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Investigation of Anti-inflammatory Activity Polyherbal Formulation (*Calotropis gigantea* and *Glycyrrhiza glabra*)

C. Girish*, V. Anjali, A. Deevan Paul

S. V. U. College of Pharmaceutical Sciences, Sri Venkateshwara University, Tirupati – 517502, Andhra Pradesh, India

ABSTRACT

Traditional medicine is defined as therapeutic practices that have been in existence, for hundreds of years before the development and spread of modern sciences. This is due to the fact that traditional medicine is the most affordable and accessible health care system available. Ayurveda mentions number of plants for the management of several diseases. Several researchers had given their contributions for finding hidden therapeutic activities of Ayurvedic drugs, but still number of plants need a comprehensive study on them. The present study is focused on the investigation of the potent medicinal herbs such as *Calotropis gigantea* belongs to family Asclepiadaceae, *Glycyrrhiza glabra* belongs to family Leguminosae for their anti-inflammatory activity in the form of polyherbal formulation. In carrageenan induced inflammation the polyherbal formulation showed a maximum anti-inflammatory effect at 120 min (71.17%) and progressively increased by 180 min (78.1%). And similarly in formalin induced inflammation the anti-inflammatory effect induced by polyherbal formulation progressively increased and reached a maximum of 72.24% at 180 min. The present investigation concluded that the plants *Calotropis gigantea* and *Glycyrrhiza glabra* in the form of polyherbal formulation having anti-inflammatory activity.

Keywords: *Calotropis gigantea, Glycyrrhiza glabra,* polyherbal formulation, anti-inflammatory activity.

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***Corresponding author**: Dr. C. Girish
Address: S. V. U. College of Pharmaceutical Sciences, Sri Venkateshwara University, Tirupati – 517502, Andhra Pradesh, India
Tel.: +91-9290534422
E-mail ✉: cgirish.svu@gmail.com
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INTRODUCTION

Ayurvedic system of healthcare has gained importance and is becoming popular. It is a comprehensive system of healthcare that originated in India has recommended a number of drugs for the treatment of various diseases. [1] Because of the effectiveness and less adverse reactions compared to the synthetic chemicals, Ayurvedic system has attained popularity globally. The classical text of Ayurveda mentions number of plants for the management of several diseases. Undoubtedly several researchers had given their contributions for finding hidden therapeutic potentials of number of

Ayurvedic drugs, but still number of plants need a comprehensive study on them. The objective of the present study is evaluation of the anti-inflammatory activity of polyherbal formulation, which is a combination of the potent medicinal herbs such as *Calotropis gigantea* and *Glycyrrhiza glabra.*

Inflammation is defined as the local response of the living mammalian tissues to injury due to any agent. It is a body defence reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necroses cells and tissues. Inflammation (latin, inflamatio=to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. [2] It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. [3]

Calotropis gigantea belongs to family Asclepiadaceae, also known as sweat akand, is used in traditional medicine for treatment of various ailments. The leaves of *Calotropis gigantea* was shown in Figure 1. It is found throughout plains and lower hills of India usually near water found growing up to an altitude of 900 m throughout India including Andamans. Various chemical constituents have been reported for various parts of this plant. Flowers contain waxy matters which has esters of resinols, α, β-calotropeol, β-amyrin, stigmasterol, giganteol, calotropin, triterpenoid flavonoid, flavonoid glycoside, wax, acids and alcohols seeds are rich in amino acids, major being phenylalanine, lysine and histidine. The leaf contains ascorbic acid, ortho-pyrocatechic acid and also contains β-amyrin, taxasterol, tarasterol and beta-sitosterol. Shoot and leaf extracts possess anti bacterial activity. Tender fresh leaves have been reported to cure fits and convulsions in children. Extracts of leaf with oil and rock salt warmed are poured into ear for ear ache. Fresh warmed leaves or poultice is bandaged on painful rheumatic plant is purgative, anthelmintic, antitumor and has been used in disease of spleen and liver. Leaves have been used in enlargement of liver and flowers are also good for liver Paracetamol induced hepatic damage in rats has been reported.

Glycyrrhiza glabra Linn is one of the most extensively used medicinal herbs from the ancient medical history of Ayurveda. It is also used as a flavouring herb. The word *Glycyrrhiza* is derived from the greek term *glykos* (meaning sweet) and *rhiza* (meaning root). *Glycyrrhiza glabra* linn, commonly known as 'liquorice' and 'sweet wood' belongs to Leguminosae family. The roots of *Glycyrrhiza glabra* was shown in Figure 2. Vernacular names for liquorice are jeshthamadh (marathi), jothi-madh (hindi), yashtimadhu, madhuka (sanskrit), jashtimadhu, jaishbomodhu (bengali), atimadhuram, yashtimadhukam (telugu), jethimadhu (gujarati) and atimadhuram (tamil). Liquorice has been recommended as a prophylactic agent for gastric and duodenal ulcers. It is employed in dyspepsia as an anti-inflammatory agent [4] during allergenic reactions. [5] It is used as a contraceptive, laxative, anti-asthmatic, emmenagogue, galactagogue, and antiviral agent in folk therapy. [6] *Glycyrrhiza* roots are useful for treating cough because of its demulcent and expectorant property. [7] It is also effective against anaemia, gout, sore throat, tonsillitis, flatulence, sexual debility, hyperdipsia, fever, skin diseases, swelling. Liquorice is effectively used in acidity, leucorrhoea, bleeding, jaundice, hiccough, hoarseness, bronchitis, vitiated conditions of vata dosha, gastralgia, diarrhea, fever with delirium and anuria. [8-9]

Therefore the present study is focused on the investigation of the potent medicinal herbs such as *Calotropis gigantea* and *Glycyrrhiza glabra* for their anti-inflammatory activity.

Fig. 1: ***Calotropis gigantea***

Fig. 2: ***Glycyrrhiza glabra*** **root**

MATERIALS AND METHODS

Collection of plant material

Aerial parts of the plants *Calotropis gigantea* and *Glycyrrhiza glabra* were collected from in and around Tirumala hills and authenticated by Dr. Madhav Chetty, Taxonomist, S. V. University, Tirupathi, India.

Animals

Healthy male wistar rats each weighing 150-200 g were used for study. The rats were housed in polypropylene cages and maintained under standard conditions (12 hours light and dark cycles at 25 ± 3°C and 35-60% humidity).

Extraction

Plant materials *(Calotropis gigantea)* were collected and shade dried for a period of seven to ten days. After conforming for through drying it was passed it for milling to obtain fine powder. Then powder was extracted with the help of methanol as extracting solvent in the soxhlet apparatus for 18 hours until the

whole meal was exhausted and then air dried for solvent evaporation. Sticky waxy extract with sweet to chocolate odour was collected and found to be weighed more than 3.5 g.

The aqueous extract of *Glycyrrhiza glabra* was prepared by taking one kilogram of powdered *Glycyrrhiza glabra* and it was extracted 2 times with boiling water then filtered, and the filtrate was evaporated until it becomes syrupy.

Preparation of Poly Herbal Formulation (PHF)

The methanolic extract of *Calotropis gigantea* is suspended in 2% gum acacia solution and the aqueous extract of *Glycyrrhiza glabra* are taken in equal proportions i.e in 1:1 ratio to get the polyherbal formulation. A stock solution was prepared for further usage. The PHF was prepared on basis of Saragadhara samhitha which involves the mixing of both the potent extracts in equal ratio. [10]

Preparation of 1% carrageenan solution

1% w/v solution was prepared by dissolving 100 mg of carrageenan in 10 ml of 0.9% w/v NaCl. A stock solution was prepared for further usage.

Preparation of Diclofenac suspension

100 mg of diclofenac sodium was weighed accurately and suspended in 10 ml of distilled water using 2% w/v of acacia as suspending agent. A stock solution was prepared containing 10 mg/ml of the drug.

Dose Administration

The normal adult rats of either sex were selected and divided into four groups each containing six animals. *Calotropis* and Liquorice extracts were dissolved in 2% gum acacia suspension. The treatment protocol was planned to study the effect of anti-inflammatory effect by administrating the drugs in right hind paw of the rat and left hind paw is made as reference, no drug is administered to it.

Carrageenan dose selected was 0.1 g/kg body weight of the animal. Diclofenac sodium dose selected was 5 mg/kg. Poly-herbal formulation dose selected was 200 mg/kg for 3rd group and 400 mg/kg for 4th group. The treatment protocol is summarized and given below.

Carrageenan-induced paw edema in rats

This model is based on the principle of release of various inflammatory mediators by carrageenan. [11] The test was carried similar to that described by Winter *et al*., [12]. Edema formation due to carrageenan in the rat paw is biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome. The subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasation, increased tissue water and plasma protein exudation along with neutrophil extravasation, all due to the metabolism of arachidonic acid.

Animals are divided into four groups (n=6) starved over night with water *ad libitum* prior to the day of the experiment. The control group receives vehicle orally, while other group receives test drug and standard drug respectively. Left paw is marked with ink at the level of lateral malleolus; basal paw volume is measured plethysmographically by volume displacement method using Plethysmometer (ugo basile 7140) by immersing the paw till the level of lateral malleolus. The increase in paw volume was measured by volume displacement method by using Plethysmometer [13]. The animals are given drug treatment one hour after dosing; the rats are challenged by a subcutaneous injection of 0.1 ml of 1% solution of carrageenan into the sub plantar side of the left hind paw. The paw volume is measured again at 1, 2 and 3 hours after challenge. The increase in paw volume is calculated as percentage compared with the basal volume. The difference of average values between treated animals and control groups is calculated for each interval and evaluated statistically.

The percentage inhibition is calculated using the formula as follows

$$\% \text{ of oedema inhibition} = [1-(Vt/Vc)] \times 100$$

Vt = oedema volume in the drug treated group

Vc = oedema volume in the control group

Grouping of animals for carrageenan induced rat paw oedema model

GROUP 1: Control: 2% gum acacia vehicle (5 ml/kg)

GROUP 2: Standard: Diclofenac sodium 5 mg/kg and after 30 min 0.1 ml carrageenan suspension.

GROUP 3: Test 1- 1:1 ratio of *Calotropis* and Liquorice dose of 200 mg/kg.

GROUP 4: Test 2- 1:1 ratio of *Calotropis* and Liquorice dose of 400 mg/kg.

The severity of inflammation was observed after 0 min, 1st, 2nd, 3rd hours.

Formalin-induced paw edema

This model based upon the ability of test drug to inhibit the edema produced in the hind paw of the mice after injection of formalin. [14] The test was carried similar to that described by Gorzalczany *et al*., [15]. The nociceptive effect of formalin is biphasic an early neurogenic component followed by a later tissue mediated response.

The animals are divided into four groups (n=6). In animals of all groups inflammation is produced by sub planter injection of 20 ml of freshly prepared 2% formalin in the right hind paw of mice. The paw thickness is measured by plethysmometrically 1 hour before and after formalin injection. The drug treatment is continued for 6 consecutive days. The increase in paw thickness and percentage inhibition are calculated and compared with control group.

Grouping of animals for formalin induced rat paw oedema model

GROUP 1: Control: 2% gum acacia vehicle (5 ml/kg)

GROUP 2: Standard: Diclofenac sodium 5 mg/kg was given after 30 min administration of 0.1 ml of 2% formalin solution.

GROUP 3: Test 1- 1:1 ratio of *Calotropis* and Liquorice dose of 200 mg/kg.

GROUP 4: Test 2- 1:1 ratio of *Calotropis* and Liquorice dose of 400 mg/kg.

Table 1: Effect of polyherbal formulation on carrageenan induced rat paw oedema

S. No	Group	Dose	Oedema volume (% of Inhibition)			
			0 min	60 min	120 min	180 min
1	Group-1 (Control)	5 ml/kg	0.35 ± 0.023	0.77 ± 0.021	0.92 ± 0.017	1.07 ± 0.021
2	Group-2 (Standard)	5 ml/kg	0.30 ± 0.026 (14.29%)	0.60 ± 0.026** (38.08%)	0.48 ± 0.017*** (69.83%)	0.50 ± 0.017*** (49.75%)
3	Group-3 (Test-1)	200 mg/kg	0.27 ± 0.021* (22.85%)	0.57 ± 0.033* (37.97%)	0.45 ± 0.022*** (58.09%)	0.36 ± 0.019*** (69.36%)
4	Group-4 (Test-2)	400 mg/kg	0.25 ± 0.023* (38.57%)	0.53 ± 0.021*** (59.17%)	0.44 ± 0.020*** (71.17%)	0.32 ± 0.016*** (78.1%)

Values are in mean ± SE; n=6 in each group. *p<0.05; ** p<0.01; *** p<0.001.

Table 2: Effect of polyherbal formulation on formalin induced rat paw oedema

S. No	Group	Dose	Oedema volume (% of Inhibition)			
			0 min	60 min	120min	180 min
1	Group-1 Control 2% gum acacia	5 ml/kg	0.35 ± 0.023	0.77 ± 0.021	0.92 ± 0.017	1.07 ± 0.021
2	Group-2 Standard Diclofenac	5 ml/kg	0.28 ± 0.017 (20%)	0.48 ± 0.006** (37.66%)	0.38 ± 0.008*** (58.7%)	0.28 ± 0.01*** (73.83%)
3	Group-3 Test-1 PHF (single dose)	200 mg/kg	0.27 ± 0.021* (22.85%)	0.57 ± 0.033* (37.97%)	0.45 ± 0.022*** (51.09%)	0.36 ± 0.019*** (66.36%)
4	Group-4 Test-2 PHF (double dose)	400 mg/kg	0.25 ± 0.023* (28.57%)	0.50 ± 0.08*** (35.07%)	0.40 ± 0.008*** (56.52%)	0.297 ± 0.01*** (72.24%)

Values are in mean ± SE; n=6 in each group. *p<0.05; ** p<0.01; *** p<0.001.

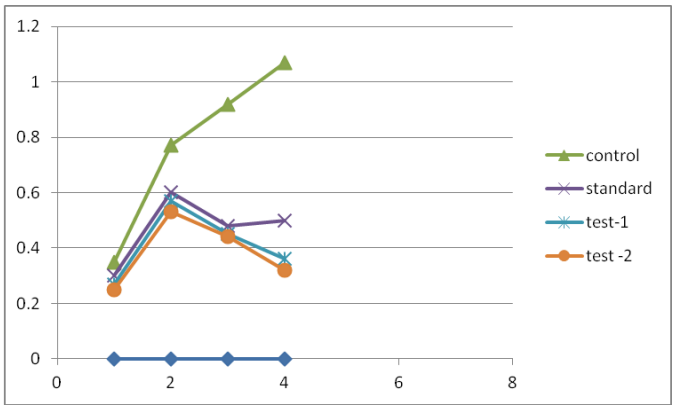

Fig. 3: Graph representing the effect of poly-herbal formulation on carrageenan induced rat paw oedema.

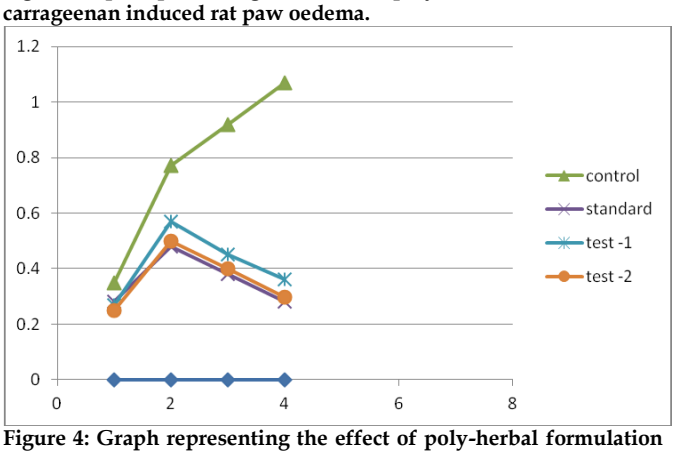

Figure 4: Graph representing the effect of poly-herbal formulation on formalin induced rat paw oedema

The severity of inflammation was observed after 0 min, 1st, 2nd, 3rd hours.

RESULTS

In the control group, the paw volume was maximum at 180 min. In the treatment group the paw volume decreased gradually and therefore readings up to third hour were recorded. Double dose of poly herbal formulation showed a maximum anti-inflammatory effect at 120 min (71, 17%) and progressively increased by 180 min (78.1%) in carrageenan induced rat paw oedema model. The results were shown in Table 1 and are graphically represented in Figure 3.

The anti-inflammatory effect induced by polyherbal formulation progressively increased and reached a maximum of 72.24% at 180 min in formalin induced rat paw oedema model. The results were shown in Table 2 and are graphically represented in Figure 4. Significant inhibition of inflammation induced by formalin was shown by polyherbal formulation in multiple dose studies.

DISCUSSION

The carrageenan test is useful in evaluation of the orally active anti-inflammatory agents especially in the acute phase of inflammation. [16] The first phase (0 –3 h after injection) results from the release of histamine, serotonin and kinin mediators on vascular permeability and the second phase is due to the high production of prostaglandins, oxygen-derived free radicals, and inducible cyclooxigenase. [17] The inhibition of Carrageenan induced inflammation in rats is an established model to screen compounds for potential anti-inflammatory activity. According to Vinegar *et al.*, 1987; the development of carrageenan induced oedema is biphasic, the first phase occurs within one hour of carrageenan administration and is attributed to the release of cytoplasmic enzymes, histamine and seratonin from the mast cells. The second phase (>1h) is mediated by an increased release of prostaglandins in the inflammatory area and continuity between the two phases is provided by Kinins.

The formalin test is model which represents persistent pain. This test is used to determine the effect of compounds on peripheral or central nociceptive pathways due to its biphasic nociceptive characteristics

which are known as the early and late phases and which result from administration of formalin. [18] The early phase is a neurogenic pain, which is an acute response observed immediately after the administration of formalin injection and may persists for 5 min (0–5 min). The late phase, is as an inflammatory pain, which is a tonic response and is due to inflammatory processes results due to the release of inflammatory mediators.

The results suggest that Diclofenac sodium possesses significant anti-inflammatory activity against phlogistic agents, under the influence of calotropis and liquorice extracts which may be mediated possibly due to inhibiting the generation of ROS/RNS by activated phagocytes and depress activation of NF-Kβ and upregulation of COX-2, INOS and adhesion molecules involved in phagocytes recruitment. Diclofenac sodium has significant anti-inflammatory activity in carrageenan and formalin induced inflammatory models. In the control group, the paw volume was maximum at 180 min in the treated group the paw volume decreased gradually and therefore readings upto third hour were recorded.

In Carrageenan induced inflammation dose of polyherbal formulation showed a maximum anti-inflammatory effect at 120 min (71.17%) and progressively increased by 180 min (78.1%).

And similarly in Formalin induced inflammation the anti-inflammatory effect induced by Test-2, Polyherbal formulation i.e PHF progressively increased and reached a maximum of 72.24% at 180 min.

Herbal based remedies serve as the important means of therapeutic medical treatment. The people are turning to usage of medicinal plants in health care. India has one of the oldest cultural traditional uses of its herbal plants since from vedic period. Ayurveda, Unani, Siddha and other traditional systems of medicine are the ancient systems of medicine and utilize numerous numbers of medicinal plants. In this present study the PHF which consists of *Calotropis gigantea* flowers and *Glycyrrhiza glabra* root shows good anti-inflammatory activity against the carrageenan and formalin induced rat paw edema. Further studies are essential for the isolation of active ingredient responsible for the anti-inflammatory activity.

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