



PRELIMINARY STUDIES, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF LEAF EXTRACT OF *Gardenia taitensis* IN EXPERIMENTAL MODELS

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ABSTRACT

Gardenia taitensis is a medium sized evergreen tree belongs to *Rubiaceae* family. The petroleum ether extract of whole plant of *Gardenia taitensis* (PGT) was investigated for the evaluation of anti-inflammatory and analgesic activity. Acute toxicity studies were performed as per OECD-423 guidelines. Toxicity signs and symptoms were not observed. Anti-inflammatory activity was established by carrageenan induced paw edema and cotton pellet induced granuloma at the dose of 200 and 400 mg/kg. Analgesic activity was carried out by tail immersion method in mice. The activity may be due to the presence of steroids in the extract. The extract exhibited significant analgesic and anti-inflammatory activity, which supports the traditional utilization of the plant. This study established analgesic and anti-inflammatory activity of the whole plant of *Gardenia taitensis*.

Key words: *Gardenia taitensis*, anti-inflammatory, analgesic, edema, granuloma.

INTRODUCTION

Gardenia taitensis is a herbaceous member of the family Rubiaceae. It is a shrub or small tree to 6 m high with conspicuous stipules. Leaves are opposite, petiolate, broadly elliptical, up to 15 cm long. Flowers are showy, white, fragrant, borne singly on stems arising from upper leaf axils. Fruit a yellowish-green sub-globose-to-ellipsoid capsule up to 5 cm long containing numerous whitish seeds surrounded by an orangish pulp. Flowers available throughout the year, fruit often rare. It is common along coastal limestone rock and occasionally in coastal forest near the shore. It is also widely cultivated in villages. It is native to the South-West Pacific Islands of Fiji, Vanuatu, Tonga, and nearby islands, and widely cultivated and naturalized on many other islands in the South Pacific [1-2]. Inflammation is local response of living mammalian tissues to injury due to any agent. Inflammation is a protective and defensive mechanism of body. Inflammation types are acute and chronic. There are various components to an inflammatory reaction such as edema formation, leukocyte infiltration and granuloma

formation that can contribute to the associated symptoms and tissue injury. Signs of inflammation as: Rubor – Redness; Tumor –Swelling or edema; Color –Heat; Dolor – Pain; 5th sign *functio laesa* –loss of function was later added by Virchow [3]. The main symptoms of the body are increased body temperature and pain. Analgesia or pain is an ill-defined, unpleasant sensation, usually evoked by an external or internal noxious stimulus [4]. Pain acts as a warning signal against disturbances either in the body or in the external environment of an individual and protective in nature. Pain receptor organs are distributed throughout the body. Clinically, pain can be considered as Superficial or cutaneous pain, deep non- visceral pain, visceral pain, Referred pain, Deafferentiation or neuropathic pain, Psychogenic or functional pain [5]. Analgesic is a drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. Analgesics are divided into two groups. Opioid analgesics- these are the products obtained

from opium poppy; relief from pain and depression of the CNS; some of them induce sleep. Non- Opioid analgesics- they do not interact with Opioid receptors and relieve pain without depression of the CNS. However there are no reports on the anti inflammatory and analgesic activity of the whole plant. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIALS AND METHODS

Plant Materials:

The *Gardenia taitensis* plants were collected in the month of September, 2010 from Thirumala hills in Chittoor district of Andhra Pradesh, India. The leaves were identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi [6].

Preparation of Extract:

The leaves of the plant were collected and dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no.40. Then the powder was extracted with petroleum ether in a Soxhlet extraction apparatus.

Phytochemical Screening:

The *Gardenia taitensis* was tested for the presence of alkaloids, glycosides, steroids, triterpenoids, flavonoids, tannins and reducing sugars by qualitative and quantitative methods [7].

Animals:

Male Wister rats weighing 160-200gm were used for the study of anti- inflammatory activity. Wister rats of either sex (19-32mg) were used for the study of analgesic activity. They were maintained under standard environmental conditions and were fed with standard pellet diet with water ad libitum.

Preparation of the drug for the experimental study:

Petroleum ether extract was administered in the form of suspension in 2% tween 80 solution and the standard drug in water was used for the study.

Acute Toxicity Studies:

Acute oral toxicity studies were performed as per OECD-423 guidelines. Male Wister mice were used for the study. The animals were divided into six groups containing six animals in each group. The extract was administered orally at the doses from 200- 2000mg/kg. There were no signs of toxicity and mortality was observed up to 2000mg/kg.

ANTI- INFLAMMATORY ACTIVITY

Carrageenan induced rat paw edema:

The animals were divided into 4 groups each group contained 6 animals. Animals were weighed and marked for

identification. All the groups were treated with extract and standard drugs used for the study. After 30min. of the treatment, 0.1ml of 1% carrageenan in saline was injected into the sub plantar region of the left hind paw of each rat to induce edema [8]. Group I: Control, Group II: PGT - 200mg/kg, Group III: PGT -400mg/kg, Group IV: Standard- Indomethacin-10mg/kg

The paw volume was measured initially and at intervals of 30, 60, 120, 180min. after carrageenan injection by volume displacement method using Plethysmometer by immersing the paw in mercury cell. The percentage inhibition of paw volume in drug treated group was compared with control group. Indomethacin (10mg/kg) was used as standard drug. The percentage inhibition of paw edema was calculated by using the following formula;

$$\text{Percentage of edema inhibition} = [(V_c - V_t) / V_c] \times 100$$

Vc- Volume of edema in control group

Vt- volume of edema in treated group

Cotton pellet granuloma

The rats are divided into 4 groups, 6 animals in each group. The cotton pellet granuloma model investigated the proliferation phase of inflammation [9]. The animals of each group were treated with standard and test drugs orally. After 30 minutes the animals were anaesthetized with diethyl ether. The 20mg of sterile cotton pellets were inserted in each axilla of rats by making small subcutaneous incision. The incisions are sutured with sterile catgut [10]. The extract and standard drugs were administered for seven days. After 8th day the animals were sacrificed with excess anaesthesia and cotton pellets were removed surgically and separated from extraneous tissues. The pellets were weighted and dried at 70°C for 6 hour and weighted again. The dry weight of the pellets was taken and granuloma formation was measured. Group I: Control, Group II: PGT - 200mg/kg, Group III: PGT - 400mg/kg, Group IV: Standard- Indomethacin- 10mg/kg. The percentage inhibition of granuloma was calculated by using the following formula;

$$\text{Percentage inhibition} = [(W_c - W_d) / W_c] \times 100$$

Wc = Pellet weight in control group

Wd = Pellet weight in drug treated group

ANALGESIC ACTIVITY

Tail immersion method:

The mice were divided into 4 groups, 6 animals in each group. Mice were weighed and 3-4cm. area of the tail was marked and immersed in the thermo- statically maintained water bath at 51⁰c. The withdrawal time of the tail from the hot water in seconds was noted as the reaction time. The maximum cut off time for immersion was 150 seconds to avoid the injury of the tail tissue. Then control group was treated with vehicle, standard group was treated with Indomethacin 10mg/kg and test groups were treated

with 200 and 400 mg/kg orally. The initial readings were taken immediately before the administration of the test and standard drugs and at then 60, 90, 120, 150 min after the administration. Tail withdrawal time difference after drug administration was used to indicate the analgesia produced by standard and test drugs [11].

RESULT AND DISCUSSION

General qualitative and quantitative tests showed the presence of steroids. The plant extract did not exhibit any mortality up to the dose level 2000mg/kg. The petroleum ether extract of leaf part of *Gardenia taitensis* was evaluated for anti-inflammatory activity in acute and chronic experimental animal models and analgesic activity was evaluated for centrally acting analgesic effect in animal model. The *Gardenia taitensis* exhibited significant anti-inflammatory and analgesic activity at the doses of 200,

400mg/kg. The *Gardenia taitensis* showed 44.23% inhibition at the dose of 200mg/kg and 50% inhibition at the dose of 400mg/kg 3hr after the drug treatment in carrageenan induced paw edema, where as the standard drug showed 53.84% of inhibition.

In chronic model i.e., cotton pellet granuloma the *Gardenia taitensis* showed decreased formation of granuloma tissue of 43.04% at the dose of 200mg/kg, 52.44% at 400mg/kg, where as standard showed 53.58% at the dose of 10mg/kg. The PGT exhibited analgesic activity at the dose 200 and 400mg/kg. The duration as well as the intensity of analgesia produced by *Gardenia taitensis* was dose dependent. It showed significant analgesic activity at 200mg/kg even in the first hour of the test after the 120min the activity began to decrease. The analgesic activity was almost comparable to that produced by Indomethacin.

Table 1. Effect of the *Gardenia taitensis* on carrageenan induced paw edema:

Treatment	Dose (mg/kg)	Paw edema volume (ml)					Percentage of inhibition
		0min	30min	60min	120min	180min	
Control	0	0.27±0.01	0.30±0.03	0.37±0.01	0.4±0.01	0.52±0.01	—
PGT	200	0.27±0.02	0.32±0.01	0.37±0.01	0.34±0.04***	0.29±0.01***	44.23
PGT	400	0.29±0.01	0.32±0.02	0.36±0.01	0.32±0.01***	0.26±0.01***	50
Indomethacin	10	0.28±0.02	0.33±0.02	0.35±0.01	0.32±0.01***	0.24±0.01***	53.84

Table 2. Effect of *Gardenia taitensis* on cotton pellet induced granuloma

Treatment	Dose Mg/kg	Weight of cotton pellet		Percentage of inhibition
		Before	After	
Control	0	20±0.00	44.16±1.01	—
PGT	200	20±0.00	25.15±0.76***	43.04
PGT	400	20±0.00	21±0.58***	52.44
Indomethacin	10	20±0.00	20.5±0.56***	53.58

Table 3. Effect of *Gardenia taitensis* in mouse tail immersion method:

Treatment	DoseMg/kg	Tail withdrawing in sec				
		Before	After			
			60	90	120	150
Control	0	1.5±0.42	1.83±0.30	1.17±0.31	1±0.38	0.7±0.21
PGT	200	1.5±0.42	5±0.25***	5.5±0.22***	4.53±0.26***	4.36±0.21***
PGT	400	1.5±0.43	5.5±0.22***	6.5±0.25***	5.56±0.31***	4.34±0.21***
Indomethacin	10	1.83±0.48	6.33±0.42***	7.5±0.34***	5.26±0.27***	4.17±0.30***

CONCLUSION

In conclusion, the results of the present study support to the traditional use of *Gardenia taitensis* in inflammation and analgesia. This study established anti-inflammatory activity of the leaf extract of *Gardenia taitensis*. Acute oral toxicity studies were performed and mortality was not observed up to 2000mg/kg. *Gardenia*

taitensis exhibited significant anti-inflammatory and analgesic activity at the doses of 200 and 400mg/kg. The results produced by *Gardenia taitensis* were comparable with that of standard Indomethacin. The activities may be due to the presence of steroids in the extract.

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