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SCREENING OF ANTI-INFLAMMATORY AND ANTI ANALGESIC ACTIVITY OF *CONVOLVULUS PLURICAULIS* CHOISY

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ABSTRACT:

Objective: This study was designed for Screening of anti-inflammatory and anti-analgesic activity of ethanolic extract of *Convolvulus pluricaulis* Choisy in various animal experimental models.

Material and Methods: Ethanolic extract of *Convolvulus pluricaulis* Choisy evaluated for anti-inflammatory activity in Carrageenan-induced paw edema, Cotton pellet-induced granuloma model. The anti-analgesic activity was evaluated by Hot Plate Method and Tail-Flick Assay.

Result: Administration of the ethanolic extract of *Convolvulus pluricaulis* Choisy at dose of 800 mg/kg p.o. showed significant ($P < 0.001$) inhibition of rat paw edema. The analgesic activity was determined on Wistar albino rats by hot plate method, tail flick assay using Morphine sulphate as standard drug at a dose of 5 mg/kg of body weight and the results were expressed as mean increase in latency after drug administration ± SEM.

Conclusion: This study shows that ethanolic extract of *Convolvulus pluricaulis* Choisy has significant anti-inflammatory and anti-analgesic activity.

INTRODUCTION: *C. pluricaulis* (family *Convolvulaceae*) is one of four plants that is referred to as Shankhapushpi, and appears to be the 'true' form of Shankhapushpi according to the Ayurvedic Pharmacopoeia with the other three herbs (*Clitorea ternatea*, *Evolvulus alsinoides* and *Canscora decussata*) being used as replacements for Convolvulus [1].

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C. pluricaulis is medicinally used for a brain tonic, nervine tonic, alternative and laxative as well as to reduce anxiety, neurosis, cognitive decline, and has some reported usage for fertility and seminal issues [2]. *C. pluricaulis* contain convolamine, Scopoletine, Ceryl alcohol, β-sitosterol, palmitic, myristic, and linoleic acid [3].

Earlier studies have shown that the extract of the plant possesses Antioxidant activity [4], Anticonvulsant activity [5], Antidepressant activity [6], Anxiolytic activity [7, 8], Learning behavior & memory enhancement activity [9, 10], Anti Thyroid activity [11], Antiulcer activity [12], Anti-obsessive activity [13], Neuroprotective activity [14, 15], Hepato-protective activity [16], Anti-bacterial activity [17],

Anti-viral activity [18], Nootropic activity [19]. So review of literature revealed that the antidiabetic activity of this plant has not been subjected to scientific evaluation. Since the leaves are one of the important parts of the plant *Convolvulus pluricaulis,* it was used for the pharmacological investigation.

METHOD AND MATERIAL:

Collection and authentication of plant material: The leaves of *C. pluricaulis* was collected from village raksa near Jhansi, (U.P) India and authenticated by Dr. Neelema Sharma, (Research Officer Incharge) in National Vrkshayurveda Research Institute (N.V.R.I.) Department of AYUSH, Ministry of Health & Family Welfare. Jhansi, with accession no.- 21706**.**

Extraction of plant material: Leaves of *C. pluricaulis* was air dried in the shade and coarsely powder by using grinder. The powder plant material (300 gm) was packed in the Soxhlet apparatus and continuously extracted by ethanol at the temperature 70°C. The percentage yield was calculated against 300 g of powder drug. It was 11.89%.

Chemicals and drugs: All the chemicals and solvents were of analytical grade and were procured from Loba Chemie Pvt. Ltd. 107, Wodehouse Road, Mumbai, India. Carrageenan was procured from Himedia laboratories Pvt. Ltd., Mumbai-400086, India. Indomethacin (10 mg/kg p.o.) sample is taken from Indian drug pharmaceutical Ltd. Rishikesh (Utrakhand), India, Morphine sulphate 5 mg/kg (i.p.).

Experimental Animals: Experiments were performed on adult male Wistar rats (body weight range 150–200 g), 10 to 11 weeks of age. Animals were housed and maintained at 22°C under a 12-h light/12-h dark cycle, with free access to food and water. Experiments were carried out during the normal light/dark cycle, always starting at the same hour (10:00 AM). Efforts were made to minimize animal suffering and to reduce the number of animals used. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity. All experiments complied with the guidelines on ethical standards for the investigation in animals. The experimental protocol was approved by the Institutional Animal Ethics Committee of the Bundelkhand University. (Reference number BU/Pharm/IAEC/12/025).

Acute oral toxicity studies: Acute toxicity studies were performed on albino wistar rats according to Organization for Economic Co-operation and Development (OECD) – 425 guidelines the animals were kept fasted for 2 hours with free access to water. The *C. Pluricaulis* extract were administered orally at a dose of 50 mg/kg. The dose at which mortality was observed in two out of three albino Wistar rats, it was considered as toxic dose [20].

However, if no mortality was observed, the procedure was repeated with higher dose such as 100, 300, 500, 2000, 5000 mg/kg body weight. Toxic manifestations like abnormal motor activity, alteration in water or food intake, respiration, sedation and moribund stages were observed for 6 h and mortality for 24 h.

There was no mortality amongst the graded dose groups of albino Wistar rats up to a dose of 5000 mg/kg for duration of 72 h. This finding probably suggests that *C. Pluricaulis* extract are relatively safe or non-toxic in albino Wistar rats at the doses used for this study.

Evaluation of Anti-inflammatory activity:

1. **Carrageenan-induced paw edema:** The rats were divided into five groups of six animals each. Acute inflammation was induced by intraplantar administration of 0.1 mL of carrageenan (1% solution in normal saline). Group A was treated with 2% gum acacia solution, Group B with indomethacin (10 mg/kg p.o.), Group C with *C. pluricaulis* extract (400 mg/kg body weight); Group D with *C. pluricaulis* extract (600 mg/kg body weight); Group E with *C. pluricaulis* extract (800 mg/kg body weight); 1 h before administration of phlogistic agent. The paw volume was measured prior to injection of phlogistic agent (0 h) and then at a predetermined interval of 60 min up to 3 h after carrageenan injection.

Paw volume was measured using Digital Plethysmometer (UGO Basil, Italy). Change in the paw volume was measured, and anti-inflammatory activity was calculated as follows:

% Inhibition of inflammation= 1− (Vt/Vc) × 100,

Where, Vt represents the change in the paw volume in *C. pluricaulis* extract treated group and Vc represents the change in the paw volume in the corresponding vehicle-treated control group [21] **[Table 1]**

2. **Cotton pellet-induced granuloma:** The rats were divided into five groups (n = 6). After shaving the fur, the rats were anesthetized with ether and 20 mg of sterile cotton pellets was surgically inserted in the groin region. Group A was treated with 2% gum acacia solution, Group B with indomethacin (10 mg/kg p.o.), Group C with *C. pluricaulis* extract (400 mg/kg body weight); Group D with *C. pluricaulis* extract (600 mg/kg body weight); Group E with *C. pluricaulis* extract (800 mg/kg body weight); respectively, for seven consecutive days from the day of cotton pellet implantation. The animals were anesthetized on the 8th day, and the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C for constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation [22] **[Table 2]**

Evaluation of Analgesic Activity: The animals were divided into five groups of 5 animals each. The control group received distilled water (p.o.), Test Group 1 was given *C. pluricaulis* extract 250 mg/kg (p.o.), Test Group 2 was given *C. pluricaulis* extract 500 mg/kg (p.o.), Test Group 3 was given *C. pluricaulis* extract 750 mg/kg (p.o.),and Standard Group was given morphine sulphate 5 mg/kg (i.p.).

1. **Hot Plate Method:** The hot plate test was used to measure analgesic activity by the method described by Eddy with minor modifications. Rats were kept on a hot plate having a constant temperature of 55±1°C.

The time taken for either paw licking or jumping was recorded. Each rat was separately placed on the hot plate in order to obtain the animal's response to electrical heat-induced pain (licking of the forepaws and eventually jumping out of the plate). Jumping out of the hot plate was taken as an indicator of the animal's response to heat-induced pain.

The time taken for each rat to jump out of the plate (i.e., reaction time) was noted and recorded in seconds. Starting before and 15 min after oral administration (p.o.) of vehicle, the test agents *C. pluricaulis* extracts, respectively, at 250, 500 and 750 mg/kg, (p.o.) and intraperitoneal (i.p.) injection of morphine sulfate at 5 mg/kg, the nociceptive response was measured every 15 min interval over a 90-minute period [23] **[Table 3]**

2. **Tail-Flick Assay:** The Tail flick assay was used to measure analgesic activity by the method described by Amour & Smith, 1941 [24] with minor modifications in the process. Tail flick method was employed to study the antinociceptive activity in albino rats. A radiant heat automatic tail flick analgesiometer was used to measure response latencies. Basal reaction time of animals to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on radiant heat source. The tail withdrawal from the radiant heat was taken as end point. The cut-off time of 10–12 s was imposed to avoid tail damage by heat.

Animal failing to withdraw its tail in 3–5 s was rejected from study. Three to five basal reaction times for each rat at an interval of 5 min were taken to confirm normal behavior of the animal. Control reaction was recorded twice with 15 min intervals between readings. Starting before and 15 min after oral administration (p.o.) of vehicle, the test agents *C. pluricaulis* extracts at 250, 500 and 750 mg/kg, p.o. and intraperitoneal (i.p.) injection of morphine sulfate at 5 mg/kg, the nociceptive response was measured every 15 min interval over a 90-minute period [24] **[Table 4]**

Statistical Analysis: Values for analgesic activity were expressed as "mean increase in latency after drug administration ± SEM" in terms of seconds whereas values for anti-inflammatory activity were expressed as "mean increase in paw volume ± SEM." The significance of difference between means was analyzed by one-way ANOVA followed by Turkey's multiple comparison tests. The difference was considered significant when $P < 0.05$. All statistical procedures were performed according to the method of Alcaraz [25].

Result of Anti-Inflammatory Activity: In studies carried out on the ethanolic and aqueous leaves extract of *C. pluricaulis*, it was found that ethanolic extract was better and potent showing significant decrease in paw volume. The results of anti-inflammatory activity are given in the Tables 1 and 2. Table 1 show that the paw volume of control group was increased rapidly after carrageenan injection, otherwise in case of rats pre-treated with ethanolic extract shows inhibition of paw edema significantly as compared to standard drug.

TABLE 1: ANTI-INFLAMMATORY EFFECTS OF C. PLURICAULIS EXTRACT IN CARRAGEENAN-INDUCED PAW EDEMA MODELS OF INFLAMMATION

Group	Dose (mg/kg)	1h	2h	3h
Control		0.61 ± 0.003	0.68 ± 0.023	0.79 ± 0.015
Indomethacin	10	0.52 ± 0.012	0.50 ± 0.013*	0.49 ± 0.083*
C. pluricaulis extract 400 mg/kg	400	0.50 ± 0.005	0.49 ± 0.010*	0.50 ± 0.008*
C. pluricaulis extract 600 mg/kg	600	0.50 ± 0.006*	0.49 ± 0.010*	0.50 ± 0.009*
C. pluricaulis extract 800 mg/kg	800	0.51 ± 0.010*	0.50 ± 0.011*	0.49 ± 0.014*

*Significant at $P < 0.001$, P-value was calculated by comparing with control by ANOVA followed by the Student t-test, Values are expressed as mean ± SEM

TABLE 2: ANTI-INFLAMMATORY EFFECT OF *C. PLURICAULIS* EXTRACT IN COTTON PELLET-INDUCED GRANULOMA MODELS OF INFLAMMATION

Group	Dose (mg/kg)	Increased weight of dry cotton pellet (mg)
Control		38.61 ± 0.10
Indomethacin	10	18.71 ± 0.44*
C. pluricaulis extract 400 mg/kg	400	23.31 ± 0.21*
C. pluricaulis extract 600 mg/kg	600	20.30 ± 0.02*
C. pluricaulis extract 800 mg/kg	800	25.31 ± 0.09*

*Significant at $P < 0.001$, P-value was calculated by comparing with control by ANOVA followed by the Student t-test, Values are expressed as mean ± SEM

Result of Analgesic Activity: In studies carried out on the analgesic activity of ethanolic and aqueous leaves extract of *C. pluricaulis*, it was found from the percentage inhibition index that ethanolic extract was better analgesic than aqueous extract when compared with standard drug morphine sulphate.

In this study, the ethanolic extract of *C. pluricaulis* at dose 750 mg/kg shows statistically significant analgesic activity compared to control, standard, 250 mg/kg and 500 mg/kg The results of treatment with the extracts of *C. pluricaulis* are comparable with the standard and it showed significant analgesic activity as shown in Tables 3 and 4.

TABLE 3: HOT PLATE METHOD: EFFECTS OF ETHANOLIC EXTRACTS OF *C. PLURICAULIS* AND MORPHINE SULPHATE ON PAIN INDUCED BY HOT PLATE METHOD

Group	Dose (mg/kg)	0 min	15 min	30 min	60 min	90 min
Control		4.05±0.01	3.89±0.16	4.02±0.32	4.11±0.02	3.78±0.15
Standard	5 (i.p)	4.01±0.12*	4.92±0.18	7.15±0.11	8.87±0.12	10.41±0.01
250 mg/kg	250	3.68±0.005#	4.25±0.01#	4.98±0.12#	5.57±0.17	6.13±0.20
500 mg/kg	500	4.01±0.02#	4.68±0.05#	6.33±0.10*	7.18±0.005	8.02±0.12
750 mg/kg	750	4.02±0.01@	4.45±0.10@	6.45±0.12@	7.20±0.10@	9.2±0.13

Symbols represent statistical significance: *$P < 0.05$, #$P < 0.01$, @$P < 0.001$.

TABLE 4: TAIL FLICK ASSAY: TIME OF MEASUREMENT OF LATENCY OF TAIL FLICK (SEC).

Group	Dose (mg/kg)	0 min	15 min	30 min	60 min	90 min
Control		2.74±0.16	2.81±0.12	2.97±0.18	3.01±0.05	2.82±0.12
Standard	5 (i.p)	2.90±0.12	4.12±0.18	6.59±0.11	9.35±0.10	9.18±0.18
250 mg/kg	250	2.80±0.12	2.99±0.20	3.67±0.17	5.47±0.20	5.11±0.06
500 mg/kg	500	2.85±0.10	3.55±0.12	5.29 ± 0.12	7.75 ± 0.23	6.92 ± 0.16
750 mg/kg	750	2.86±0.11	3.56±0.14	5.35 ± 0.16	7.80 ± 0.35	8.0 ± 0.7

DISCUSSION: In this study, pharmacological evaluation of anti-inflammatory and analgesic activity of ethanolic extract of *C. pluricaulis* was carried out using different experimental models. Variety of indigenous drugs is used for relief in inflammation. The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local edema induced in the rat paw by an injection of an irritant agent. The development of edema in the paw of the rats after the injection of carrageenan has been described as a biphasic event.

The initial phase, observed around 1 h, is attributed to the release of histamine and serotonin; the second accelerating phase of swelling is due to the release of prostaglandin-like substances. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and nonsteroidal anti-inflammatory agents. Significant anti-inflammatory activity was observed for ethanolic extract of *C. pluricaulis* in carrageenan- and serotonin-induced edema model. Since prostaglandins are involved in swelling and are inhibited by flavonoids, it could be suggested that reduced availability of prostaglandins by flavonoids of ethanolic extract of *C. pluricaulis* might be responsible for its anti-inflammatory effect.

Due to nociceptive stimulation, various mediators are produced like prostaglandin, cytokinin, bradykinin, and so forth, producing acute pain and inflammation. The ethanolic extract of *C. pluricaulis* prevents the nociceptive component which may be due to the inhibition of the production of prostaglandin and related compounds. Experimental evidence suggests that the extract reduced the rate of edema in carrageenan-induced rat paw edema model. Similarly, it significantly delayed the reaction time of animals to the heat stimulus.

So it is concluded from the previous study that the extract has potent analgesic and anti-inflammatory effect.

In conclusion, this study demonstrates that ethanolic extract of *C. pluricaulis* has marked antipyretic and moderate anti-inflammatory activities.

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