rootstock. This genus is rich in triterpenoids, flavonoids, anthocyanins, tannins, resins, starch, proteins, carbohydrates and steroids [6]. *C. ternatea* is a bioactive plant and used in various ailments as a folklore medicine. In the traditional Indian system of medicine, the roots, seeds and leaves have been used as a brain tonic and promote memory enhancer [7].

Over the last few years, this plant possess various pharmacological effects like anti-asthmatic, anti-depressant, anti-convulsant, anti-stress, memory enhancer, nootropic, anxiolytic like activities [8-11]. It is also used in treating diabetes mellitus [12], burning sensation, inflammations, skin diseases, pulmonary tuberculosis, eye infections, urinogenital disorders and as an anti-dote for treating toxicity [13].

To best our knowledge, no study has been investigated on comparison of different extracts of *C. ternatea* leaves on analgesic and anti-inflammatory activity in acute inflammation using a rat model.



GRAPHICAL ABSTRACT

MATERIALS AND METHODS:

Chemical and drugs: Drugs used in this study were of pharmaceutical grade. Carrageenan and Complete Freund's adjuvant were purchased from Sigma Chemicals (St Louis, USA). Pure sample of diclofenac sodium were obtained as a gift from Ind. Swift Laboratories (Baddi, India). All other reagents and chemicals used in the study were of analytical grade.

Plant material: Leaves of *Clitoria ternatea* were collected in September 2009 from Botanical garden, (Forest Research Institute Dehradun, Uttaranchal, India).

The specimen plant (NISCAIR/RHMD/consult/-2009-10/1337/139) was identified with the help of literature and authenticated by Dr. H. B. Singh, Scientist F & Head, Raw Materials Herbarium and Museum, N.I.S.C.A.I.R, New Delhi, India. The fresh plant material was cleaned with distilled water to remove debris and dried at 35°-40ºC for 10 days and, pulverized in the electric grinder; the powder was passed through sieve No. 60 and used for further extraction.

Preparation of extracts: The dried powder of the leaves (3 kg) was successively extracted with Soxhlet apparatus using petroleum ether (60-80°C) and ethanol for 72 h each.

Crude aqueous extract of these leaves were prepared separately by maceration for 24 h. The last trace of solvent was removed by vacuum dried. The extracts were stored below 4ºC until further used. When needed, the extract was suspended/dissolved in desired solvent and used. The extracts were concentrated by performing the qualitative chemical tests to determine various phytochemical constituents.

Animals: Albino male rats (Wistar strain) of about 180-200 g were used after obtaining the approval of the Institute Animal Ethics Committee (MMCP/IEC/10/08). The animals were housed under standard conditions of temperature (24 ± 28°C) and relative humidity (60-70%) with a 12:12 light-dark cycle. The animals were fed standard pellet diet (Lipton India, Ltd) and water *ad-libitum.*

Drugs and dosage: The doses were selected on the basis of acute studies according to the OECD guidelines [17]. Extracts were administered orally in different doses (100 mg/kg, 200 mg/kg & 400 mg/kg) in the form of suspension prepared in 0.2 ml of 2% w/v carboxy methyl cellulose with 2.0% tween 80. Diclofenac sodium (10 mg/kg) was administered orally in the form of suspension.

Experimental design: Animals were divided into 12 groups. Each group consists of 6 rats:

Group 1 – Normal (N)

Group 2 – Positive Control {Carrageenan}(C)

Group 3 – Standard group {Carrageenan + Diclofenac sodium 10 mg/kg b. w p. o (S)}

Group 4 – Test group: {Carrageenan + Petroleum ether extract 100 mg/kg b.w p.o ($Ptet_{100}$)}

Group 5 – Test group: {Carrageenan + Petroleum ether extract 200 mg/kg b.w p.o ($Ptet_{200}$)}

Group 6 – Test group: {Carrageenan + Petroleum ether extract 400 mg/kg b. p.o ($Ptet_{400}$)}

Group 7– Test group: {Carrageenan + Ethanol extract 100 mg/kg b.w p.o (Et_{100})}

Group 8– Test group: {Carrageenan + Ethanol extract 200 mg/kg b.w p.o (Et_{200})}

Group 9– Test group: {Carrageenan + Ethanol extract 400 mg/kg b.w p.o (Et_{400})}

Group 10 – Test group: {Carrageenan + Aqueous extract 100 mg/kg b.w p.o (Aq_{100})}

Group 11 – Test group: {Carrageenan + Aqueous extract 200 mg/kg b.w p.o (Aq_{200})}

Group 12 – Test group: {Carrageenan + Aqueous extract 400 mg/kg b.w p.o (Aq_{400})}

Experimental models (Acute Inflammation):

a) **Carrageenan induced model [14]:** Each 0.1 ml of 1% w/v carrageenan was injected into the rat paw of all the groups except normal group after one week of the oral administration of drugs. The paw oedema volume was measured with digital plethysmometer (Model 7140, UGO Basile, Italy) after a gap of 1 h, 2 h, 3 h and 4 h. The percentage inhibition of oedema compared with that of the control was taken as anti-inflammatory activity. The percentage inhibition of oedema was calculated by the formula:

Percentage inhibition of oedema = (A-B)/A X 100.

Where, A represents the paw volume of the control group and B represents the paw volume of the test drug treated group.

b) **Tail flick method (Analgesic activity) [14]:** Analgesic effect in rats was assessed using analgesiometer. The instrument has a nichrome wire, which is heated to the required temperature and maintained by means of heat regulators. The strength of the current passing through the naked nichrome wire was kept constant (4 Amps).

The rats were kept in a rat holder with only the tail portion protruding out. The tail was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire but without touching it. The latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting. A cut-off time of 10 sec was planned to avoid any tissue damage in the animal.

The reaction time for each group was measured at 30, 60, 90 and 120 min on the seventh day after one week oral administration of drugs.

Statistical analysis: The data were expressed as mean ± SEM. The statistical analysis of the ankle joint diameter, paw oedema, % inhibition and soft tissue thickness were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. An associated probability of error, $p<0.05$ was considered statistical significant.

RESULTS & DISCUSSION: Researches on plants with medicinal properties and identification of the chemical components responsible for their activities have corroborated the traditional uses of ancient healing wisdom. They have proven the enduring healing potential of many plant medicines even in today's hi-tech community. Therefore, the present study was aimed at evaluating the scientific basis for the traditional use of *C. ternatea* using analgesic and inflammatory rat model. The extracts were screened for phytochemical studies to confirm the presence of triterpenoids, alkaloids, flavonoids, anthocyanins, tannins, resins, starch, proteins, carbohydrates and steroids which were also reported in the previous investigations in different part of plant [15].

The results of qualitative analysis depicted that pet ether extract of *C. ternatea* showed the presence of steroids, carbohydrates and triterpenoids but failed to show proteins, glycosides, flavonoids, saponins and tannins whereas ethanol and aqueous extracts showed positive response for all constituents except triterpenoids, carbohydrates and steroids. Different constituents are found in different parts of the plant. The main active constituents present in the roots were taraxerol and taraxerone reported by Banerjee *et al*., 1963 [16] & Kumar *et al.,* 2008 [17] through HPTLC and also investigated the scopoletin through TLC [18-19].

The leaves and flowers contained flavonoid glycosides β-sitosterol, kaempferol-3-monoglucoside, kaempferol-3-rutinoside, kaempferol-3-neohesperiodoside, kaempferol-3-O-rhamnosyl-(1,6)-glucoside, kaempferol-3-O-rhamnosyl-(1,6)-galactoside and kaempferol-3-O-rhamlnosyl-(1,2)-O-chalmnosyl-(1,2)-O-[rhamnosyl-(1,6) glucoside [20] which were further confirmed through UV, NMR and Mass spectroscopy data, the presence of Kaempferol-3-glucoside, Kaempferol-3-rutinoside and Kaempferol-3-neohesperidoside was characterized as Kaempferol-3-o-rhamnosyl glucoside called as Clitorin [21].

The constituents reported in the mucilage are alkaloids and anhydrogalacatan, anhydropentosan and methylpentosan. Another study reported on the different active constituents present in flowers are delphinidin glycosides, anthocyanins (ternatins C1, C2, C3, C4, C5, D3, preternatins A3, ternatin A1, A2, B1, B2, D1 and D2), malvidin glucoside, delphindin-3, 5-diglucoside, Delphinidin 3-glucoside, 3-methyl ether of delphinidin glucoside, major flavonol glycosides of kaempferol, quercetin and myricetin are also present in small quantities [22].

In the carrageenan-induced model, among different time intervals of all the extracts, maximum inhibition (23.80%) was observed at 6^{th} h with ethanol extract (400 mg/kg) but the early onset of action showed at 3^{rd} h with only petroleum ether extract (400 mg/kg) and remained constantly statistical significant.

Among different extracts, only petroleum ether extracts (100 mg/kg, 200 mg/kg & 400 mg/kg) showed statistical significant inhibition from 4^{th} to 6^{th} h (**Table 1**) which was also similar to previous study reported by Kumar *et al.,* 2012. Another study has also been reported about the inhibition effect on the different inflammation markers like hyaluronidase, elastase and matrix-metalloproteinase-1 inhibitor with methanolic and ethyl extracts [23].

Other species of this plant also possess anti-inflammatory activity [24]. This anti-edematous effect was significantly observed during the first phase of inflammation indicating the inhibition of histamine release. In addition to this, the extracts were also significantly maintained during the second and third phases of oedema development. In analgesic activity, all the three extracts (200 mg/kg & 400 mg/kg) showed analgesic effect after 30 m but ethanol extract remain statistical constant up to end of the experiment after treatment of acute inflammation (**Table 2**).

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TABLE 1: EFFECTS OF CLITORIA TERNATEA LEAVES EXTRACTS ON CARRAGEENAN INDUCED RAT PAW MODEL

Sr. No.	Groups (n=6)	1st hr	2nd hr	3rd hr	4th hr	5th hr	6th hr
1.	Normal control	0.688±0.03 (-1.51%)	0.690±0.09 (17.4%)	0.682±0.08 (19.75%)	0.682±0.02 (15.68%)	0.694±0.14 (14.67%)	0.678±0.032 (5.89%)
2.	Positive control	0.673±0.06	0.712±0.02	0.822±0.13	0.839±0.03	0.832±0.05	0.815±0.01
3.	Standard (Diclofenac 15mg/kg)	0.679 ± 0.05 ns (-0.89%)	0.705±0.07 ns (0.983%)	0.657± 0.02 * (20.07%)	0.602±0.08*** (28.24%)	0.600±0.04*** (27.88%)	0.583±0.07*** (28.46%)
4.	Pet ether extract (100mg/kg)	0.681±0.04 ns (-1.18%)	0.731±0.09 ns (-2.66%)	0.752±0.09 ns (8.51%)	0.748±0.06** (10.84%)	0.731±0.01*** (12.13%)	0.712±0.05*** (12.63%)
5.	Pet. ether extract (200mg/kg)	0.685 ± 0.03 ns (-1.78%)	0.712± 0.08 ns (0%)	0.704± 0.08 ns (14.35%)	0.701± 0.08 ** * (16.44%)	0.692± 0.08 ** (16.82%)	0.631±0.08*** (22.57%)
6.	Pet. ether extract (400mg/kg)	0.675 ± 0.12 ns (-0.297%)	0.701±0.04 ns (1.54%)	0.689± 0.08 * (16.18%)	0.673±0.04 *** (19.78%)	0.644±0.02 *** (22.59%)	0.641±0.05 *** (21.34%)
7.	Ethanol extract (100mg/kg)	0.682±0.03 ns (-1.33%)	0.742±0.02ns (-4.21%)	0.730±0.05ns (11.19%)	0.720±0.02*** (14.18%)	0.713±0.04** (14.30%)	0.703±0.06** (13.74%)
8.	Ethanol extract (200mg/kg)	0.662 ± 0.15 ns (1.63%)	0.777±0.09 ns (-9.12%)	0.785± 0.03 ns (0.851%)	0.779± 0.06 * (6.0%)	0.731±0.21 ns (-12.13%)	0.727±0.10* (-10.79%)
9.	Ethanol extract (400mg/kg)	0.692 ± 0.05 ns (-2.82%)	0.767±0.08 ns (-7.72%)	0.710± 0.03 ns (4.50%)	0.676± 0.03 *** (19.42%)	0.634±0.02*** (23.79%)	0.621±0.03 *** (-23.80%)
10.	Aqueous extract (100mg/kg)	0.676±0.03 ns (-0.44%)	0.733±0.05ns (-2.94)	0.774±0.02 ns (9.48%)	0.748±0.07** (10.84%)	0.730±0.02*** (12.25%)	0.721±0.08 ns (11.53%)
11.	Aqueous extract (200mg/kg)	0.680±0.11 ns (-1.04%)	0.710±0.03 ns (0.28%)	0.726± 0.01 ns (11.67%)	0.733± 0.03 *** (12.63%)	0.721±0.06 ** (13.34%)	0.721±0.16 ns (11.53%)
12.	Aqueous extract (400mg/kg)	0.699±0.15 ns (-3.86%)	0.734±0.08 ns (-3.08%)	0.712± 0.03 ns (13.38%)	0.701± 0.05*** (16.44%)	0.687±0.13* (17.42%)	0.674±0.01*** (17.30%)

TABLE 2: EFFECTS OF CLITORIA TERNATEA LEAVES EXTRACTS ON ANALGESIC ACTIVITY

Sr. No.	Groups (n=6)	After 30 min. (sec.)	After 60 min. (sec.)	After 90 min. (sec.)	After 120 min. (sec.)
1.	Positive Control	3.01±0.90	3.54±0.76	4.05±0.96	3.15±0.17
2.	Standard (Diclofenac 15mg/kg)	11.4±1.60***	10.3±2.0***	10.0±0.81** *	7.75±1.20***
3.	Pet. Ether extract (100mg/kg)	3.13±1.20 ns	5.19±0.47**	4.13±1.03 ns	3.94±0.64*
4.	Pet. ether extract (200mg/kg)	6.0±2.41*	8.3±1.45***	8.03±1.89**	4.25±2.98 ns
5.	Pet. Ether extract (400mg/kg)	9.55±2.70***	7.05±1.62***	8.15±1.60***	4.5±2.70 ns
6.	Aqueous extract (100mg/kg)	3.76±0.22ns	4.02±0.40 ns	3.8±0.22 ns	3.18±1.31ns
7.	Aqueous extract (200mg/kg)	5.13±1.58 *	4.55±1.32 ns	5.35±0.92*	3.11±0.37 ns
8.	Aqueous extract (400mg/kg)	4.4±1.32 *	4.67±0.31 **	6.12±0.40*	4.1±1.19 ns
9.	Ethanol extract (100mg/kg)	4.32±1.38 ns	4.793±0.56**	6.14±1.21***	3.15±1.64ns
7.	Ethanol extract (200mg/kg)	5.25±0.25 ***	6.21±0.93 ***	5.25±0.45 *	4.05±0.95 *
8.	Ethanol extract (400mg/kg)	7.2±1.22 ***	6.05±1.25**	6.44±2.56*	5.15±2.11 *

Similar results were also reported in previous study [16] with pet ether extract. Both pain and inflammation are the results of chemical signals from the point of invasion to the brain. The therapeutic effect of petroleum ether is mechanistic inhibition of NF-κB activation which can occur by different mechanisms including;

a) Inhibiting,

b) Targeting the proteasomal degradation,

c) Interfering the translocation of NF-κB to the nucleus or the binding of NF-κB to DNA.

This inhibitory effect may be presence of high content of triterpenoids like taraxerol and taraxerone [25] which is reported by other researchers also [26]. Various secondary enzymes are also responsible for inflammations such as phosphlolipase A_2, cycloxygenase and lipo-xygenase and the nitric oxide synthase. The inhibition of these enzymes exerted by flavonoids is definitely one of the important cellular mechanisms of anti-inflammation [27].

However polyphenols has also been shown to suppress the expression of TNF-α induced MMP-13 in primary chondrocytes. Thus many other natural compounds involved the same pathway and showed different therapeutic activity which needs to be further investigated.

The present experimental findings of above models suggested that pet ether and ethanol extracts of this plant may give promising results in the long run practice and have a high potency as an anti-inflammatory and analgesic effect in the treatment of acute inflammatory conditions. Hence, it is necessary to evaluate its anti-inflammatory activity on humans in clinical conditions specially inflammations.

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Conflict of interest statement: There are no conflicts of interest.

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