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## ANTI-INFLAMMATORY ACTIVITY OF *CAMELLIA SINENSIS* BY USING COTTON PELLET GRANULOMA IN RAT

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### ABSTRACT

*Camellia sinensis* is the ethno medicinal plant widely used traditionally for the treatment of many inflammatory diseases because of plenty of source of flavonoid, saponin and terpenes. The present study has been focused to assess the anti-inflammatory activity of *Camellia sinensis* by using cotton pellet granuloma in rat for the better therapeutic activity. The aqueous extract of above plant was administered orally at a dose of 400 mg/kg and 800 mg/kg body weight for 7 days after implantation of sterilized cotton pellet in axilla and groin region of the rat. A significant ( $P \leq 0.01$ ) reduction of wet weight and dry weight of cotton pellet at 31.77%, 45.10% and 19.51%, 48.15% was observed. A significant reduction ( $P \leq 0.01$ ) in total leukocyte number, ESR and spleen weight was found at the same dose. A better activity was observed at 800mg/kg body weight.

**KEYWORDS:** Cotton pellet, Flavonoids, ESR, Inflammation.

### INTRODUCTION

Since time immemorial, indigenous plants have been a major source of medicine. In folk medicine, they are used, in single or in combined forms for treating different types of inflammatory and arthritic conditions. Prolonged administrations of steroidal and nonsteroidal anti-inflammatory drugs are known to be associated for their adverse effects. Herbal drugs have lesser side effects and are largely replaced by synthetic drugs. 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<sup>1</sup>.

Inflammation is a common clinical condition<sup>2-5</sup> 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.<sup>6</sup> Inflammatory signals greatly enhance COX-2 expression, particularly in inflammatory cells such as monocytes, macrophages, endothelial cells, and fibroblasts.<sup>7</sup> Numerous trials to develop promising anti-inflammatory drugs currently target the suppression of PGE2 production and COX-2 expression.<sup>8</sup> 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, Ibuprofen etc.), Disease-modifying anti-inflammatory drugs (DMARDs) (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.<sup>9-10</sup> 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.<sup>10-13</sup> 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.

Our study has been focused on to evaluate the anti-inflammatory activity of aqueous extract of *Camellia sinensis* (Green Tea). The anti-inflammatory properties have, however, not been studied so far of corresponding plant.

## **MATERIALS AND METHODS**

### *Collection of plant materials:*

In the present study mature plant material of *Camellia sinensis* purchased from Jillimudi Apparao and Sons Ayurvedic Shop, Visakhapatnam, India. They were identified and

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authenticated by Dr. M. Veraiah, Retd Professor in Botany, Andhra University, Visakhapatnam. After authentication, the Plant materials were powdered to 40 # mesh particle size and subjected to standardization with the different parameters.

*Preparation of extracts and Phytochemical Screening:*

*Preparation of aqueous extract of Camellia sinensis:*

The leaves of *Camellia sinensis* were purchased from local market of Visakhapatnam. The dried leaves were powdered and about 100gm of coarse powder was subjected to hot maceration with water for 24 hrs with frequent agitation. After filtration, the filtrate was concentrated to dryness under reduced pressure and controlled temperature 50°-60° C.

*Animals:*

Male albino rats of Wistar strain weighing 160-180 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/CPCSEA dated 08-04-2011) of Yalamarty Pharmacy College.

*Acute oral toxicity study:*

Acute toxicity test were performed on rat of either sex weighing 160-180 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 (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The aqueous extract at different doses up to 4000 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 observed at 4000 mg/kg. So 400 mg/kg and another higher (800mg/kg body weight) doses was selected as the therapeutic dose.

*Induction of Inflammation by Cotton pellet Granuloma method<sup>14</sup>*

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 extract for consecutive 7 days, once per day. They were sacrificed on day 8<sup>th</sup> by cervical dislocation and the pellets were removed and immediately the wet weight was taken, freed from extraneous tissue and dried at 60<sup>o</sup>C for 24 hrs. The percentage inhibition of the wet weight and dry weight of the granuloma were calculated and compared.

$$\text{Percentage inhibition (\%)} = \frac{\text{Control - Treated}}{\text{Control}} \times 100$$

#### *Experimental set up*

##### *Experimental Design for Cotton pellet granuloma model*

Group-I : Vehicle control received distilled water (10 ml/kg).

Group-II : Animals treated with Dexamethasone (0.5 mg/kg).

Group-III : Animals treated with *Camellia sinensis* (400mg/kg).

Group-IV : Animals treated with *Camellia sinensis* (800mg/kg).

##### *Biochemical Assays and spleen weight*

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. <sup>15</sup> 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. <sup>16</sup> Spleen weight was measured for individual animals of different groups.

##### *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  $\pm$  SEM for six animals in each group.

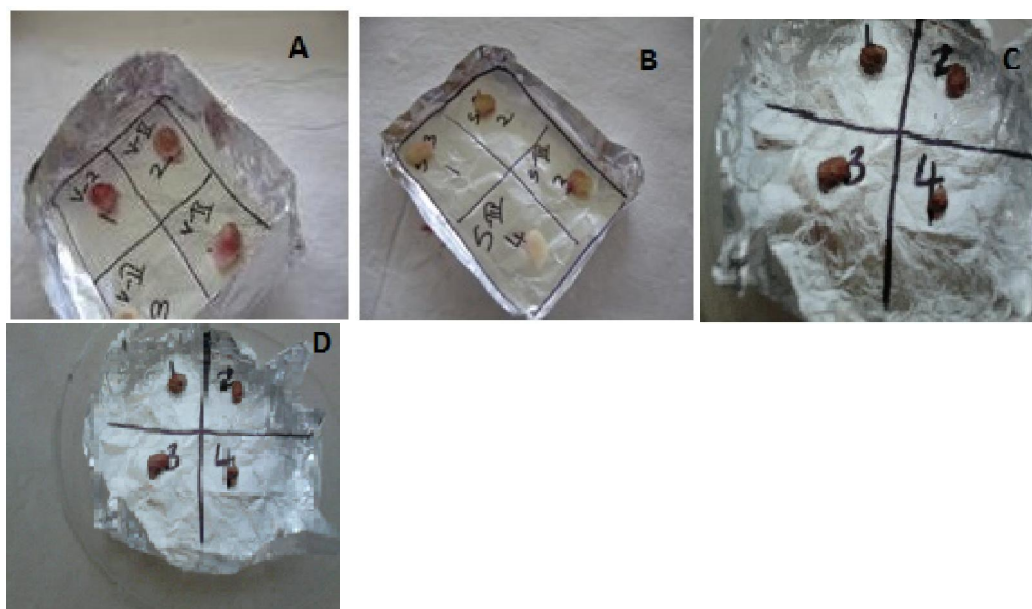
## **RESULTS**

### *Phytochemical analysis*

Preliminary phytochemical screening showed the presence of Phenolic compounds, Tannins phytosterol, flavonoids and Cardiac glycosides in aqueous extracts of *Camellia sinensis*.

#### *Cotton pellet induced granuloma formation*

The aqueous extract at different doses and standard drug was evaluated by cotton pellet induced granuloma formation to understand its potential in sub-acute inflammatory phase. Fig.1 and Table-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 86.16% and 78.85% respectively. The aqueous extract at different dose (400 and 800mg/kg body weight) showed significant ( $P \leq 0.01$ ) reduction of wet weight and dry weight of cotton pellet at 31.77%, 45.10% and 19.51%, 48.15% respectively.



**Fig.1:** Cotton pellet granuloma induction in rat, A- Vehicle control (maximum granuloma), B- Standard Dexamethasone (0.5mg/kg), C-Herbal Extract (400mg/kg), D- Herbal Extract (800mg/kg) showing inhibition of the granuloma.

**Table 1:** Effect of Herbal Extract on wet and dry weight of cotton pellets

Group	mean wet weight(mg)	% of Inhibition	mean dry weight(mg)	% of Inhibition
Vehicle control	89.72 ± 2.17	-	22.80 ± 0.62	-
Dexamethasone (0.5 mg/kg)	12.41 ± 0.32**	86.16	4.82 ± 0.75**	78.85
Aqueous extract 400 mg/kg	61.21 ± 2.15**	31.77	18.35 ± 0.67**	19.51
Aqueous extract 800 mg/kg	49.25 ± 1.83**	45.10	11.82 ± 0.42**	48.15

Values are expressed as mean  $\pm$  SEM (standard error of the mean) of 6 determinants. \*\* $P \leq 0.01$ , compared to vehicle control.

#### *Effect of Herbal Extract on Total WBC count*

In vehicle control maximum increase in WBC was found as compare to normal (Table-2). The standard drug and different dose of test formulation significantly ( $P \leq 0.01$ ) reduced the migration of WBC as compare to vehicle control.

**Table 2:** Effect of Herbal Extract on total WBC count

Group	WBC no. (Day-0)	WBC no. (Day-8)
Vehicle control (Acacia 1%)	9100 $\pm$ 194.4	16400 $\pm$ 943.5
Dexamethasone (0.5 mg/kg)	7580 $\pm$ 127.64**	7820 $\pm$ 31.69**
Aqueous extract 400 mg/kg	7860 $\pm$ 106.87**	8620 $\pm$ 583.43**
Aqueous extract 800 mg/kg	7260 $\pm$ 98.70**	8180 $\pm$ 78.83**

Values are given in mean  $\pm$  SEM (n=6), \*\* $P \leq 0.01$ , compared to vehicle control

#### *Spleen weight / 100g body weight*

During inflammation the enlargement of spleen was found in vehicle control (Table-3). The Standard drug Dexamethasone (0.5 mg/kg) and Aqueous extract at different doses (400 and 800mg/kg) produced significant ( $P \leq 0.01$ ) suppression of the spleen weight.

#### *Erythrocyte sedimentation rate (ESR)*

The oral administration of Herbal Extract at the dose of 400 and 800 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-4).

**Table 3:** Average spleen weight / 100gm body weight of rat in control and treated group on 8<sup>th</sup> day in cotton pellet granuloma model

S.No.	Group	Spleen weight in gm / 100 gm body weight
1	Vehicle control (Acacia 1%)	0.706 $\pm$ 0.025
2	Dexamethasone (0.5 mg/kg)	0.344 $\pm$ 0.016**
3	Aqueous extract 400 mg/kg	0.648 $\pm$ 0.013**
4	Aqueous extract 800 mg/kg	0.568 $\pm$ 0.015**

Values are expressed as mean  $\pm$  SEM (standard error of the mean) of 6 determinants.  $**P \leq 0.01$ , compared to Vehicle control.

**Table 4:** Erythrocyte sedimentation rate of different groups and percentage of inhibition in rate of sedimentation by different treatment groups.

Groups	ESR (mm)
Vehicle control	2.55 $\pm$ 0.086
Dexamethasone (0.5 mg/kg)	1.24 $\pm$ 0.013**
Aqueous extract 400 mg/kg	1.46 $\pm$ 0.238**
Aqueous extract 800 mg/kg	1.38 $\pm$ 0.024**

Values are expressed as mean  $\pm$  SEM (standard error of the mean) of 6 determinants.  $**P \leq 0.01$ , compared to Vehicle control.

## DISCUSSION AND CONCLUSION

There are a multitude of approaches for identifying new pharmaceuticals. The treatment of inflammatory conditions with plants is widely reported.<sup>17</sup> 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).<sup>18</sup>

Daily administration of aqueous Herbal Extract of *Camellia sinensis* (test substances) at 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 Extract at dose (400 and 800mg/kg body weight) showing significant ( $P \leq 0.01$ ) reduction of wet weight and dry weight of cotton pellet at 31.77%, 45.10% and 19.51%, 48.15% respectively (Table-1 and Fig.1). The standard drug dexamethasone produces maximum activity by inhibiting the wet weight and dry weight of cotton pellet 86.16% and 78.85% respectively.

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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-2). In our study it was found that the administration of herbal Extract at dose 400 and 800 mg/kg body weight leads to inhibition of leukocyte migration. The activity may be due to presence of flavonoid, saponin and terpenes. 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 herbal extract and standard drug dexamethasone.

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 repellent surface charges on red cells and cause them to aggregate into rouleaux and hence the sedimentation rate increases.<sup>19</sup> 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.

In conclusion aqueous herbal Extract of *Camellia sinensis* revealed significant anti-inflammatory activity in both cotton pellet granuloma in rat at dose 400 and 800 mg/kg body weight. Whereas 800 mg/kg proved to be better therapeutic effect.

#### **Conflict of interest statement**

The authors declare that they have no conflict of interest.

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