

## EVALUATION OF IN VITRO AND IN VIVO ANTI- INFLAMMATORY ACTIVITY OF *GARCINIA COMBOGIA* L.

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### ABSTRACT

Objective: *Garcinia combogia* is a plant grows through India and southeast Asia. The fruit of the plant is a commonly used diet ingredient in India. The major objective of the study was to evaluate the in vitro and in vivo anti-inflammatory activity of the ethanolic extract of *Garcinia combogia*.

Methods: In vitro anti-inflammatory activity was evaluated using hRBC membrane stabilization method. The extract possessed moderate membrane stabilization activity for all the concentrations tested. Diclofenac was used as the standard. To study the anti-inflammatory activity carrageenan induced paw oedema model was used. Ibuprofen 100mg/kg was used as the standard. Doses of 200 mg/kg and 400 mg/kg were tested.

Results: In hRBC membrane stabilization method the extract showed moderate in vitro anti-inflammatory activity. Significant ( $P < 0.05$ ) reduction in the paw volume of the standard and test treated group compared to the control was observed when measured at the third hour.

Conclusion: It can conclude that the ethanolic extract of *Garcinia combogia* posses significant in vivo anti-inflammatory activity, and moderate in vitro anti-inflammtory activity as per the present study.

### INTRODUCTION

Medicinal plant sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of India [1]. Inflammation and pain has become the focus of global scientific research because of its implication in virtually all human and animal diseases[2]. *Garcinia combogia* is a common plant cultivated throughout India. It belongs to the family *Guttifererae*. Various activities of the plants like Diuretic Activity[3] anti-ulcerogenic potency[4], and Attenuation of colitis injury [5] have been reported. Moreover it is the source for a natural diet ingredient, which is currently a range in America, Japan, Europe and other western countries[3]. In the present study the effect of the plant in acute inflammation has been evaluated using carrageenan induced paw oedema model using wistar albino rats. The free radical scavenging potential of the ethanolic extract of the plant was evaluated by reductive ability assay.

### MATERIALS AND METHODS

#### Plant material

The leaves of *Garcinia combogia* had been collected from Malappuram dt, Kerala, India during the month of November 2012 and were dried under shade. The coarsely powdered shade dried leaves of *Garcinia combogia* was charged in an aspirator bottle and extracted with ethanol by cold maceration method for 3 days. After decantation and filtering, nearly 80% of the solvent was removed by distillation over boiling water bath and the remaining under reduced pressure. The extracts so obtained, were further dried in vacuum desicator and the extract so obtained was used for further studies. The extracts were dissolved in distilled water using 1% CMC as suspending agent.

#### In vitro Anti-inflammatory activity

The HRBC membrane stabilization method was used to study the in-vitro anti-inflammatory activity. Blood was collected under aseptic condition from a healthy male volunteer. Blood was mixed with sterilised Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). It was then centrifuged at 3000 rpm and packed cell were washed and suspension was made with isosaline (0.85%, pH 7.2). Varying concentrations of the drug was mixed with 1 ml of phosphate buffer (0.15M, pH 7.4), and 2ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension. Diclofenac was used as standard. 2 ml of

distilled water was used in the control. The mixtures were incubated at 37°C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated spectrophotometrically at 560 nm. The percentage of haemolysis was calculated using the formula % Haemolysis = O.D. of drug treated sample \*100 / O.D. of control. [6]

#### In vivo aanti inflammatory study

Albino Wister rats of either sex weighing 200-250 g, were used for the studies. The animals were procured from small animal breeding unit, Mannuthy, Thrissur. The whole procedure was approved by Institutional animal ethics committee. Animals were housed under standard conditions of temperature (23±1°C), 12 hours light/dark cycle and fed with standard pellet diet and water *ad libitum*. The animals were deprived of food for 24 hours before experimentation. But allowed free access to water. Rats of either sex were divided into four groups of six animals each.

Oedema was induced by the sub-planter injection of carageenan in to the right hind paw of three groups of 6 animals each. In acute toxicity study, the single oral dose 4000mg/kg was found to be safe for the animal. The two test groups received the alcoholic extract of *Garcinia combogia* 400mg/kg and 200mg/kg respectively. The standard group received ibuprofen(100mg/kg) and the control animals received the vehicle(1% w/v CMC suspension) only. Paw volumes were measured using Plethysmometer at intervals. The drug sample were administered orally by suspending in 1%w/v CMC suspension using oral tubes.[7]

### RESULTS AND DISCUSSION

Table:1 shows absorbance values and calculated percentage protection for various concentrations of the extract and for 50 µg/ml of diclofenac. There is increase in percentage protection for all the concentrations tested, indicating the hRBC membrane stabilization potency of the extract, even though it was much less than that of the standard drug diclofenac.

The paw volume at different time rates (1hr, 2hr and 3hr) after the administration of carageenan was determined. The paw volume of the animals in the standard drug treated and extract treated groups were found to be less than that of the control group. At the first hour, the paw volume was found to be 0.230±0.040, for control group 0.17±0.03 and 0.192±0.046, 0.166±0.047 respectively for drug treated and extract treated groups.

**Table 1: In vitro anti-inflammatory activity of ethanolic extract of *Garcinia combogia*.**

Concentration ( $\mu\text{g/ml}$ )	Absorbance	Percentage protection
Control	0.51 $\pm$ 0.012	.....
100	0.24 $\pm$ 0.004	52.6 $\pm$ 0.003
200	0.21 $\pm$ 0.004	57.6 $\pm$ 0.002
300	0.19 $\pm$ 0.003	60.5 $\pm$ 0.002
400	0.17 $\pm$ 0.001	65.3 $\pm$ 0.001
500	0.15 $\pm$ 0.005	69.9 $\pm$ 0.005
Diclofenac(50 $\mu\text{g/ml}$ )	0.13 $\pm$ 0.002	73.9 $\pm$ 0.002

Values are, mean $\pm$  SEM, of three parallel measurements

**Table 2: Effects of Ethanolic extract of *Garcinia combogia* on paw oedema.-**

Treatment/ Particulars	Paw volume at different time intervals after the administration of Carageenan.		
	1 hr	2 hr	3 hr
Control	0.236 $\pm$ 0.04	0.258 $\pm$ 0.047	0.285 $\pm$ 0.12
Ibuprofen (100mg/kg)	0.17 $\pm$ 0.03	0.155 $\pm$ 0.032	0.132 $\pm$ 0.037***
Ethanolic extract of <i>Garcinia combogia</i> (200mg/kg)	0.192 $\pm$ 0.046	0.183 $\pm$ 0.046	0.167 $\pm$ 0.041*
Ethanolic extract of <i>Garcinia combogia</i> (400mg/kg)	0.166 $\pm$ 0.047	0.153 $\pm$ 0.038	0.138 $\pm$ 0.041*

Values are, mean $\pm$  SEM, n =6, \*P < 0.05 compared with control (One way ANOVA followed by Dunnet's test)

There was increase in the paw volume of the control group of animals when measured at the second hour. It was 0.258 $\pm$ 0.047. While the paw volume of animals in the standard and test treated groups were found to decrease. It was 0.155 $\pm$ 0.032 and 0.183 $\pm$ 0.046, 0.153 $\pm$ 0.038 respectively.

Significant (P < 0.05) reduction in the paw volume of the standard and test treated group compared to the control was observed when measured at the third hour. For control the paw volume was 0.285 $\pm$ 0.12. For ibuprofen treated group the value was 0.132 $\pm$ 0.037 and 0.167 $\pm$ 0.041, 0.133 $\pm$ 0.041 for the extract treated groups

#### CONCLUSION

In recent years there has been growing interest in therapeutic use of natural products, especially those derived from plants. *Garcinia combogia* is very common dietary ingredient in many parts of India. The present study was to evaluate the in vitro and in vivo anti-inflammatory potency of the leaves of *Garcinia combogia*. From the results it can be concluded that the leaves of the plant *Garcinia combogia* possess appreciable anti-inflammatory activity especially against carrageenan induced paw oedema in rats. It also possessed moderate in vitro anti-inflammatory action in hRBC membrane stabilization method. Only a few works have been done on this plant as per literature review. This work suggests more studies which involves the isolation of the active principle and the development of new therapeutic agents from this plant.

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