Anti-Inflammatory Activity Of Polyherbal Formulation By Using Cotton Pellet Granuloma In Rat And Xylene Induced Mice Ear Edema Model

Nafiza Bhanu, Yalamarty Pharmacy College, YalamartyNagar, Tarluwada, Visakhapatnum, A.P, India
N. K. Mishra, Yalamarty Pharmacy College, YalamartyNagar, Tarluwada, Visakhapatnum, A.P, India
J. R. Panda, Roland Institute of pharmaceutical sciences, Ambapua, Khodasingi, Berhampur, Odisha, India
S. Patra, University Department of Pharmaceutical Sciences, Utkal University, Vanivihar, Bhubaneswer, India

Abstract
Andrographis paniculata, Alpinia galanga, Boswellia serrata, Curcuma longa and Withania somnifera are the ethno medicinal plants widely used traditionally for the treatment of many inflammatory diseases because of plenty of source of flavonoid, saponin, steroids and terpenes. The present study has been focused to assess the anti-inflammatory activity of Poly herbal mixture in syrup base by using cotton pellet granuloma in rat and xylene induced mice ear edema model for the better therapeutic activity. The Poly herbal formulation consists of methanolic extract of above plants at equal proportion. The herbal formulation was administered orally at a dose of 150 mg/kg, 200 mg/kg and 250 mg/kg body weight for 7 days after implantation of sterilized cotton pellet in axilla and groin region of the rat. A significant (P ≤ 0.01) reduction of wet weight and dry weight of cotton pellet at 31.78%, 53.45%, 75.08% and 11.75%, 28.83%, 40.28% was observed. A significant reduction (P≤0.01) in total leukocyte number, ESR and spleen weight was found at the same dose. Similarly in xylene induced mice ear edema model the herbal formulation at doses (300, 400 and 500 mg/kg) reveled a significant (P≤0.01) reduction of the inflammation. A better activity was observed at 250 mg/kg and 500mg/kg body weight in rat and mice model respectively.

Keywords: Cotton pellet, Flavonoids, ESR, Inflammation

1. Introduction
Medicinal plants are an important element of indigenous medical systems in South American countries and elsewhere and these resources are usually regarded as part of a culture’s traditional knowledge. For many years, Europe has profited from exchange with other continents, and many of the pure natural products and some of the phototherapeutic preparations used today are derived from plants used in indigenous cultures. The role of the ethno botanist in the search for new drugs was of continuous importance until the second half of the 20th century, when other approaches became more “fashionable”. However, in recent years, the use of such information in medicinal plant research for drug development has again received considerable interest in the media and in some segments of the scientific community.

Inflammation is a common clinical condition and is a response of a tissue to injury. The cardinal signs of inflammation are swelling (tumor), heat (calor), redness (rubor), pain (dolor) and loss of function (function laesa) and occurs due to the movement of plasma fluids, proteins, and inflammatory cells from the lumen of the vascular system out into the tissues. However, if untreated, it may lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma).

Cyclooxygenase 2 (COX-2) is an inflammatory enzyme that catalyzes the production of prostaglandin E2 (PGE2) from the substrate lipid, arachidonic acid. Inflammatory signals greatly enhance COX-2 expression, particularly in inflammatory cells such as monocytes, macrophages, endothelial cells, and fibroblasts. Numerous trials to develop promising anti-inflammatory drugs currently target the suppression of PGE2 production and COX-2 expression. Therefore, an improved understanding of the mechanisms underlying COX-2 and PGE2 generation should facilitate the identification of target inflammatory signaling pathways for the development of effective drugs.

Presently, the drugs commonly in use for the treatment of inflammation are Glucocorticoids (e.g. Cortisone and Prednisolone), NSAIDS (e.g. Diclofenac, Ibufrofen etc.), Disease-modifying anti-inflammatory drugs (DMIRDs) (e.g Methotrexate, Leflunomide) and Biological response modifiers (e.g Tumor necrosis factor, Alpha blocking agents). Because of their high cost, the prolonged use of many of these drugs is associated with severe adverse reactions and toxicity, including some risk of infections in subsets of patients being treated with biological response modifiers. As a result, alternative treatments based on natural plant products and herbal mixtures belonging to the realm of Polyherbal formulation, complementary and alternative medicine (CAM) are becoming increasingly popular in the India, US and other countries. Comparatively, the use of herbal and other naturally based medicine has a long history with minimum or no side effects. A large number of plants are used in Ayurveda for the treatment of inflammation. The growing popularity of natural and herbal medications, easy availability of raw materials, cost-effectiveness and paucity of reported adverse reaction, prompted us to formulate a poly herbal oral preparation and assess its anti-inflammatory effects.
The present paper was undertaken in order to investigate the prepared poly herbal formulation in syrup base for better anti-inflammatory activity.

Our study has been focused on Poly herbal mixture in syrup base contains *Andrographis paniculata*, *Alpinia galanga*, *Boswellia serrata*, *Curcuma longa* and *Withania somnifera* in order to evaluate the anti-inflammatory activity. The anti-inflammatory properties in this mixture in syrup base have, however, not been subject to any scientifically controlled investigations so far.

2. Materials And Methods

2.1. Collection of plant materials:
In the present study mature plant material of *Andrographis paniculata*, *Alpinia galanga*, *Boswellia serrata*, *Curcuma longa* and *Withania somnifera* were purchased from Jillimudi Apparao and Sons Ayurvedic Shop, Visakhapatnam, India. They were identified and authenticated by Dr. M. Veraiah, Retd Professor in Botany, Andhra University, Visakhapatnam, by studying morphological features of leaf arrangement, bark, flower inflorescence arrangement, fruit and seed (Specimen No-104 for further studies). After authentication, plants were shade dried at room temperature until they become free from moisture. The Plant materials were powdered to 40 # mesh particle size and subjected to standardization with the different parameters.

2.2. Preparation of extracts and Phytochemical Screening:

2.2.1. Preparation of extract of *Andrographis paniculata*:
The leaves of *Andrographis paniculata* were purchased and dried in shade. The dried leaves were powdered and about 200gm of coarse powder was subjected to macerate in 95% ethanol and kept at room temperature for three days. After filtration, the filtrate was concentrated to dryness under reduced pressure and controlled temperature 50°-60°C.14

2.2.2. Preparation of extract of *Alpinia galanga*:
Roots of *Alpinia galanga* were obtained, shade dried at room temperature and subjected to size reduction to get coarse powder. The powdered material 240 gm was subjected to extraction by Soxhlet extractor using absolute alcohol as solvent. After extraction the extract was concentrated to dryness under reduced pressure and controlled temperature 50°-60°C.15

2.2.3. Preparation of extract of *Boswellia serrata*:
200 gm of *Boswellia serrata* olio-gum-resin was purchased and the extraneous matter was removed. It was then coarsely powdered and 200 gm of olio-gum-resin powder was treated with 600 ml of methanol in a glass jar and stirred to form a homogenous mixture then allowed to stand for 72 hrs at room temperature. The supernatant was filtered by decantation and evaporated under elevated temperature and under reduced pressure until a constant weight was obtained.14

2.2.4. Preparation of extract of *Curcuma longa*:
Roots of *Curcuma longa* were obtained and shade dried at room temperature. The dried roots were cut into small pieces and powdered by using hand mill and passed through sieve 40 # mesh particle size. About 500 g of coarse powder was subjected to extraction by Soxhlet extractor using absolute alcohol as solvent. After extraction the extract was concentrated to dryness under reduced pressure and controlled temperature 50°-60°C.15

2.2.5. Preparation of extract of *Withania somnifera*:
*Withania somnifera* roots were procured and made into coarse powder. Its aqueous extract was prepared by suspending 15 gm root powder in 100 ml distilled water, followed by shaking (150 rpm, at 60°C, for 24 h).16 The extract was filtered and evaporated under reduced pressure to get a concentrate product.

2.3. Preparation of Polyherbal Mixture in Syrup base:
Accurately weighed quantities of extracts were taken in glass vessel (Table – 1). 2% Gum Acacia was added and mixed. The Simple syrup I.P containing sucrose (66.7%w/w. Fisher Scientific India Pvt. Ltd.) was added to make up the volume to 100 ml. The preparation was transferred to a clean and dry amber coloured bottle and stored at room temperature. The final stock syrup contains 20 mg/ml of Poly herbal mixture in syrup base. The formulation was administered orally as suspension. The product prepared was evaluated for pH, colour, odour and redispersibility. The results are presented in Table - 2.

2.4. Animals:
Male albino rats of Wistar strain weighing 160-180 g and albino Swiss mice 20-25 g was taken from Mahaveer Enterprises, Hyderabad. The animals were housed in solid-bottomed polypropylene cages and acclimatized to animal house conditions. The rats were fed with commercial pellets and water ad libitum. The standard pellet diet was supplied by Rayan’s biotechnologies Pvt. Ltd, Hyderabad, (A.P.). The experiments were designed and conducted in accordance with the ethical norms. The study protocol was submitted before Institutional Animal Ethics Committee (IAEC) (Regd. No. 1430/PO/a/CPCEA dated 08-04-2011) of Yalamarty Pharmacy College. The IAEC has approved this protocol (Approval No.YLMV-002 dated on 04-08-2012).

2.5. Acute oral toxicity study:
Acute toxicity test were performed on rat of either sex weighing160-180 g body weight. The procedure was
performed as per the Organization for Economic Co-operation and Development (OECD guidelines 2000), received draft guidelines 423, from the committee for the purpose of control and supervision of experiments on Animals (CPSEIA), Ministry of Social Justice and Empowerment, Government of India. The herbal extract with 1% gum acacia at different doses up to 2000 mg/kg body weight was administered and the animals were observed for behavioral changes, toxicity and motility up to 48 hrs. There was no mortality was observed at 2000 mg/kg. So 200 mg/kg and another two (One lower dose 150mg/kg and another higher dose 250mg/kg body weight) doses were selected as the therapeutic dose.

2.6. Induction of Inflammation
2.6.1. Cotton pellet Granuloma method
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 0.5 mg/kg and various doses of the herbal formulation for consecutive 7 days, once per day. They were sacrificed on day 8th by cervical dislocation and the pellets were removed and immediate the wet weight was taken, freed from extraneous tissue and dried at 60°C for 24 hrs. The percentage inhibition of the wet weight and dry weight of the granuloma were calculated and compared.

\[
\text{Percentage inhibition (\%)} = \left( \frac{\text{Control - Treated}}{\text{Control}} \right) \times 100
\]

2.6.2. Xylene induced mice ear edema
Xylene induced mice ear edema is one of the acute inflammation study. One hour after oral administration of distilled water, Diclofenac and herbal extract to different groups i.e. vehicle control, standard and test respectively, 50µl of xylene was applied to the anterior and posterior surfaces of the right ear under light ether anesthesia. The left ear was considered as control. Four-hours later xylene application, mice were sacrificed by cervical dislocation and both ears were removed. Ear lobes were punched out in circular disc using metal punch (6 mm diameter) and weighed. The difference in the weight of discs from right treated and left untreated was calculated and was used as measure of edema. The level of percentage inhibition was calculated using the following formula.

\[
\text{Percentage inhibition (\%)} = \left( \frac{\text{Control - Treated}}{\text{Control}} \right) \times 100
\]

2.7. Experimental set up
2.7.1. Experimental Design for Cotton pellet granuloma model
Group-I: Vehicle control received 1% Acacia (10 ml/kg).
Group-II: Animals treated with Dexamethasone (0.5 mg/kg).
Group-III: Animals treated with herbal formulation (150mg/kg).
Group-IV: Animals treated with herbal formulation (200mg/kg).
Group-V: Animals treated with herbal formulation (250mg/kg).

2.7.2. Experimental Design for Xylene induced mice ear edema
Group-I: Vehicle control received 1% Acacia
Group-II: Animals treated with Diclofenac (50mg/kg).
Group-III: Animals treated with formulation (300mg/kg).
Group-IV: Animals treated with formulation (400mg/kg).
Group-V: Animals treated with formulation (500mg/kg).

2.8. Biochemical Assays And Spleen Weight
2.8.1. Cotton pellet granuloma model
Different biochemical parameters like WBC count and Erythrocyte sedimentation rate (ESR) were estimated. For the estimation of Total WBC count blood samples were added with WBC diluting fluid and by the help of Neubauer’s chamber total numbers of WBC was Calculated by using the formula, Total WBC count = Total no. of cells X Volume correction factor X 20. Erythrocyte sedimentation rate (ESR) was determined by using Westergren method where the blood is drawn into a Westergren-Katz tube to the 200 mm mark. The tube is placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The calculation is done by the measurement of distance of fall of RBC in 1 hour. Spleen weight was measured for individual animals of different groups.

2.9. Statistical analysis:
All the grouped data were statistically evaluated with Microsoft excel. Hypothesis testing methods include one way analysis of variance (ANOVA) followed by Dunnett’s. \( P \leq 0.05 \) and 0.01 were considered to indicate statistical significance. All the results were expressed as Mean ± SEM for six animals in each group.

3. Results
3.1. Phytochemical analysis
Preliminary phytochemical screening showed the presence of phytosterol, saponins, flavonoids, alkaloids and glycosides in different extracts of selected plants for polyherbal formulation.
3.2. Cotton pellet induced granuloma formation
The Polyherbal formulation in syrup base at different doses and standard drug was evaluated by cotton pellet induced granuloma formation to understand its potential in sub-acute inflammatory phase. Table-3 and Fig.1 indicating the significant ($P \leq 0.01$) reduction of wet weight and dry weight of cotton pellet. The standard drug dexamethasone at dose (0.5mg/kg) produced maximum activity by inhibiting the wet weight and dry weight of cotton pellet 90.61% and 76.78% respectively. The herbal formulation at different dose (150, 200 and 250mg/kg body weight) showed significant ($P \leq 0.01$) reduction of wet weight and dry weight of cotton pellet at 31.78%, 53.45%, 75.08% and 11.75%, 28.83%, 40.28% respectively.

3.2.1. Effect of Herbal formulation on Total WBC count
In vehicle control maximum increase in WBC was found as compare to normal (Table-4). The standard drug and different dose of test formulation significantly ($P \leq 0.01$) reduced the migration of WBC as compare to vehicle control.

3.2.2. Spleen weight / 100g body weight
During inflammation the enlargement of spleen was found in vehicle control (Table-5). The Standard drug Dexamethasone (0.5 mg/kg) and Herbal formulation at different doses (150, 200 and 250mg/kg) produced significant ($P \leq 0.01$) suppression of the spleen weight.

3.2.3. Erythrocyte sedimentation rate (ESR)
The oral administration of Poly herbal mixture in syrup base at the dose of 150, 200 and 250 mg/kg body weight exhibited significant ($P \leq 0.01$) and a better reduction of Erythrocyte sedimentation rate (ESR) in comparison to the control group (Table-6).

3.3. Xylene induced mice ear edema
Similarly, in case of xylene model the mean ear edema of different groups is presented in Table-7, Fig.2 and ear lobe of different groups are shown in Fig.3. The standard drug, diclofenac sodium at dose 50 mg/kg showed significant anti-inflammatory activity by reducing ear edema 42.22% as compared to vehicle control group. Herbal formulation at different doses (300, 400 and 500mg/kg) produced significant ($P \leq 0.01$) reduction of the inflammation 22.22%, 26.66%, and 44.44% respectively.

4. Discussion And Conclusion
There are a multitude of approaches for identifying new pharmaceuticals. The treatment of inflammatory conditions with plants is widely reported. In the field of natural product biology, ethnopharmacological as well as bioprospecting approaches have received renewed attention in recent years. The concept of ethnopharmacology specifically aims to develop plant-based drugs and their Poly herbal formulation more widespread local use either as pure compounds or plant extracts (phytotherapy). Concurrent with this analysis is the ancestral use of plants by indigenous people (ethnobotany).

Such data aids an ethno pharmacological approach by allowing in order to develop a poly herbal formulation in syrup base for the better and safety therapy. In our experiments we have focused on Poly herbal mixture in syrup base (Table-1) contains Andrographis paniculata, Alpinia galanga, Boswellia serrata, Curcuma longa and Withania somnifera for the evaluation of anti-inflammatory activity. Accelerated stability study of Poly Herbal mixture in syrup base proved to be better physically stable (Table-2).

Daily administration of herbal formulation (test substances) of different doses for consecutive 7 days orally has shown significant ($P \leq 0.01$) inhibition of inflammation as compare to control in cotton pellet granuloma technique. Cotton pellet granuloma is one of the exudative of inflammation and the cotton pellet granuloma is taken as proliferate phase of inflammation. The wet weight and dry weight of cotton pellets has reduced significantly ($P \leq 0.01$) in the treatment groups as compare to vehicle control. In case of wet weight and dry weight, herbal formulation at dose (150, 200 and 250mg/kg body weight) showing significant ($P \leq 0.01$) reduction of wet weight and dry weight of cotton pellet at 31.78%, 53.45%, 75.08% and 11.75%, 28.83%, 40.28% respectively (Table-3 and Fig.1). The standard drug dexamethasone produces maximum activity by inhibiting the wet weight and dry weight of cotton pellet 90.61% and 76.78% respectively.

Increased white blood cell counts are a common feature of inflammatory reactions, especially those induced by microbial infection. So in vehicle control group an increase in total leukocyte number was found. A significant reduction ($P \leq 0.01$) in total leukocyte number was found in case of treated groups (Table-4). In our study it was found that the administration of poly herbal formulation in syrup base at dose 150, 200 and 250 mg/kg body weight leads to inhibition of leukocyte migration. The activity may be due to presence of steroidal glycoside and steroidal saponine. Enlargement of spleen occur during inflammation as spleen has the phagocyte nature which is marked in vehicle control group. The spleen weight also significantly decreases at all doses in the treated groups with poly herbal formulation and standard drug dexamethasone (Table-5).

Erythrocyte sedimentation rate (ESR) in the vehicle control group several fold high compared to drug treated groups. This may be due to the flavonoid content of the poly herbal formulation. These flavonoids are having the surface charge neutralizing effects. ESR is strongly related.
with the ability of red cells to aggregate into orderly stacks or rouleaux. Proteins are thought to affect the repellant surface charges on red cells and cause them to aggregate into rouleaux and hence the sedimentation rate increases. The rate of sedimentation was increased in vehicle control group where as in case of treated groups the ESR level was significantly decreased in Cotton pellet granuloma model (Table-6).

Similarly in case of xylene model the mean ear edema of different groups is presented in Table-7, Fig.2 and ear lobe of different groups are shown in Fig.3. The standard drug, diclofenac sodium at dose 50 mg/kg showed significant anti-inflammatory activity by reducing ear edema 42.22% as compared to vehicle control group. Herbal formulation at different doses (300, 400 and 500mg/kg body weight) produced significant (P<0.01) reduction of the inflammation 22.22%, 26.66%, and 44.44% respectively. In conclusion Poly herbal mixture in syrup base revealed significant anti-inflammatory activity in both cotton pellet granuloma in rat at dose 150, 200 and 250 mg/kg body weight and xylene induced mice ear edema models at dose 300, 400 and 500mg/kg body weight. Whereas 250 mg/kg in rat and 500 mg/kg in mice proved to be better therapeutic effect.

Conflict Of Interest Statement
The authors declare that they have no conflict of interest.

Acknowledgement
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References


Figures

Fig.1: Cotton pellet granuloma induction in rat, A- Vehicle control (maximum granuloma), B-Standard Dexamethason (0.5mg/kg), C-Herbal formulation (150mg/kg), D-Herbal formulation (200mg/kg), E-Herbal formulation (250mg/kg) showing inhibition of the granuloma.

Fig. 2: Effect of Herbal formulation in albino Swiss mice using Xylene induced mice ear edema
Values are expressed as mean ± SEM (standard error of the mean) of 6 determinants. **P≤0.01, compared with vehicle control.

Fig. 3: Xylene induced mice ear edema, A-Vehicle control (more inflammation and swelling of ear), B-Standard Diclofenac (50mg/kg), C-Herbal formulation (300mg/kg), D-Herbal formulation (400mg/kg), E-Herbal formulation (500mg/kg) showing reduction of inflammation and swelling of ear
Table 1: Formula for Poly Herbal Mixture in syrup base

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpinia galangal</td>
<td>400 mg</td>
</tr>
<tr>
<td>Andrographis paniculata</td>
<td>400 mg</td>
</tr>
<tr>
<td>Boswellia serrata</td>
<td>400 mg</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>400 mg</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>400 mg</td>
</tr>
<tr>
<td>Acacia</td>
<td>2 gm</td>
</tr>
<tr>
<td>Simple syrup base</td>
<td>q.s 100 ml</td>
</tr>
</tbody>
</table>

Table 2: Accelerated stability study of Poly Herbal mixture in syrup base

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial</th>
<th>I month</th>
<th>II month</th>
<th>III month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
<td>40°C*</td>
<td>RT</td>
<td>40°C*</td>
</tr>
<tr>
<td>Odour</td>
<td>Characteristic</td>
<td>Characteristic</td>
<td>Characteristic</td>
<td>Characteristic</td>
</tr>
<tr>
<td>pH</td>
<td>7.1</td>
<td>7.1</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td>Redispersibility</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
</tbody>
</table>

RT-Room temperature: 40 ± 2°C; RH – 65 ± 5 %. / * – Temp – 40 ± 2°C; RH – 80%.

Table 3: Effect of Poly herbal mixture on wet and dry weight of cotton pellets

<table>
<thead>
<tr>
<th>Group</th>
<th>mean weight(mg)</th>
<th>wet % of Inhibition</th>
<th>mean weight(mg)</th>
<th>dry % of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control (Acacia 1%)</td>
<td>89.72 ± 2.17</td>
<td>-</td>
<td>20.80 ± 0.62</td>
<td>-</td>
</tr>
<tr>
<td>Dexamethasone (0.5 mg/kg)</td>
<td>8.42 ± 0.32**</td>
<td>90.61</td>
<td>4.82 ± 0.75**</td>
<td>76.78</td>
</tr>
<tr>
<td>Herbal formulation 150 mg/kg</td>
<td>61.21 ± 2.15**</td>
<td>31.78</td>
<td>18.35 ± 0.67**</td>
<td>11.75</td>
</tr>
<tr>
<td>Herbal formulation 200 mg/kg</td>
<td>41.76 ± 1.83**</td>
<td>53.45</td>
<td>14.80 ± 0.42**</td>
<td>28.83</td>
</tr>
<tr>
<td>Herbal formulation 250mg/kg</td>
<td>22.36 ± 1.28**</td>
<td>75.08</td>
<td>12.42 ± 0.38**</td>
<td>40.28</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (standard error of the mean) of 6 determinants. **P≤0.01, compared to vehicle control.

Table 4: Effect of Polyherbal mixture on total WBC count

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC no. (Day-0)</th>
<th>WBC no. (Day-8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control (Acacia 1%)</td>
<td>9100 ± 194.4</td>
<td>16400 ± 943.5</td>
</tr>
<tr>
<td>Dexamethasone (0.5 mg/kg)</td>
<td>7580 ± 127.64**</td>
<td>5320 ± 151.69**</td>
</tr>
<tr>
<td>Herbal formulation 150 mg/kg</td>
<td>7260 ± 106.87**</td>
<td>7620 ± 3.43**</td>
</tr>
<tr>
<td>Herbal formulation 200 mg/kg</td>
<td>7260 ± 98.70**</td>
<td>7180 ± 78.83**</td>
</tr>
<tr>
<td>Herbal formulation 250mg/kg</td>
<td>7460 ± 69.57**</td>
<td>6580 ± 127.64**</td>
</tr>
</tbody>
</table>

Values are given in mean ± SEM (n=6), **P≤ 0.01, compared to vehicle control.
Table 5: Average spleen weight / 100gm body weight of rat in control and treated group on 8th day in cotton pellet granuloma model

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Group</th>
<th>Spleen weight in gm / 100 gm body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle control (Acacia 1%)</td>
<td>0.706 ± 0.025</td>
</tr>
<tr>
<td>2</td>
<td>Dexamethasone (0.5 mg/kg)</td>
<td>0.344 ± 0.016**</td>
</tr>
<tr>
<td>3</td>
<td>Herbal formulation 150 mg/kg</td>
<td>0.581 ± 0.013**</td>
</tr>
<tr>
<td>4</td>
<td>Herbal formulation 200 mg/kg</td>
<td>0.468 ± 0.015**</td>
</tr>
<tr>
<td>5</td>
<td>Herbal formulation 250 mg/kg</td>
<td>0.400 ± 0.008**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (standard error of the mean) of 6 determinants. **P≤0.01, compared to Vehicle control.

Table 6: Erythrocyte sedimentation rate of different groups and percentage of inhibition in rate of sedimentation by different treatment groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ESR (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>2.55 ± 0.086</td>
</tr>
<tr>
<td>Dexamethasone (0.5 mg/kg)</td>
<td>1.24 ± 0.013**</td>
</tr>
<tr>
<td>Herbal formulation 150 mg/kg</td>
<td>1.52 ± 0.238**</td>
</tr>
<tr>
<td>Herbal formulation 200 mg/kg</td>
<td>1.78 ± 0.024**</td>
</tr>
<tr>
<td>Herbal formulation 250 mg/kg</td>
<td>1.6 ± 0.015**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (standard error of the mean) of 6 determinants. **P≤0.01, compared to Vehicle control.

Table 7: Weight of the ear edema (mg) and percent of inhibition by different treated groups

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Ear edema(mg)</th>
<th>(%) inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control (10 ml/kg)</td>
<td>4.5 ± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac sodium (50 mg/kg)</td>
<td>2.66 ± 0.1**</td>
<td>42.22</td>
</tr>
<tr>
<td>Herbal formulation (300 mg/kg)</td>
<td>3.5 ± 0.1**</td>
<td>22.22</td>
</tr>
<tr>
<td>Herbal formulation (400 mg/kg)</td>
<td>3.31 ± 0.1**</td>
<td>26.66</td>
</tr>
<tr>
<td>Herbal formulation (500 mg/kg)</td>
<td>2.46 ± 0.1**</td>
<td>44.44</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (standard error of the mean) of 6 determinants. **P≤0.01, compared to Vehicle control.