

ANTIINFLAMMATORY AND ANTIBACTERIAL ACTIVITIES OF DIFFERENT EXTRACTS OF *GYNANDROPSIS GYNANDRA*

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ABSTRACT

Objective: The present study was carried out to evaluate the Antiinflammatory and Antibacterial activities of different extracts of *Gynandropsis gynandra*.

Methods: The different extracts of *G. gynandra* at doses 100, 200 and 400mg/kg b.w. were tested for their antiinflammatory activity against Carrageenan Induced Rat paw Oedema Model in rats and Antibacterial was tested against Gram +ve and Gram -ve bacterial strains using cup plate method.

Results: The extracts of *G. gynandra* showed a dose dependent antiinflammatory. Among all the extracts, methanolic extract produced maximum inhibition of increased paw oedema (38.66 ± 1.5) at a dose of 400mg/kg and different extracts showed good zone of inhibitions on tested bacterial strains.

Conclusion: The results of the present investigation indicate that all the extracts of *G. gynandra* possess antiinflammatory and anti bacterial activities and this effect was may be due to the presence of various chemical constituents.

Keywords: *Gynandropsis gynandra*, Antiinflammatory activity, Carrageenan and Antibacterial activity.

INTRODUCTION

The use of plant compounds for pharmaceutical purposes has gradually increased in world. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs [1]. The lacks of toxicity of many plants have been scientifically proved in the recent years. The global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use. Development of modern drugs from plants should be emphasized for the control of various diseases. [2-5]. In view of the traditional use of medicinal plants as a source of medicine in many diseases, folklore and claims made by the people in different countries for *Gynandropsis gynandra* [6-9]. Now, the present work has been undertaken to evaluate the antiinflammatory and antibacterial activities of different extracts of the *G. gynandra*.

MATERIALS AND METHODS

Plant material and Preparation of extract

Gynandropsis gynandra was collected at Marteru region, A.P. India and authenticated by Prof. M. Venkaiah, Department of Botany, Andhra University. Freshly collected plant material was dried under shade and was made into coarse powder. Coarse powder of *G. gynandra* was extracted separately with 70% v/v ethanol, Methanol, Ethyl acetate and Hexane using a soxhlet apparatus. The extracts thus obtained were dried under reduced pressure at a room temperature not exceeding 40°C to get the extracts.

Drug and Chemicals

Indomethacin, Carboxy Methylcellulose Sodium (CMC Na) and Carrageenan were purchased from Sigma chemicals, USA. All other chemicals used were of analytical grade.

Test Organisms

Eight bacterial species were used. The bacterial species were purchased from National collection of industrial micro organisms (NCIM), Pune. The Bacterial species were maintained in the nutrient broth medium on placing shaker in separate culture tubes for each species separately. Out of eight, four are Gram positive Organisms (*Bacillus pumillis*, *Bacillus megaterium*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*) four are Gram Negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhimurium*).

Animals

Adult Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200-250gm were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of $23 \pm 2^\circ\text{C}$ having $50 \pm 5\%$ relative humidity with 12-h light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute Toxicity Study

The acute toxicity study was conducted for Hydro-alcoholic, methanolic, ethyl acetate and hexane extracts of *G. gynandra* as per OECD (Organisation for Economic Co-operation and Development) guidelines 420 (OECD.2001).

Acute inflammation model: carrageenan induced rat paw Oedema [10, 11]

Fourteen groups of rats were treated orally with 1% Sodium CMC, 5 mg/kg Indomethacin, 200, 400 and 800 mg/kg of hydro-alcoholic, methanolic, ethyl acetate, hexane extracts of *G. gynandra* respectively. Sixty minutes later, an injection of 1% carrageenan in normal saline was made into the sub plantar region of the right hind paw of each rat in each group. Before induction of oedema, the dorsiventral thickness of both the paws of each was measured using Zeitlin's apparatus which consists of a graduated micrometer, combined with a constant loaded lever system to magnify the small changes in the paw thickness during the course of experiment. The measurements were taken at 1 hour intervals after induction of oedema for up to 6 hrs. Oedema was monitored as the percentage increase in paw thickness in the Carrageenan injected paw. To assess the oedema in control paw (right) saline was injected subcutaneously.

The percent inhibition of paw thickness is calculated using the formula:

$$\text{Percentage inhibition} = 100[1 - (Y_t / Y_c)]$$

Y_t = Average increase in paw thickness in groups tested with test compounds.

Y_c = Average increase in paw thickness in control.

Statistical Analysis

Data of Paw thickness was analyzed by using One-Way ANOVA followed by post hoc test Dunnett's test using Graph pad Prism-5 software. The results are expressed as Mean \pm S.E.M. $P < 0.05$ was considered as significant.

Antibacterial activity [12]

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds. A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculum. These cups were spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of dimethyl sulphoxide and water which were used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

RESULTS

Acute Toxicity Study

Acute toxicity studies in mice revealed that the extracts up to 2000mg/kg produced no sign of toxicity or mortality.

Anti-inflammatory Activity in Carrageenan Induced Rat paw Oedema Model

The Indomethacin at a dose of 5 mg/kg and different extracts of *G. gynandra* at doses 100, 200 & 400 mg/kg significantly (** $P < 0.001$) inhibited the maximal oedema response (Table 1) at 4th h.

The hydro-alcoholic extract of *G. gynandra* at doses 100, 200 & 400 mg/kg significantly inhibited the maximal oedema response and the percentage inhibition was found to be 62.92 \pm 1.5%, 23.68 \pm 1.4%, 31.09 \pm 2.1% and 34.63 \pm 2.4% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 68.4 \pm 1.2, 34.5 \pm 1.1, 39.94 \pm 1.5 and

44.82 \pm 2.1 respectively (Figure 1) over 6 h when compared to the control group treated with drug vehicle.

The methanolic extract of *G. gynandra* significantly inhibited the maximal oedema response and percentage inhibition was found to be 29.52 \pm 1.1%, 34.62 \pm 1.2% and 38.66 \pm 1.5% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 39.15 \pm 1.5, 43.02 \pm 2.1 and 46.86 \pm 2.1 respectively (Figure 1) over 6 h when compared to the control group treated with drug vehicle.

The ethyl acetate extract of *G. gynandra* significantly inhibited the maximal oedema response and percentage inhibition was found to be 17.81 \pm 0.5%, 22.95 \pm 1.2% and 28.9 \pm 1.4% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 30.5 \pm 1.2, 35.22 \pm 1.1 and 39.84 \pm 1.5 respectively (Figure 1) over 6 h when compared to the control group treated with drug vehicle.

The hexane extract of *G. gynandra* significantly inhibited the maximal oedema response and percentage inhibition was found to be 16.2 \pm 0.5%, 21.11 \pm 1.2% and 26.41 \pm 1.5% respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 28.23 \pm 1.2, 32.86 \pm 1.4 and 37.09 \pm 2.1 respectively (Figure 1) over 6 h when compared to the control group treated with drug vehicle.

Antibacterial activity

All the tested extracts at different concentrations have shown significant antibacterial activity against gram -ve organisms than gram +ve organisms along with standard drug (Table No.2).

Hexane showed highest zone of inhibition (16mm) against *Pseudomonas aeruginosa* at a concentration of 1200 μ g/cup. Ethyl Acetate and Hydro-alcoholic (Ethanol 70% v/v) extracts showed moderate zones of inhibition on tested bacterial strains. Ethyl Acetate extract showed maximum zone of inhibition (15mm) on *Escherichia coli* at a concentration of 1200 μ g/cup. Hydro-alcoholic extract showed highest zone of inhibition (13mm) *Pseudomonas aeruginosa* at a concentration of 1200 μ g/cup.

The methanolic extract showed zones of inhibition against tested bacterial strains and it showed maximum zones of inhibition (16mm) on *Pseudomonas aeruginosa* at a concentration of 1200 μ g/cup.

Table 1: Percentage inhibition of Carrageenan induced paw oedema in rats by prophylactic treatment with the different extracts of *G. gynandra* and Indomethacin. * $P < 0.001$.**

Name of the Extract	Dose (mg/kg b.w)	Percentage inhibition of the maximal paw oedema during 6 hours	Percentage inhibition of total AUC paw oedema during 6 hours
	Group I (Control)	0.0	0.0
	Group II (Indomethacin)	62.92 \pm 1.5***	68.4 \pm 1.2***
Hydroalcoholic	Group III (100 mg/kg)	23.68 \pm 1.4***	34.5 \pm 1.1***
	Group IV (200 mg/kg)	31.09 \pm 2.1***	39.94 \pm 1.5***
	Group V (400 mg/kg)	34.63 \pm 2.4***	44.82 \pm 2.1***
Methanolic	Group VI (100 mg/kg)	29.52 \pm 1.1***	39.15 \pm 1.5***
	Group VII (200 mg/kg)	34.62 \pm 1.2***	43.02 \pm 2.1***
	Group VIII (400 mg/kg)	38.66 \pm 1.5***	46.86 \pm 2.1***
Ethyl acetate	Group IX (100 mg/kg)	17.81 \pm 0.5***	30.5 \pm 1.2***
	Group X (200 mg/kg)	22.95 \pm 1.2***	35.22 \pm 1.1***
	Group XI (400 mg/kg)	28.9 \pm 1.4***	39.84 \pm 1.5***
Hexane	Group XII (100 mg/kg)	16.2 \pm 0.5***	28.23 \pm 1.2***
	Group XIII (200 mg/kg)	21.11 \pm 1.2***	32.86 \pm 1.4***
	Group XIV (400 mg/kg)	26.41 \pm 1.5***	37.09 \pm 2.1***

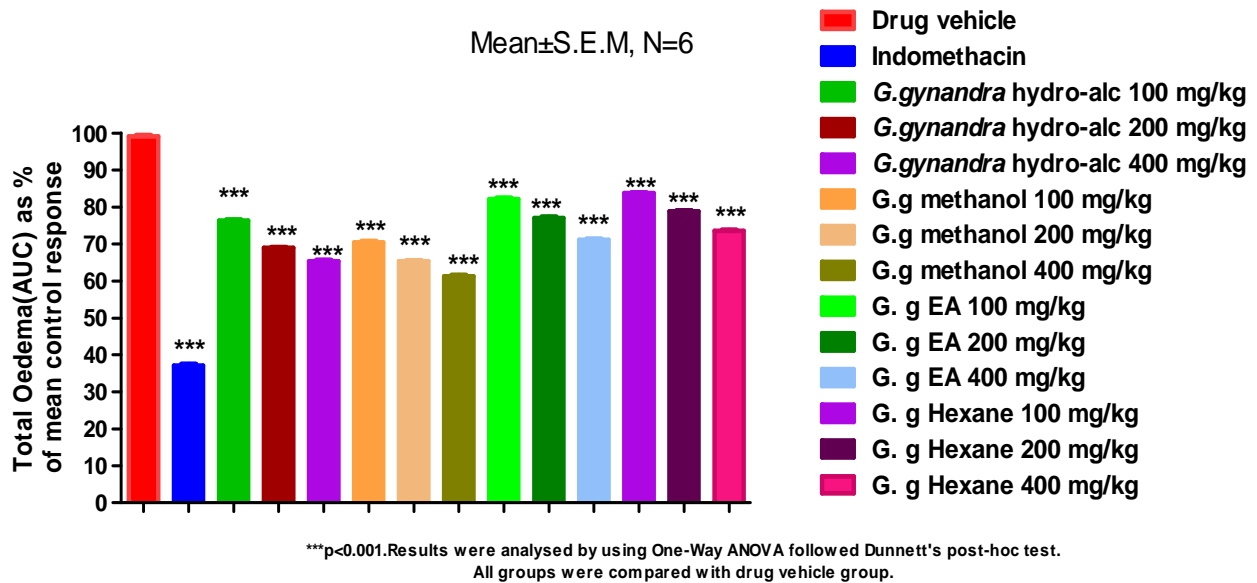


Fig. 2: Effect on total paw oedema (AUC) response of the different extracts of *G. gynandra*, at 100, 200 & 400 mg/kg along with Indomethacin (2.5×10^{-5} moles/kg body wt) in carrageenan induced rats.

Table 2: Antibacterial activity of different extracts of *Gynandropsis gynandra*.

<i>Gynandropsis gynandra</i> Extracts	Dose (µg/cup)	Zone of inhibition [#] (diameter in mm)							
		Gram +ve				Gram -ve			
		<i>B.p.</i>	<i>B.m.</i>	<i>S.e.</i>	<i>S.p.</i>	<i>E.c.</i>	<i>P.a.</i>	<i>K.p.</i>	<i>S.t.</i>
Hexane	150	-	-	-	-	6	7	-	-
	300	-	6	-	6	8	9	6	6
	600	5	8	6	8	10	12	8	8
	1200	6	11	8	10	12	16	10	9
Ethyl acetate	150	-	-	5	-	6	7	5	5
	300	5	5	7	6	9	9	7	6
	600	6	7	9	8	12	12	9	8
	1200	8	10	12	10	15	14	12	10
Hydro-alc.ext (Ethanol 70% v/v)	150	-	-	-	-	-	6	5	-
	300	-	-	5	6	5	8	7	6
	600	7	6	7	8	8	10	9	8
	1200	10	9	10	11	10	13	12	10
Methanolic	150	-	-	5	-	-	6	-	-
	300	7	-	7	-	-	9	6	7
	600	10	-	9	5	6	12	8	10
	1200	14	7	12	6	8	16	10	12
Chloramphenicol	10	14	15	15	14	15	16	16	17
DMSO	-	-	-	-	-	-	-	-	-

B.a=*Bacillus pumillis*, *B.m*=*Bacillus megaterium*, *S.e*= *Staphylococcus epidermidis*, *S.p*=*Streptococcus pneumonia*; *E.c*=*Escherichia coli*, *P.a*=*Pseudomonas aeruginosa*, *K.p*= *Klebsiella pneumonia*, *S.t*=*Salmonella typhimurium*

- =No activity; #Values are the average of triplicate; Includes the cup diameter (4mm)

DISCUSSION

The inflammation has long been recognized; the tissues become red, swollen, tender, or painful, there is local heat and the patient may be febrile. Inflammation can be categorized mainly as Chronic and Acute inflammatory disease. Acute and chronic inflammatory diseases are still one of the most important health problems in the world. Although several agent known to treat inflammatory disorders, their prolonged use often leads to gastric intolerance, bone marrow depression, water and salt retention. For this reason there is a need to find and develop new antiinflammatory drugs with low side effects. Now in the present study, the systemic anti-inflammatory effect was tested at 100, 200 and 400 mg/kg for hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *G. gynandra* using carrageenan induced paw oedema model during 6h [13]. The tested extracts showed significant ($p < 0.001$) inhibition of paw oedema during 6 h along with the standard drug indomethacin.

Indomethacin is a cyclooxygenase inhibitor, the extracts has activity which is comparable to indomethacin and can be said to inhibit the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant antiinflammatory activity against carrageenan induced paw edema, so inhibition of carrageenan induced paw edema by the extracts could also be due to its inhibitory activity on the lipoxygenase enzyme [11, 14-17]. Among all extracts methanol extract produced significant activity compared to other extracts.

The hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *G. gynandra* also showed the good antibacterial activity (Zone of Inhibition) on tested bacterial strains. The extracts showed more activity on G-ve bacteria than G+ve bacteria. Plants are rich in a wide variety of secondary metabolites belonging to chemical classes (tannins, terpenoids, alkaloids, and polyphenols) represent different biological activities that depend on the diversity and quantity [18]. Therefore, from the results of present study it may be estimated that

the different phytochemical compounds present in the plant extracts may be responsible for antibacterial activity.

In conclusion, the present study provides the rationale for the traditional use of the extracts of *G. gynandra* in the management of different diseases. Further studies would be worthwhile for isolation and characterization of the common constituents (Bio active molecules) of all extracts of the *G. gynandra* with the help of numerous advanced technologies such as GC-MS, LC-MS, IR and NMR.

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