

## RESEARCH ARTICLE

### Study of immunomodulatory activity of aqueous extract of *Carica papaya* in Wistar rats

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#### ABSTRACT

**Background:** Various parts of *Carica papaya* (CP) Linn. have been traditionally used as ethnomedicine for a number of disorders including cancer. There have been anecdotes of patients with advanced cancers achieving remission following consumption of tea extract made from CP leaves. However, the precise cellular mechanism of action of CP tea extracts remains unclear. **Aims and Objectives:** The aim of this study is to study the immunomodulatory activity of aqueous extract of CP in Wistar rats. **Materials and Methods:** The effect of test extract and standard drug on the delayed type hypersensitivity response in Wistar rats using sheep red blood cells as antigen, administration of aqueous extract of CP at the dose of 400 and 800 mg/kg and levamisole 50 mg/kg, treatments which were given orally for 14 days. **Results:** We observed that aqueous extract of CP showed a significant increase in paw edema compared to control group. The standard drug levamisole showed the maximum increase in paw volume compared to all groups. **Conclusion:** The effect of this extract was comparable to the standard drug levamisole. CP leaf extract may potentially provide the means for the treatment and prevention of selected human diseases such as cancer, various allergic disorders, and may also serve as immunoadjuvant for vaccine therapy.


**KEY WORDS:** Carica Papaya, Immunomodulatory, Delayed Type Hypersensitivity

#### INTRODUCTION

Immunology as a science probably began with the observations by Metchnikoff in 1882 that starfish when pierced by a foreign object (a rose thorn) responded by coating it with cells (later identified as phagocytes).<sup>[1,2]</sup> Immunology, the study of the way in which the body defends itself against invading organisms or internal invaders (tumors) has developed

rapidly over the last 40 years, and particularly during the last 10 years with the advent of molecular techniques. It is now a rapidly moving field that is contributing critical tools for research, diagnosis, and therapeutics for the treatment of a wide range of human diseases.<sup>[3-5]</sup>

*Carica papaya* (CP) Linn. commonly called as paw-paw and it belongs to the family Caricaceae. Papaya possesses excellent medicinal properties for treatment of different ailments. The different parts of the CP plant including leaves, seeds, latex, and fruit exhibited to have medicinal value.<sup>[6,7]</sup> The stem, leaf, and fruit of papaya contain plenty of latex. The latex from unripe papaya fruit contains enzymes papain and chymopapain; other components include a mixture of cysteine endopeptidases, chitinases, and an inhibitor of serine protease. Phytochemical analysis of CP leaf extract revealed

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the presence of alkaloids, glycosides, flavonoids, saponins, tannins, phenols, and steroids. CP possesses different activities such as antioxidant and free radical scavenging activity, anticancer activity, anti-inflammatory activity, anti-diabetic activity, wound healing activity and antifertility effects.<sup>[8-11]</sup> Thus, CP acts as a multi-faceted plant. It is also imperative to identify the mechanism of the plant compounds and study the active principle of the extract. Thus, we should include the papaya in our diet as fruit salads, fruit juice, leaf extract, decoction prepared through papaya leaves, etc. However, including papaya seeds in any of the form should be avoided for young men and pregnant women, since it possesses antifertility effects that were demonstrated well in animal models.<sup>[12,13]</sup> Likewise, the aqueous extract demonstrated antitumor activity and immunomodulatory activity in tumor cell lines and it proved upregulation of immunomodulatory genes by microarray studies.<sup>[12-14]</sup> Various parts of CP Linn. have been traditionally used as ethnomedicine for a number of disorders, including cancer. There have been anecdotes of patients with advanced cancers achieving remission following consumption of tea extract made from CP leaves. However, the precise cellular mechanism of action of CP tea extracts remains unclear. The aim of this study is to study of immunomodulatory activity of aqueous extract of CP in Wistar rats.

## MATERIALS AND METHODS

The sheep red blood cells (SRBCs) were procured from Veterinary College (KVAFSU), Hebbal, Bengaluru, Karnataka, India. The levamisole (Cipla Limited - India) was purchased from Local Pharmacy, Bengaluru, Karnataka, India. The leaves of CP belonging to the family Caricaceae were collected from plants sourced from rural Bengaluru, Karnataka, India. The leaves were air dried powdered, and the resultant powder was taken for extraction. This plant was identified and authenticated from FRLHT, Yelahanka, Bengaluru, Karnataka, India.

The fresh water extract was prepared by suspending 100 g of the finely blended dried leaves in 200 ml of distilled water. This was then agitated using the blender after which another 300 ml of distilled water was added. The mixture was stirred every 3 min for 30 min and then allowed to stand for 24 h. The extract was then decanted and filtered through a Whatman filter paper. The filtrate was then concentrated with the rotary evaporator at 45°C. This extract was then stored in the refrigerator at 4°C until use. The yield was 12 g.

### Acute Toxicity Studies

The CP extract was tested for acute toxicity studies as per procedure given in OECD<sup>[15,16]</sup> guidelines. Rats ( $n = 6$ ) were starved for overnight and fed orally with the following doses of extracts - 50, 100, 200, 400, 800 and 1600 mg/kg.

Animals were observed for next 14 days for behavioral changes and mortality. The 400 mg/kg and 800 mg/kg dosages were selected for this study. The experiment was carried out by using albino Wistar rats (weighing between 150 and 250 g), which were procured from central animal house of the institute. They were maintained under standard laboratory conditions. They were provided with a standard diet supplied by Pranav Agro Industries Ltd., India and water ad libitum at the central animal house.

The experimental protocol has been approved by institutional animal ethics committee, 24 rats were divided into four groups of six animals each. Group I: Control, Group II: CP aqueous extract - 400 mg/kg/day by oral route for 14 days, Group III: CP aqueous extract - 800 mg/kg/day by oral route for 14 days, Group IV: Standard-Levamisole - 50 mg/kg/day by oral route for 14 days. The parameter assessed was delayed type hypersensitivity (DTH) response.

## RESULTS

The effect of test extract and standard drug on the DTH response in Wistar rats using SRBCs as antigen, administration of aqueous extract of CP at the dose of 400 mg/kg and 800 mg/kg and levamisole 50 mg/kg treatments which were given orally for 14 days showed significant increase in paw edema compared to control group. The standard drug levamisole showed the maximum increase in paw volume compared to all groups. The results are shown in Table 1 and Figure 1.

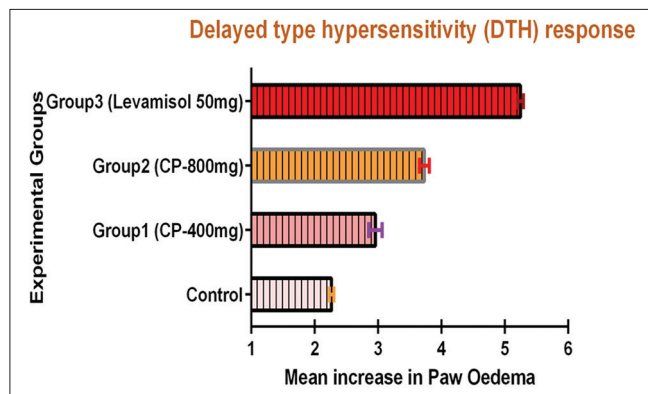
## DISCUSSION

The results of this study revealed that the aqueous extract of leaves of CP generally showed Immunostimulatory effect on the cell-mediated immunity in Wistar rats. It is possible that

**Table 1: DTH response (increase in paw edema) in Group I-IV**

Group	Treatment	Dose	DTH response, increase in paw edema (mm), mean±SEM (n=6)
I	Control		2.26±0.037
II	Test extract I	<i>Carica papaya</i> 400 mg/kg	2.95±0.107*
III	Test extract II	<i>Carica papaya</i> 800 mg/kg	3.73±0.077***
IV	Standard	Levamisole - 50 mg/kg	5.24±0.045***

Dunnet *t*-test and *P* values as significant \*if  $P < 0.05$ , highly significant \*\*if  $P < 0.01$ , and extremely highly significant \*\*\*if  $P < 0.001$  as compared to control. DTH: Delayed type hypersensitivity, SEM: Standard error of mean



**Figure 1:** Delayed-type hypersensitivity response (increase in paw edema) in Group I-IV

the presence of phytoconstituents such as saponins, tannins, cardiac glycosides, and alkaloids (caprine, pseudocarpaine, and dehydrocarpaine I and II) as reported by previous studies<sup>[16,17]</sup> might be responsible for the observed immune stimulatory ability. Further, Studies are required to gain more insights into the possible mechanism of action. The study was undertaken to carry out the immunomodulatory activity of aqueous extract of CP. For the experimental work, the air dried leaves were powdered and were extracted with distilled water and was freeze dried.

The aqueous extract of CP in two different doses 400 and 800 mg/kg was tested for their immunomodulatory action, out of which the higher dose of 800 mg/kg showed statistically significant Immunomodulatory activity. The effect of this extract was comparable to the standards drug levamisole.

CP leaf extract may potentially provide the means for the treatment and prevention of selected human diseases such as cancer, various allergic disorders, and may also serve as immunoadjuvant for vaccine therapy.

## CONCLUSION

The effect of this extract was comparable to the standards drug levamisole. CP leaf extract may potentially provide the means for the treatment and prevention of selected human diseases such as cancer, various allergic disorders, and may also serve as immunoadjuvant for vaccine therapy.

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