

## EVALUATION OF IN VITRO ANTI ARTHRITIS ACTIVITY OF GARCINIA CAMBOGIA FRUIT AGAINST THE DENATURATION OF ALBUMIN

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### ABSTRACT

To evaluate the anti-arthritis activity in vitro in chloroform extract of Garcinia cambogia fruit against albumin denaturation. In controlled experimental condition egg albumin was incubated in with different concentration of chloroform extract and subjected to determination of absorbance to evaluate the percentage of protein inhibition for Anti-arthritis activity using Diclofenac sodium as the standard drug. Dose dependent increase in the percentage inhibition was found as a result. Inhibition of albumin denaturation was observed for all the concentration of the chloroform extract of Garcinia Cambogia fruit. From the current study it can be concluded that Garcinia cambogia fruit possessed marked In vitro Anti-arthritis activity effect against denaturation of albumin. The effect was due to xanthone present as one of the phyto chemical components in of Garcinia cambogia fruit.

## INTRODUCTION

Rheumatoid Arthritis is auto immune diseases <sup>[1]</sup> in which there are a joint inflammation synovial proliferation and destruction of articular cartilage. Antigen antibody immune complexes consist of IgM involved in activation and cytokine release <sup>[2]</sup> which are chemotactic for neutrophils. Inflammation of cells produces lysosomal enzymes involved in cartilage damage and bone erosion. Arthritis inflammation and tissue injury is due oxidative stress has been implicated in immune disease <sup>[3]</sup>. In Rheumatoid arthritis disease the production of auto antigens may be due to protein denaturation. <sup>[4]</sup>

Production of auto antigens are controlled in certain arthritic diseases and tissue proteins denaturation inhibition and lysis of membrane in rheumatic disease leads to anti-arthritic activity or anti-inflammatory activity <sup>[5]</sup>. Presently many non-steroidal and immunosuppressive drugs are used to control inflammatory symptoms and pain they are associated with certain undesirable side effects. With these difficulties, the field of arthritis research has progressed exponentially towards herbal therapies <sup>[6]</sup> that have been considered safe and effective in elevating chronic pain associated with arthritis.

Herbal medicine contains a large variety of different compound, several of which have biological activity. There is a great demand for herbal medicine in the developed as well as developing countries like India, <sup>[7]</sup> because of their wide biological activities higher safety of margin perceived <sup>[8]</sup> efficacy, low incidence serious adverse effects and lesser cost. Herbal medicine deals with plants and plant extract in treating disease. Garcinia cambogia fruit related species of guttiferace family. Garcinia cambogia (Garcinia gummi gutta) commonly known as kodampuli or Malabar tamarind. <sup>[9]</sup>. Garcinia cambogia is a tiny, sweetish, exotic fruit found in South india and south east asia .The fruit is small in size and looks like yellow or reddish pumpkin or purple pumpkin.

The Garcinia cambogia fruit has been customarily used in preparation of food and cooking with distinctive taste .The bark is given as decoction, in use to treat rheumatism <sup>[10]</sup> and gastrointestinal disorders. The phytochemical test was performed showed the presence of alkaloids, tannins, phenolic flavonoids, carbohydrates and proteins <sup>[11]</sup>. Benzophenones <sup>[12]</sup> are the one of the important secondary metabolite in Garcinia gummi gutta followed by xanthenes and bioflavonoid. Phytochemical test confirmed

the presence of xanthenes as one of the main constituent present in guttiferae family. Naturally occurring xanthone seem to possess remarkable pharmacological and biological activities ant arthritis, antimicrobial,<sup>[13]</sup> anti-inflammatory, analgesic, anti-viral. Denaturation of protein is one of the main causes of inflammation and rheumatoid arthritis<sup>[14, 15]</sup>.

Upon literature survey no reports are investigated for in-vitro anti-arthritis activity Garcinia cambogia fruit. Hence the current study was done to evaluate the possible in vitro Anti-arthritis activity in chloroform extract of Garcinia cambogia fruit against albumin denaturation

## **MATERIALS AND METHODS**

### **Collection of fruits**

The Garcinia cambogia fruit was collected in the month of July from Thrisur Kerala State India. The Garcinia cambogia fruit and plant material was recognized and authenticated by GKVK, Bangalore. The voucher specimen bearing the number Reference NO. UASB-4551 is acknowledged in the herbarium file of the department.

Cleaning of fruits were washed with water and cut into slices using stainless steel knife. The pericarp of the Garcinia cambogia

species was removed and the seeds were separated. The pericarp was shade dried for a period of 60 days. Pericarp was dried it was powdered coarsely in a mixer grinder.

### **Drugs and chemicals.**

Diclofenac sodium was procured from Sigma Aldrich Mumbai India. All other chemicals were of analytical grade obtained commercially. Double distilled water from all- glass still was used throughout the study.

### **Preparation of the crude extract:**

The pericarp powder was stored in an airtight container and used for further extraction about 100gm of powder of pericarp was extracted using 1000mL chloroform for a period of 6 hours separately using a Soxhlet apparatus. The chloroform crude extract were evaporated to dryness in a rotatory vacuum evaporator under reduced pressure and were stored at 4°C for further use.

### **Phytochemical test for Xanthone**

One ml Garcinia cambogia extract was added with 1ml of alcoholic ferric chloride. The formation of brown colour indicates the presence of xanthenes.

### **Evaluation of in vitro Anti-arthritis activity**

Garcinia cambogia chloroform extract were dissolved in minimum amount of dimethyl

formamide (DMF) and diluted with phosphate buffer saline (PBS (0.2M, pH 6.4) final concentration of DMF in all solutions was less than 2.5%. Test solution (1ml) containing different concentration of Garcinia cambogia chloroform extract in the range of 31.25,62.5,125 µg,250 ,500,1000 µg/ml was mixed in 1ml of mM albumin solution in phosphate buffer saline<sup>[8]</sup> and incubated in BOD incubator for 15 min. The reaction mixture was kept in a water bath for 10 min at 70 °C for denaturation .After cooling ,the absorbance of turbidity was measured at 660nm (SHIMADZU,UV 1800) using dimethyl formamide(DMF) as blank .Diclofenac sodium in the concentration range of (31.25,62.5,125 µg,250 ,500,1000 µg/ml) was used as standard drug and treated in a similar manner for determination of absorbance. Calculated percentage of inhibition of denaturation from control when drug was not added. Average of triplicate of each experiment was taken. Determination of absorbance standard diclofenac sodium was done in a similar manner. The percentage inhibition of albumin denaturation was calculated by using the following formula:

%inhibition =  $100 \times (V_t / V_c - 1)$  where  $V_t$  = absorbance of test sample,  $V_c$ = absorbance of control.

## EXPERIMENTAL RESULTS

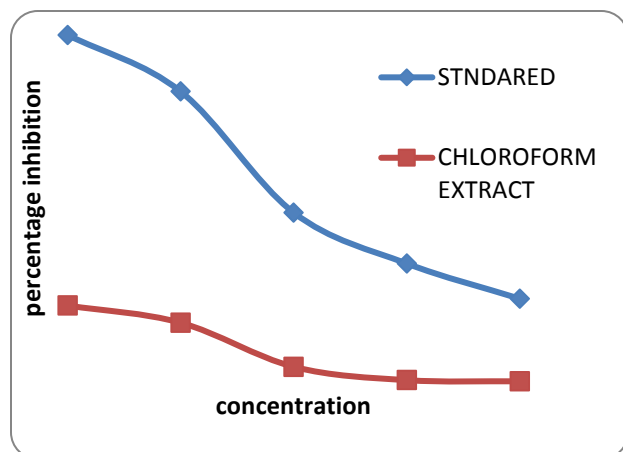
In the current study investigation, the In Vitro Anti-arthritic activity effect of chloroform extract of Garcinia cambogia fruit was determined against egg albumin denaturation. The summary of the results given in Table.1. The present findings exhibited concentration dependent inhibition of albumin (protein) denaturation by Garcinia cambogia chloroform fruit extract throughout the concentration range of 31.25 to 1000 µg/ml. Diclofenac sodium at the concentration range of 31.25 to 1000 µg/ml was used as reference drug .Which also exhibited concentration dependent inhibition of protein denaturation results are summarized in Table 2. Graphical representation of percentage inhibition against various concentrations of standard and Garcinia cambogia fruit extract are shown in Figure.1.

**Table1. Effect of Garcinia Cambogia on Albumin denaturation**

Concentration(µg/mL)	%inhibition
Control	
62.5	0.78
125	1.07
250	4.28
500	14.91
1000	19.04

**Table2. Effect of Diclofenac sodium on Albumin denaturation**

Concentration( $\mu\text{g/mL}$ )	%inhibition
Control	
62.5	20.68
125	29.17
250	41.44
500	70.61
1000	84.24

**Figure 2. Percentage of inhibition against different concentration of Standard and Garcinia cambogia.**

## DISCUSSION

Problems are encountered in using animals for pharmacological experimental research, such as Animal ethical committee issues and difficult to rationalize [16]. Hence, in the existing study the denaturation of protein bioassay was selected for in vitro assessment of Anti-arthritis activity in chloroform extract

of Garcinia cambogia fruit. Inflammation and arthritic disease are caused due to denaturation of tissue proteins. Auto antigens are produced in inflammatory arthritic disease may be due to in vivo protein denaturation [17], therefore would be worth file for rheumatoid arthritis and drug development. Protein denaturation means loses of biological properties of protein molecule [18]. As a part of examination the method of anti-arthritis and anti-inflammatory activity of the plant to inhibit protein denaturation was studied. Denaturation of protein is a cause of inflammation [19] and Rheumatoid arthritis. Thus protection against protein denaturation would be main mechanism against inflammation and Rheumatoid arthritis.

The test sample increased in absorbance with respect to control indicated protein stabilization i.e. inhibition of heat induced protein (albumin) was investigated. It has been reported that one of the features of several NSAID is their ability to stabilize (prevent denaturation) heat treated albumin at physiological [20] pH6.2-6.5.

So analysis of our study reveals that the chloroform extract of the Garcinia cambogia fruit exhibits strong invitro anti-arthritis activity against albumin denaturation.

Therefore anti-inflammatory drugs produced dose dependent inhibition of albumin denaturation.

## CONCLUSION

Due to global alarm against side effects caused by allopathic drugs there is trend of returning back to nature. Large number of herbal species has been traditionally or as folk medicines <sup>[21]</sup> against inflammatory disorders.

The current research is to explore the usage of *Garcinia cambogia* fruit against arthritis and inflammation experimentally representing significant activity. Hence *Garcinia cambogia* fruit can be used as a potent natural anti-arthritic activity and anti-inflammatory agent. The anti-arthritic and anti-inflammatory activity might be due to xanthenes present as one of the secondary metabolite. Further isolation of xanthone is necessary to ascertain mechanism behind Anti-arthritis activity and anti-inflammatory activity.

The exploration is based on the need for anti-arthritis and anti-inflammatory agents from natural herbal sources with potent activity and fewer side effects. The source of new Anti-arthritis and Anti-inflammatory drugs

from herbal medicine is a fruitful and follows logical research strategy in evaluation.

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