



Study of Leucopoiesis Activity of Caricapapaya in Wistar Rats

KEYWORDS

Carica papaya, immunity, aqueous extract.

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ABSTRACT *Background: Literature has shown that ingestion of drugs can alter normal range of hematological parameters. However, such studies dealing with the effects of Carica papaya on hematological parameters are scarce. Hence the present study was planned to assess the effect of aqueous extract of Carica Papaya leaves in wistar rats, on hematological parameters such as total leucocyte count (TLC) & differential leucocyte count (DLC), which serve as markers of immunostimulation. Materials & Methods: The effect of test extract and standard drug on TLC & DLC in wistar rats, administration of aqueous extract of Carica Papaya at the dose of 400mg/Kg and 800mg/Kg and Levamisole 50mg/Kg treatments which were given orally for 14 days. After an overnight fast, the rats were sacrificed and blood collected for analysis. Results: The results showed a dose dependent statistically significant ($p < 0.05$) increased in TLC & DLC. It is therefore concluded that aqueous extract of leaves of Carica papaya may have a stimulatory effect on immune system which may have resulted from the available bioactive phytochemicals.*

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)^{1,2}. 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 treatment of a wide range of human diseases^{3,4,5}.

Carica Papaya Linn, commonly called as paw-paw and it belongs to the family Caricaceae. Papaya possess excellent medicinal properties for treatment of different ailments. The different parts of the Carica Papaya plant including leaves, seeds, latex and fruit exhibited to have medicinal value^{6,7}. The stem, leaf and fruit of papaya contain plenty of latex. The latex from unripe papaya fruit contain enzymes papain and chymopapain; other components include a mixture of cysteine endopeptidases, chitinases and an inhibitor of serine protease. Phytochemical analysis of C.papaya leaf extract revealed the presence of alkaloids, glycosides, flavanoids, saponins, tannins, phenols and steroids. Carica papaya possesses different activities such as antioxidant and free radical scavenging activity, anticancer activity, anti-inflammatory activity, antidiabetic activity, wound healing activity and antifertility effects^{8,9,10,11}. Thus C.papaya 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 possess antifertility effects that was demonstrated well in animal models^{13,14}. Likewise, the aqueous extract demonstrated antitumor activity and immunomodulatory

activity in tumor cell lines and it proved upregulation of immunomodulatory genes by microarray studies^{12,13,14}. Various parts of Carica papaya Linn(CP) 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.

Literature has shown that ingestion of drugs can alter normal range of hematological parameters. However, such studies dealing with the effects of *Carica papaya* on hematological parameters are scarce. Hence the present study was planned to assess the effect of aqueous extract of Carica Papaya leaves in wistar rats, on hematological parameters such as total leucocyte count and differential leucocyte count, which serve as markers of immunostimulation.

Methodology: The Levamisole (Cipla Limited- India) was purchased from local pharmacy, Bangalore. The total leucocyte count and differential leucocyte count tests were performed in SYN diagnostic laboratories, Bangalore. The leaves of Carica papaya belonging to the family Caricaceae were collected from plants sourced from rural Bangalore. The leaves were air dried powdered, and the resultant powder was taken for extraction. This plant was identified and authenticated from FRLHT, Yelahanka, Bangalore

The fresh water extract was prepared by suspending 100grams of the finely blended dried leaves in 200ml of distilled water. This was then agitated using the blender after which another 300ml of distilled water was added. The mixture was stirred every 3 minutes for 30 minutes and then allowed to stand for 24 hours. 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 gm.

Acute Toxicity studies: The CP extract was tested for acute toxicity studies as per procedure given in OECD^{15,16} 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 400mg/Kg and 800mg/Kg dosages were selected for this study. The experiment was carried out by using Albino Wistar rats (weighing between 150 to 250 gms), 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 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: *Carica papaya* aqueous extract-400mg/kg/day by oral route for 14 days, Group-III: *Carica papaya* aqueous extract- 800mg/kg/day by oral route for 14 days, Group-IV: Standard – Levamisole-50mg/kg/day by oral route for 14 days. The parameters assessed were total white blood cell count & differential leucocyte count.

Results:

Total Leukocyte Count

The lower dose of *Carica Papaya* 400 mg/Kg showed mild increase and higher dose of 800 mg/kg aqueous extract of *Carica Papaya* showed a highly significant increase in the mean total leukocyte count, as compared to control. The results were highly significant for the standard drug levamisole 50mg/kg.

Table 02: Differential Leucocyte Count in Group I-IV

Group	Treatment	Dose	Mean % of lymphocyte	Mean % of eosinophils	Mean % of neutrophils
I	Control		28.12 ± 0.1621	4.44±0.159	60.457± 0.532
II	Test extract-1	<i>Carica papaya</i> 400mg/kg	30.16± 0.372*	5.06±0.042**	61.572± 0.524
III	Test extract-2	<i>Carica papaya</i> 400mg/kg	32.16±0.792***	5.59±0.142***	69.062±.227***
IV	Standard	Levamisole 50mg/kg	33.75±0.418***	6.26 ± 0.82***	71.395±.297***

Dunnett 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

Table 01: Total Leucocyte count in Group I-IV

Group	Treatment	Dose	Mean total leukocyte count ± SEM
I	Control		4.83×10 ³ /Cu.mm ± 0.019
II	Test extract-I	<i>Carica papaya</i> 400mg/kg	5.88×10 ³ /Cu.mm ± .038**
III	Test extract-II	<i>Carica papaya</i> 800mg/kg	8.042×10 ³ /Cu.mm ± 0.060***
IV	Standard	Levamisole-50 mg/kg	9.240×10 ³ /Cu.mm± 0.027***

DUNNETT 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, SEM-Standard Error of Mean.

Differential Leukocyte Count

The lower dose of *Carica Papaya* 400 mg/Kg showed mild increase and higher dose of 800 mg/kg aqueous extract of *Carica Papaya* showed a highly significant increase in the differential leukocyte count, as compared to control. The results were highly significant for the standard drug levamisole 50mg/kg.

Discussion:

The use of herbal preparations as a treatment for diseases is very common. In Nigeria, rural communities use herbs as food and traditional medicine. *Carica papaya* extract has been used traditionally by some population as digestive agent, and for skin wound healing. Some of these usages have been studied in vitro and in animal models. Scientific evidence for their effect on hematological parameters is lacking. Reports of plant extract inducing increase white blood cells and lymphocytes have pointed out their ability to stimulate immune system.

The results of present study revealed that the aqueous extract of leaves of *Carica Papaya* generally showed increase in total leucocyte count and differential leucocyte count, which serve as markers of immunostimulation. It is possible that the presence of phytoconstituents like saponins, tannins, cardiac glycosides and alkaloids (caprine, pseudocarpaine and dehydrocarpaine I and II) as reported by previous studies^{16,17} 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 effect of aqueous extract of *Carica Papaya* on total leucocyte count and differential leucocyte count, which serve as markers of immunostimulation. For the experimental work the air dried leaves were powdered and were extracted with distilled water and were freeze dried.

The aqueous extract of *Carica Papaya* in two different doses 400 mg/kg and 800 mg/kg was tested, out of which the higher dose of 800mg/kg showed statistically significant increase in total and differential leucocyte counts. The effect of 800 mg/kg of aqueous extract of *Carica Papaya* were comparable to standard drug Levamisole.

It is therefore concluded that aqueous extract of leaves of *Carica papaya* may have a stimulatory effect on immune system which may have resulted from the available bioactive phytochemicals.

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