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EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *WATTAKAKA VOLUBILIS* LEAVES

Dr.Amit Singh, K.Vamshi SharathNath*, Dr.L.SatyaNarayana.
Department of Pharmaceutics, Monad University, Hapur, UttarPradesh-245101.
Nalla Narasimha Reddy Educational Society's Group Of Institutions School of Pharmacy.

Abstract:
The anti-inflammatory activity of methanolic extract of Wattakaka volubilis(L). Leaves (200 and 400 mg/kg. p.o.) in albino wistar rats was assessed by using various types of in-vivo pharmacological screening methods such as Carageenan induced paw oedema and Cotton pellet induced granuloma technique.At the dose of 400mg/kg, methanolic extract of Wattakaka volubilis L. significantly (p<0.01)decreased the paw oedema from the second hour of carageenan induced paw oedema method and a significant (p<0.01) decrease in granuloma formation was observed in the cotton pellet technique. However, the significant (p<0.01) decrease in the paw oedema was also observed even at the dose of 200mg/kg of methanolic extract of Wattakaka volubilis L. after four hours carageenan induction.The anti-inflammatory activity of the methanolic extract may be due to the inhibition of prostaglandin synthesis and by the stabilization of the lysosomal membrane as evidenced from its efficacy against acute, sub acute inflammation.

Key Words: *Anti-inflammatory activity, Wattkaka volubilis, carageenan, cotton pellet.*

Corresponding author:
K.Vamshi SharathNath,
Department of Pharmaceutics,
Monad University, Hapur, UttarPradesh-245101.
Nalla Narasimha Reddy Educational Society's
Group Of Institutions School of Pharmacy.

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INTRODUCTION:

History of medicine goes back practically to the existence of human being. The current accepted modern medicine or allopathy has gradually developed over the years of scientific and observational efforts of scientists. However, the basis of its development remains in the roots of traditional medicine and therapies[1]. Inflammation is the complex biological protective response of vascular tissue to harmful stimuli such as pathogens, damaged cells or irritants. The release of chemicals from WBC, increase the blood flow to the injured area and may result in redness, warmth, swelling and pain[2]. Inflammatory response occurs in three distinct phases, each mediated by different mechanisms Acute Transient phase, Delayed sub-acute phase & Chronic proliferation phase . The inflammation may cause due to the physical agents like heat, cold, radiation or mechanical trauma, chemical agents like organic and inorganic poisons, infective agents like bacteria, viruses and their toxins or immunological agents like cell mediated antigen-antibody reaction[3]. Various mediators are involving in inflammatory response such as serotonin, histamine and importantly metabolites of arachidonic acid namely prostaglandins (PGs), Interleukin-1 (IL-1) and Tumour necrosis factor (TNF) are the main mediators playing a pivotal role in inflammation[4]. Thus inflammation is distinct from infection, the former being a protective response of the body while the later is invasion into the body by harmful microbes and their resultant ill effects by toxins.

The objective of of the present study was to investigate anti-inflammatory activity of methanolic extract of *Wattakaka volubilis* Linn by Carageenan induced paw oedema method and Cotton pellet induced granuloma technique.

MATERIALS AND METHODS:

Collection of Plant Material:

The whole plant of *Wattakaka volubilis* Linn. was collected from different regions of Chittor district, after proper identification by an expert taxonomist Prof. Madhava Shetti, Department of Botany, Sri. Venkateshwara University, Chittor. The sample specimen (SAM/10/2014) was deposited at Nalla Narasimha Reddy Educational Society Group Of Institutions, School of Pharmacy for future reference.

Preparation of extracts:

The plant leaves were shade dried, and made into a coarse powder. It was then passed through a 40 mesh sieve for extraction purpose. A weighed quantity (1 kg) of the powder was subjected to continuous hot extraction by using methanol as a solvent in Soxhlet apparatus for 72 h. The extract was evaporated under reduced pressure and then dried in air (yield 8.24%w/w). The extract was administered to the animals by dissolving in Tween 80 (2% v/v).

Animals:

Male Albino wistar rats weighing about 200-250 g were used for the present study. The animals were housed in polypropylene cages, maintained under standard conditions (12 h light 12 h dark cycle; 27±1° C; 60% humidity). They were fed with standard rat pellet diet and water ad libitum.

Drugs and Chemicals:

The inflammation was induced by using Carrageenan obtained from Sigma (USA) and other chemicals used were of analytical grade.

Acute toxicity studies:

Male Albino Wistar rats weighing 200-250 g selcted by random sampling technique was performed as per OECD-423 guidelines (acute class method)[5]. The animals were fasted overnight, provided only water, after which the MEAG was administered to the respective groups orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups observed for 14 days. If mortality was observed in 2 or 3 animals, then the administered was assigned as a toxic dose. If mortality was observed in 1 animal then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300, 2000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 h.

Assessment of anti-inflammatory activity:

1. Carageenan induced paw oedema.

The animals were divided into four groups (n=6) and Group I animals served as control receiving 2% of tween 80 p.o. Group II and Group III were administered with MEAG at doses of 200 and 400 mg/kg, p.o. respectively. Group IV served as drug control receiving indomethacin 20mg/kg/i.p., one hour prior to carageenan administration. The swelling of the carageenan injected paw was quantitated every hour up to 5 hours by mercury displacement method using Plethysmograph. Percentage inhibition of oedema was calculated as-

$$\% \text{ inhibition} = \frac{V_C - V_T}{V_C \times 100}$$

Where V_T is the inflammatory increase in paw volume in drug treated rats and V_C is the inflammatory increase in paw volume in control group of rats. Percentage inhibition of oedema is proportional to anti-inflammatory activity[6,7].

2. Cotton pellet induced granuloma.

The Albino wistar rats were divided into four groups (n=6) and treatment has initiated. The assay was performed using groups of five animals each under aseptic condition and anesthesia using diethyl ether, and a ventral longitudinal scission was made in each animal. Then, four rolls of hydrofoils white cotton (Johnson & Johnson), measuring 5mm of length and weighting 40 mg each, were implanted through the diffusion of the subcutaneous tissue at four equidistant sites of the incision. The cotton rolls were previously autoclaved and treated with 0.4mL of 5% ampicilin (Ariston) aqueous solution[8, 9]. Group II, III (200 mg/kg, 400mg/kg/p.o) and Group IV receive standard drug Phenylbutazone (100mg/kg/i.p) once daily for 7 days starting with the day of implantation. Group I animals served as control receiving 2% of tween 80 p.o. On 8th day, the rats were killed and the pellets were removed. The cotton pellets along with granular tissue formed around were removed surgically and freed from extraneous tissue[10]. The pellets were weighed immediately for wet weight, and then pellets were dried in an incubator at 60 °C until constant dry weight was obtained (all the exudates dried up). The granulation tissue formation (dry weight of granuloma) was calculated after deducting the weight of cotton pellet (10mg) from constant dry weight of pellet and taken as measure of granuloma.

$$\% \text{ inhibition} = \frac{W_C - W_T}{W_C \times 100}$$

Where W_T is weight of granuloma in test drug treated rats and W_C is the weight of granuloma in control group of rats[11]

RESULTS:

Effect of MEAG on Carageenan induced paw oedema in rats:

The MEAG (400mg/kg/p.o) decreased the paw oedema significantly ($p<0.01$) from the 2nd hour after carageenan administration, when compared to the control group. The effect was comparable to the activity ($p<0.01$) produced by standard drug Indomethacin at 2nd hour after administration. However at the dose of 200mg/kg/p.o. MEAG also significantly ($p<0.01$) decreased paw oedema after 4th hour of administration. The Results are shown in the Table 1 and Figure 1.

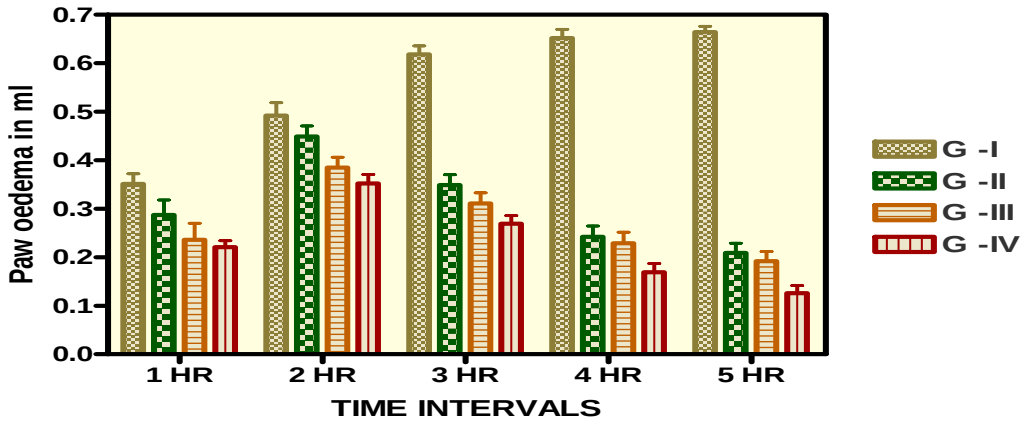
Table -1: Effect of MEAG on Carageenan induced paw oedema in rats.

Treatment	Paw Oedema volume (ml)				
	1 hr	2 hr	3 hr	4 hr	5 hr
Control	0.351±0.0211	0.492±0.0265	0.618±0.0179	0.652±0.0181	0.664±0.0120
MEAG 200mg/kg	0.287±0.0310* (18.2%)	0.449±0.0218 * (8.7%)	0.349±0.0210* (43.5%)	0.242±0.0224** (62.8%)	0.209±0.0197* (68.5%)
MEAG 400mg/kg	0.236±0.0340* (32.7%)	0.385±0.0212** (21.7%)	0.311±0.0216** (49.6%)	0.229±0.0228** (64.8%)	0.192±0.0197** (71.0%)
Indomethacin 20mg/kg/p.o	0.221±0.0130** (37.0%)	0.352±0.0186** (28.4%)	0.269±0.0169** (56.4%)	0.169±0.0181** (74.0%)	0.126±0.0158** (81.0%)

The values are expressed as mean ± SEM, and n=6. Statistical significance test for comparison was done by ANOVA, followed by Dunnet's test.

Comparison groups II, III, and IV vs. group I.
* $p < 0.05$, ** $p < 0.01$.

Fig-1.Carrageenan induced paw oedema in rats



Effect of MEAG on Cotton pellet granuloma:

The MEAG (400mg/kg/p.o) produced a significant ($p<0.01$) inhibition of granuloma formation when compared to the control group ($p<0.01$). Results were shown in Table 2 and Figure 2.

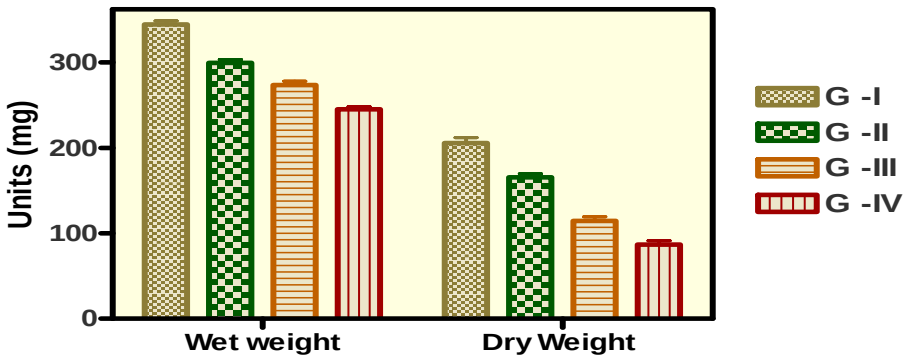
Table 2: Effect of MEAG on cotton pellet induced granuloma in rats.

Group	Treatment	Wet weight (mg)	Dry Weight (mg)
I	Control (2% Tween 80, 5ml/kg/p.o)	344.50 ± 4.210	205.71 ± 6.20
II	Test I MEAG -200mg/kg/p.o	299.50 ± 3.81*	165.41 ± 4.21*
III	Test II MEAG-400mg/kg/p.o	273.50 ± 4.50**	114.47 ± 4.87**
IV	Standard (Phenylbutazone 100mg/kg/i.p)	245.0 ± 2.98**	86.61 ± 4.65**

The values are expressed as mean ± SEM, and n=6. Statistical significance test for comparison was done by ANOVA, followed by Dunnet's test.

Comparison groups II, III, and IV vs. group I.
* $p < 0.05$, ** $p < 0.01$.

Fig-2: Cotton pellet induced granuloma in rats



Oedema induced was biphasic, the first phase (1st hr) involves release of Histamine and Serotonin, and second phase (3rd hr) was mediated by Cyclooxygenase product, Prostaglandins, producing oedema dependant in mobilization of neutrophils and continuity between two phases were provided by Kinins[10]. Carageenan induced paw oedema is an accepted useful phologistic tool to investigate

systemic anti-inflammatory agents[11]. Injection of carageenan into rat paw produces plasma extravasation and inflammation. This is characterized by increased tissue fluid & plasma protein exudation with neutrophil extravasation and metabolism of arachidonic acid by cyclooxygenase & lipooxygenase pathways[12]. MEAG showed more significant reduction in the paw oedema only during the 2nd to 5th hour whereas it has not shown any significant inhibition during the 1st and 2nd hours. The probable mechanism of anti-inflammatory action of MEAG may be due to its influence in the cyclooxygenase pathway rather than the lipooxygenase pathway. Since it was interfering with prostaglandins biosynthesis as evidenced by the maximum anti-inflammatory activity at the end of the 3rd and 5th after the challenge with carrageenan which indicates.

The cotton pellet granulomatous tissue formation is related to the chronic inflammatory process, which is characterized by several phases and this method has been widely employed to assess the transudative and proliferative components of chronic inflammation[13, 14]. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma[15]. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic sources for granuloma formation. Hence, decrease in weight of granuloma indicates that the proliferative phase was effectively suppressed by the 2nd dose of methonolic extract of *Wattkaka volubilis* and also it was indicating that the extract at doses tested was shown significant dose dependent activity.

CONCLUSION:

The carrageenan induced paw oedema and cotton pellet induced granuloma are two standard experimental models of acute and sub acute inflammation respectively. The methanolic extract was effective in both the models of inflammation, our study concludes that MEAG has significant anti-inflammatory activity and its possible mechanism may be due to the inhibition of prostaglandin synthesis and by the stabilization of the lysosomal membrane as evidenced from its efficacy against acute & sub acute inflammation. Further studies are required to identify the active principles responsible for the therapeutic action, before it is being investigated for its precise mechanism of action, kinetics and clinical efficacy.

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