



Screening of anti-inflammatory activity and dose selection of *Mollugo pentaphylla* by using cotton pellet induced granuloma in rat

N.K. Mishra^{1*}, R.K¹Panda, Gitanjali Mishra², V. Rajakumar¹, Saroj Kumar¹ and K. Tejonidhi¹

1, Department of Pharmacology, Roland Institute of pharmaceutical sciences, Berhampur, Orissa – (India)

2, Departments of Zoology, Berhampur University, Berhampur, Ganjam, Orissa – (India)

Abstract

Anti-inflammatory activity of the plant *Mollugo pentaphylla* (Ethanolic Extract) was studied by using sub acute model like cotton pellet induced granuloma technique. Sterilized raw cotton was implanted in the axilla and growing region of the rat. The ethanolic extract of the plant was administered orally (at dose 200mg/kg and 400 mg/kg body weight) by inhibiting wet weight and dry weight of the cotton pellet 47.81%, 30.08% and 30.74%, 27.81 respectively. The standard drug dexamethasone at 0.5 mg/kg body weight also inhibits the wet weight and dry weight of cotton pellet 54.45% and 46.36% respectively. The spleen weight and total WBC count was reduced significantly ($p < 0.05$). After studying three parameters (granuloma weight, spleen weight and total WBC count) herbal extract 200mg/kg was selected the best and safety dose for anti-inflammatory activity. The preliminary phytochemical investigation revealed the presence of steroidal saponins which may be responsible for tit's anti-inflammatory activity.

Key-Words: Dexamethasone, Total WBC count, Spleen weight, Granuloma, Flavonoids, Saponins

Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli; such as pathogens, damaged cell or irritants. It is the basic response of body to injury, a sequence of complicated, interrelated events work to define the body, ultimately bringing the plasma proteins & phagocytes to the injured area, for the purpose of irritating tissue repair. This study has been taken for the evaluation of Anti-inflammatory activity of the herbal extract (Ethanolic) of the plant *Mollugo Pentaphylla* (Molluginaceae) containing different compounds (Saponin, steroids tannins etc.)

It has been found from literature survey, that previously analgesic activity has been already proved (Jena and Nayak, 2009). The extract of this plant has produced a significant analgesia in the dose of 100 & 200 mg/kg body wt.

Due to this activity, further study of anti-inflammatory activity of this plant was undertaken. *Mollugo Pentaphylla* is widely used in India to treat enteritis, hepatitis, appendicitis, eye disease and cancer. Mollugogenol-A triterpenoid saponin is the active constituent responsible for anti-fungal activity². In our laboratory it was observed that crude ethanolic extract of *Mollugo Pentaphylla* exert anti-inflammatory action in sub acute model.³

Material and Methods

Collection of plant materials

The aerial parts of fresh *Mollugo Pentaphylla* were collected from Puri district, Orissa, India. The plant was made shade dried in the room temperature. The plant was authenticated by Dr. M.K. Mishra, PhD, Prof. Botany, Berhampur University, Orissa.

Preparation of leaf extract

The dry plant were made coarse powder and subjected to extraction with 95% ethanol by Soxhlet apparatus for 15 h. After filtration with cotton wool, the filtrate was concentrated in water bath at 40⁰ C. The yield was 3.2 g/100 g. The extract was stored in a refrigerator until further use.

* Corresponding Author:

E-mail: montu.phd@gmail.com

Mob.: 09438784415

Animals used

Male albino rats of Wister strain weighing around 160–180 g were procured from Ghosh Enterprise Govt. Contractor and general order supplier 6-C, Kolkata for the present study. The animals were housed in solid-bottomed polypropylene cages and acclimatized to animal house conditions. Animals were kept at temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of 30–60 %. Animal were allocated to different treatment groups and they were marked accordingly on their fur. The rats were fed with commercial rat diet (Rayans Biotechnology Pvt.Ltd, 12-13-483/A street no-14, Ternaka, Hyderabad -17, and A.P.) and water ad libitum. The experiments were designed and conducted in accordance with the ethical norms approved by Institutional Animal Ethical Committee Guidelines (IAEC No. 43/ 2010, Chairman IAEC, RIPS). Once inflammation developed by cotton pellet, food was served on the bottom of the cages as severely inflammatory rats have difficulty in feeding from the cage top.

Acute oral toxicity study

As per the OECD guidelines, the herbal formulation at different doses up to 2000mg/kg was administered and the animals were observed for behavioral changes, toxicity and motility up to 48h.

Induction of Inflammation⁸

Inflammation was induced by cotton pellet granuloma model (Sub acute). This method was adopted by D'Arcy (1960) which was carried out by using sterilized cotton pellet implantation method in rats. Under light ether anesthesia by using blunted forceps and subcutaneous tunnel was made and sterilized cotton pellets (10 ± 1 mg) were implanted in the axilla and groin region of the rat.

After recovering from Anaesthesia, animals were treated orally with vehicle control (Distilled water 10 ml / kg), Dexamethasone and various doses of the herbal extract for consecutive 7 days, once per day. They were sacrificed on day 8th by cervical dislocation and the pellets were removed, freed from extraneous tissue and dried at 60°C for 24 hrs. The percentage inhibition of the dry weight of the granuloma were calculated and compared.

Standard Drugs

Dexamethasone Sodium (Dexona, Cadila Healthcare Ltd., Batch no- LI1155) was used for experiments. All other experimental chemicals and solvents used were of analytical grade.

Experimental setup

Animals were divided into four groups of six animals in each group as follows:

Group I: Vehicle control received distilled water.

Group II: Animal treated with Dexamethasone (Dose: - 0.5 mg / kg)

Group III: Animals treated with extract (Dose:-200 mg / kg)

Group IV: Animal treated with extract (Dose: - 400 mg / kg)

At the end of the experimental period, rats were fasted overnight and the anesthetized rats were sacrificed by cervical decapitation. The blood sample was collected for Total W.B.C count and spleen weight.

Statistical Analysis

All the grouped data were statistically evaluated with Microsoft excel software. Hypothesis testing methods included two tailed one way analysis of variance (ANOVA) followed by Standard Error Mean (SEM). P-Values of less than 0.05 and 0.001 were considered to indicate statistical significance. All the results were expressed as Mean \pm SEM for six animals in each group.

Results and Conclusion**Cotton pellet induced granuloma formation**

The herbal extract at different doses and standard drug was evaluated by cotton pellet induced granuloma formation to understand its potential in sub-acute inflammatory phase. Table-1, Fig-1, Fig- 2 and Fig-5 indicating the significant ($p < 0.05$) reduction of wet weight and dry weight of cotton pellet. The standard drug dexamethasone produces maximum activity by inhibiting the wet weight and dry weight of cotton pellet 54.45% and 46.36% respectively. Two different dose (200,400mg/kg) showing significant reduction of wet weight and dry weight of cotton pellet at 47.81%, 30.74% and 30.08%, 27.81% respectively.

3.2. Effect of M. Pentaphylla on Total WBC count

In vehicle control maximum increase in WBC was found as compare to normal (Table-2 and Fig-4). The standard drug and different dose of test significantly ($p < 0.05$) decreases the migration of WBC as compare to vehicle control.

Spleen weight / 100g body weight

During inflammation the enlargement of spleen was found in vehicle control (Table-3 and Fig-3). The standard drug and all the doses of the plant produce significant ($p < 0.05$) suppression of the spleen weight.

Preliminary phytochemical Screening of ethanolic extract of Mollugo pentaphylla revealed the presence of flavonoids, saponins, steroid, tannins, and carbohydrate. Cotton pellet granuloma is one of the exudative of inflammation and the cotton pellet granuloma is taken as proliferate phase of inflammation. During the inflammatory process migration of WBC takes place which is the biological marker. Enlargement of spleen occur as spleen has the

phagocyte nature. It was observed that the extract of the *Mollugo pentaphylla* significantly reduced the granuloma formation in rat. It was found that the extract at dose 200mg/kg and 400mg/kg produced a significant anti-inflammatory activity by reducing the dry weight and wet weight of granuloma, inhibiting the migration of WBC. The spleen weight also significantly decreases by the extract. The anti-inflammatory potential may be attributed to the presence of phytoconstituents such as polyphenolic compound (flavonoids) and steroidal saponin⁵. The better suggestion is that the lower dose (200mg/kg body weight) produces a better activity than the higher dose.

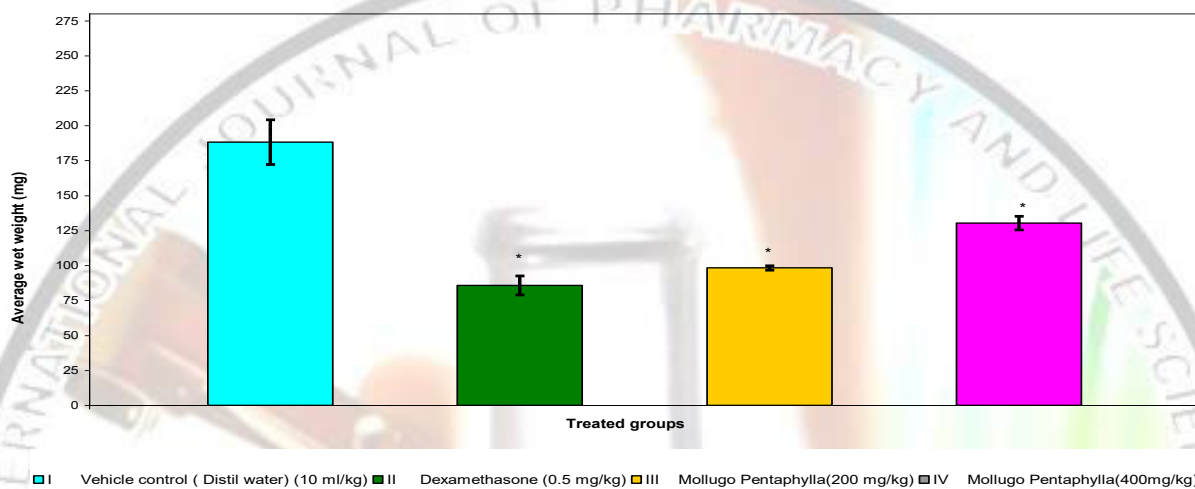
The plant *M. pentaphylla* was traditionally claimed for a large number of pharmacological action and medicinal use. In the present study it was found that the ethanolic extract of the aerial parts of plant is safe up to 2000mg/kg. The significant and better anti-inflammatory activity was attributed at lower dose 200mg/kg to the phytoconstituents present in it. Further phytochemical studies are needed to isolate the active compounds responsible for these pharmacological activities.

Acknowledgements

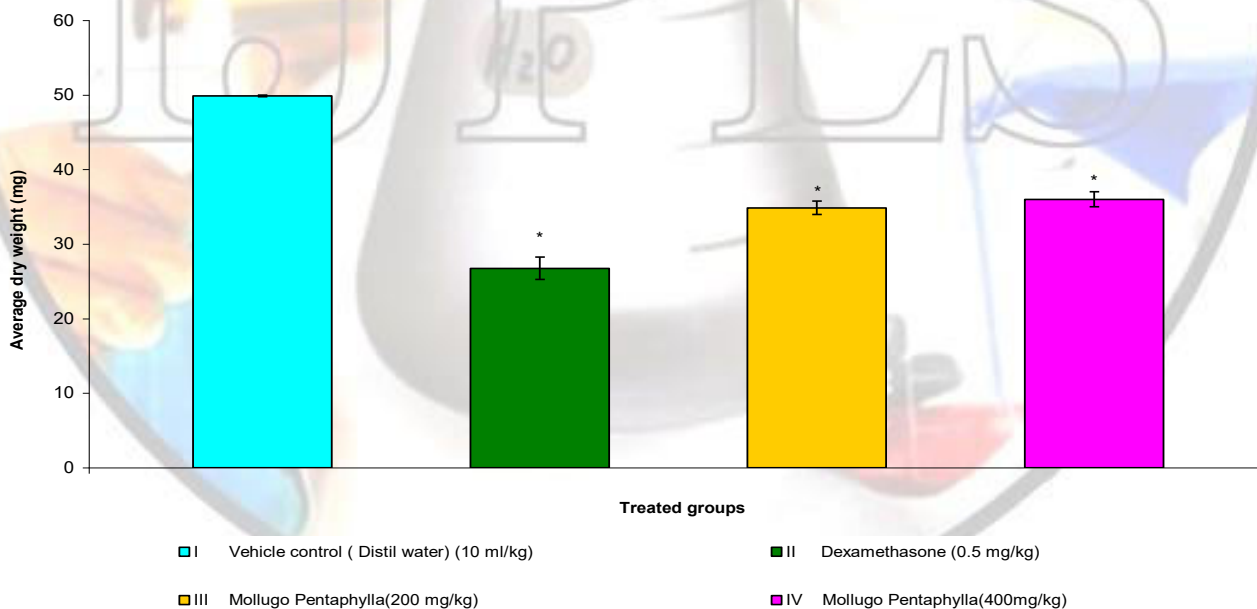
I am grateful to Dr J Surya Rao, Director and Dr. M.E. Bhanoji Rao Principal; Roland Institute of pharmaceutical sciences, Berhampur, Orissa for providing the necessary facilities for study.

References

1. Jena P.K. and Nayak B.S. (2009). Analgesic activity of *Mollugo pentaphylla* by tail immersion method. *Asian J Pharmaceutical and Clinical Research*, **2**(1), 61-63.
2. Rajasekaran M., Nair A. G. R., Hellstrom W. J. G. and Sikka S.C. (1993). Spermicidal activity of an antifungal saponin obtained from the tropical herb *Mollugo pentaphylla*. *Contraception*, **47**, 401-412.
3. Srinivas K., Rao M.E.B. and Rao S. S. (2000). Anti-inflammatory activity of *Heliotropium indicum* Linn and *Leucas aspera* Spreng in albino rats. *Indian J Pharmacol.*, **32**: 37-38.
4. Narendhirakannan R.T., Subramanian S. and Kandaswamy M. (2007). Anti-inflammatory and lysosomal stability actions of *Cleome gynandra* studied in adjuvant induced arthritic rats. *Food and chemical Toxicology*, **45**:1001-1012.
5. Akram M.T. and Dhenge R.M. (2009). Anti-inflammatory activity of ethanolic extract of *Ventilago denticulate*. *Indian J. Nat. Prod.*, **25**(2): 22-24.
6. Vogel W. H., Scholkens B. A., Sandoe J., Muller J. and Vogel W. F. *Drug discovery and evaluation*. Springer Publication. 2nd edition: 751-769.
7. Sharma A., Mann A. S., Gajbhiye V. and Kharya M.D. (2007). Phytochemical profile of *Boswellia serrata*. *J. of Pharmacognosy Reviews*, **1**: 137-142.
8. Kaneria M. S., Naik S. R. and Kohli R. K. (2007). Anti-inflammatory, antiarthritic and analgesic activity of a herbal formulation (DRF/AY/4012) *Indian J of Experimental-Biology*, **45**: 278-285.
9. Bhutia Y. D., Vijayaraghavan R. and Pathak U. (2010). Analgesic and anti-inflammatory activity of amifostine, DRDE-07 and analogs, in mice. *Indian J Pharmacol.*, **42**: 17-20.
10. Kohli K., Ali J., Ansari M. J. and Raheman Z. (2005). Curcumin: A natural anti-inflammatory agent. *Indian J. Pharmacology*, **37**(3): 141-147.

Fig. 1: Effect of the average wet weight of cotton pellet with respect to different treat group

* $p \leq 0.05$ Treated groups Vs Vehicle control

Fig. 2: Effect of the average dry weight of cotton pellet with respect to different treat group

* $p \leq 0.05$ Treated groups Vs Vehicle

Fig. 3: Showing the average spleen weight/100gm body weight on day 8th. 1= Vehicle control showing maximum weight, 2 = Dexamethasone (0.5mg/kg), 3 = *Mollugo pentaphylla* (200mg/kg), 4 = *Mollugo pentaphylla* (400mg/kg) showing significant reduction of spleen weight.

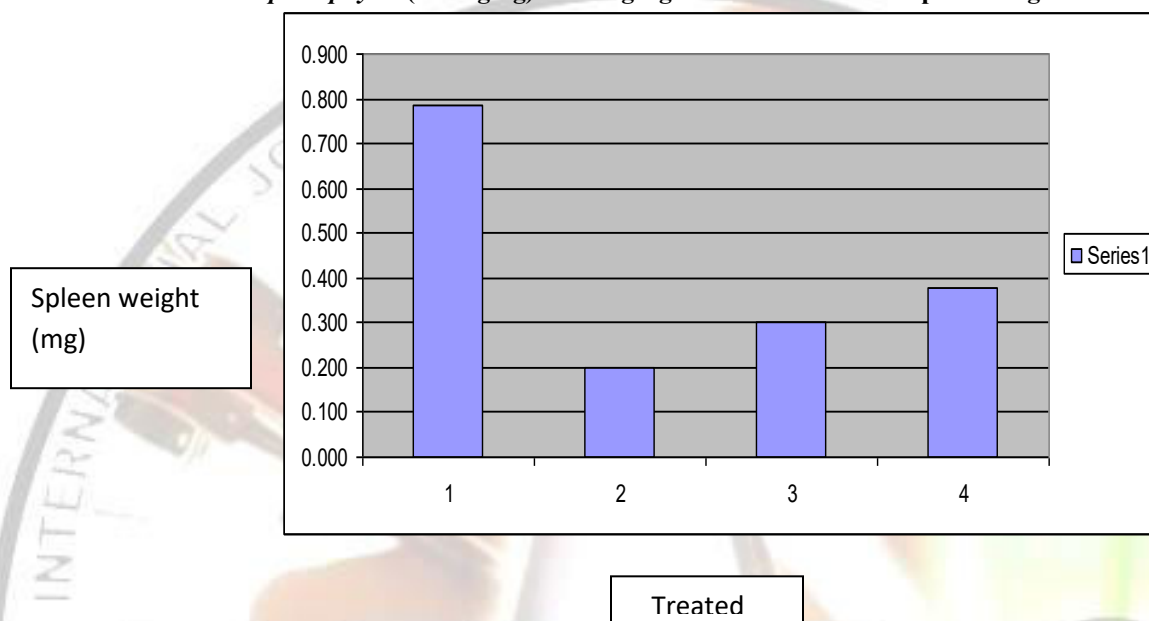


Fig. 4: Showing number of migration of W.B.C during day-0 (series-1) and day-8 (series-2). 1= Vehicle control, 2 = Dexamethasone (0.5mg/kg), 3 = *Mollugo pentaphylla* (200mg/kg), 4 = *Mollugo pentaphylla* (400mg/kg). A marked migration of W.B.C was observed in vehicle control on day-8 and a significant reduction of migration was found in other groups at the level of $p < 0.05$

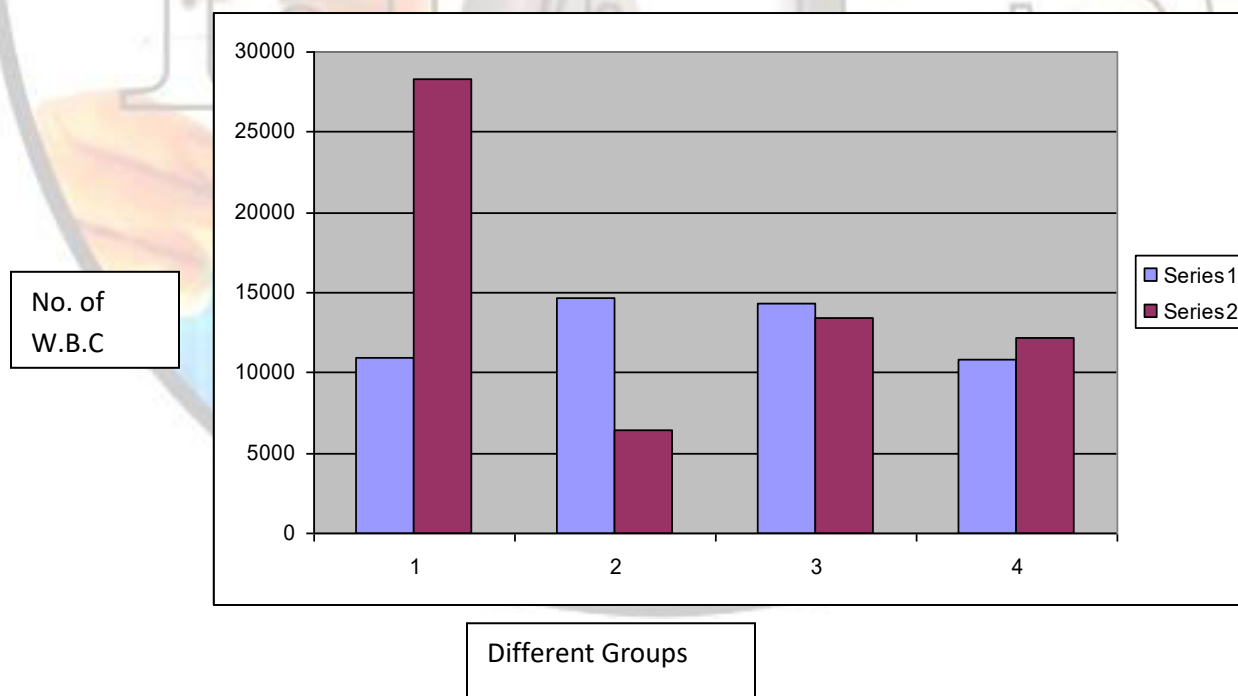


Fig. 5: Cotton pellet granuloma induction in rat, showing vehicle control (maximum granuloma), Standard Dexamethason, test-1 (200mg/kg) and Test-2 (400mg/ kg) showing inhibition of the granuloma.



(Vehicle control)



(Standard Dexamethasone)



(Test-1, 200mg/ kg)



(Test-2, 400mg/ kg)