A systematic Review on *Carica papaya* and Dengue Infection

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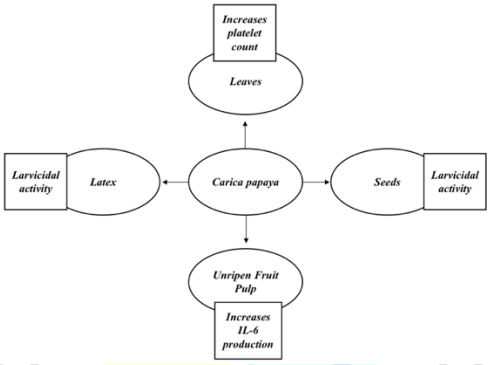
Abstract: Dengue is a terrible viral fever caused by dengue virus that belongs to Flaviviridae family. DV-1, DV-2, DV-3 and DV-4 are the four different serotypes in dengue virus. Mostly, it is spread by Aedes mosquitoes. Symptom includes asymptomatic fever, joint pain, muscle pain, hemorrhagic fever, and circulatory shock syndrome. In the extreme level, it causes death. Nowadays, it becomes a major health issue in the tropical and subtropical areas. From ancient times, in India, many herbal plants are used for herbal formulation. *Carica papaya* is a well-studied plant for its medicinal properties. Papain, chymopapain, lycopene, carotenoids, alkaloids, monoterpenoids, flavonoids (quercetin), mineral and vitamins are some of the biological active compounds present in its leaves, seeds, latex, ripe and unripe fruits. Clinical trials showed an increase in platelet count when leaf extract of *Carica papaya* was orally administrated to dengue virus infected patients. Other plant parts also showed an increased production of cytokines and larvicidal activity. This article provides a detailed view on usage of the *Carica papaya* plant parts to treat and control dengue virus infection.



***Index Terms - Carica papaya*, dengue virus, platelets, hemorrhagic fever, larvicidal activity**

I. INTRODUCTION

Dengue fever is a mosquito born dreadful disease which is caused by any one of the four-dengue virus (flavivirus) serotypes. Basically, it is an endemic infectious disease highly seen in tropic and subtropical areas. Every year, around 390 million infections occur globally [1]. Dengue virus (DV) belongs to Flaviviridae family and four serotypes of the virus referred as DV-1, DV-2, DV-3 and DV-4 are identified so far. It is a RNA (+ strand) virus which encodes 3 structural proteins and seven non-structural (NS) proteins. Three structural proteins include nucleocapsid or core (C) protein, a membrane-associated (M) protein, an enveloped (E) glycoprotein. Nowadays, Dengue infection is being rapidly expanded and viewed as a global issue. Totally around 2.5 billion people are living in dengue risk region in 100 different countries [2]. Symptoms of dengue disease vary from mild fever to severe hemorrhagic fever (DHF). Thrombocytopenia is a dengue-associated condition which leads to DHF. Extremely, it can cause dengue shock syndrome (DSS). Usage of anti-viral agents against dengue virus is challenging since virus needs host cells for replication. Host cells are damaged to certain level when the drug is administrated [3]. Vaccine development against dengue virus is difficult since there are 4 different serotypes. Sanofi Pasteur's chimeric live-attenuated dengue vaccine was developed and approved for usage in population with an age ranging from 9 to 45 years. This vaccine is efficacious for infants and naive individuals [4]. Because of the restricted usage of dengue vaccine, an alternative strategy is necessary to prevent or treat dengue disease. So far, no exclusive treatment is identified to avoid the decrease in platelet count which leads to death in dengue patients predominantly. In India, 23 different plants were reported for their activity against dengue virus. But only *Carica papaya*, *Azadirachta indica*, *Hippophae rhamnoides* and *Cissampelos pareira* are the major well studied plants for their efficiency in treating dengue symptoms [1].

In countries like Pakistan, Malaysia, Sri Lanka and other Asian countries, Leaf extract of *Carica papaya* (Fig.1) has been used to treat dengue fever. Botanical classification of *Carica papaya* is as in Table 1.

Aqueous extract of *Carica papaya* leaves contains several active compounds like papain, chymopapain, cystatin, L-tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates. Ethanol, methanol, n-butanol, dichloromethane, ethyl acetate, and n-hexane extracts of leaves, pulp and peel of *C. papaya* was analyzed for antioxidant and antibacterial potential. Extracts of leaves, bark, roots and pulp were showed >75.0 % scavenging activity and for extract of leaves and pulp, it was 84.9 and 80.9 % respectively. Pulp extract showed antibacterial activity. Both Ethanol and methanol extracts were found to have more amounts of

phenolic and flavonoids [6]. Many queries were raised against the usage of *Carica papaya* leaf extract [7] and its side effects. Aim of this paper is to consolidate the studies on the effect of extracts from different parts of *Carica papaya* plant.

II. *CARICA PAPAYA* LEAF

One of the flavonoids present in the leaf extract is quercetin (Fig.2). Highest binding energy was observed in docking studies between nonstructural 2B and 3 (NS2B-NS3) protease complex of dengue 2 virus and quercetin [8].

To elucidate the action mechanism of *Carica papaya* leaf extract, 2 groups of 60 healthy and 60 dengue infected patients were selected. Platelet-rich Plasma (PRP) and Platelet Poor Plasma were collected from the both groups. *Carica papaya* leaf extract was incubated with plasma collected from dengue patient and healthy person was kept separately. Then it was added to platelets. Platelet aggregation was reduced when the *Carica papaya* leaf extract was pre-incubated with dengue plasma. But no change in aggregation was observed when leaf extract pre-incubated control plasma was added to control RPR. Reduced platelet aggregation was noted when leaf extract was directly added to dengue infected Platelet Rich Plasma and control PRP. From the study, it was identified that the leaf extract has a factor to neutralize dengue virus [10]. *In vitro* hemolytic assay was performed with the *C. papaya* leaf extracts. *C. papaya* leaf extracts were prepared from 3 different maturity levels of leaf. Hemolysis was induced using heat and hypotonicity. The extracts were taken in different concentrations with 40% erythrocytes suspension. More than 25% inhibition was observed at a concentration of 37.5 µg/ml and there was no significant difference in the activity of differently matured leaves. Heat induced hemolysis is not suitable method to demonstrate the hemolysis inhibition activity when in hypotonicity induced hemolysis sample, dose dependent inhibition was observed [11]. So, it was found that the cell destruction was avoided by the biologically active components in leaf extract. To check the presence of bioactive components in *Carica papaya* leaf extract, HPTLC was performed. In the results, presence of myricetin (280.16 ± 5.99 µg/g), caffeic acid (370.18 ± 6.27 µg/g), trans-ferulic acid (1110.86 ± 2.97 µg/g) and kaempferol (160.53 ± 2.48 µg/g) were witnessed. UPLC-qTOF/MS results showed presence of 24 different metabolites.

2.1. Animal Studies:

Carica Papaya leaf extract (150 mg/kg) was administrated to a group of cyclophosphamide induced thrombocytopenic Wister rats for 14 days. Rats showed significant increase ($p < 0.01$) in thrombocytes count. As immunomodulatory assessment delayed-type hypersensitivity (DTH), haemagglutination titre (HT) and *in vivo* carbon clearance tests were conducted in albino mice. Increased DTH and phagocytic index were observed in *Carica Papaya* leaf extract administrated mice group and less fibrosis was observed in spleen [12]. In another study, a suspension of powdered *Carica papaya* leaves in palm oil (15 mg of powdered leaves per kg body weight) was administrated in 5 mice and thrombocyte counts before and at 1, 2, 4, 8, 10, 12, 24, 48 and 72 hours after dosing were monitored. At 1, 2, 4, 8, 10 and 12 hours after dosing, a significant increase in thrombocyte was observed than before dosing count. The saline solution injected group of mice showed that no significant increase in thrombocyte count was observed. Palm oil administrated group of mice established significant platelet count increase after 8 and 48 hours dosing [13].

2.2. Clinical Trails:

A study in 2016 showed the effect of *Carica Papaya* leaf extract in dengue patients and the influence in platelet count increase. It was performed on 300 patients across 5 centers. It is a double blind, multi-centric, randomized, placebo controlled study, where the total population was divided into two groups. One group is kept as control and the another group of *Carica Papaya* leaf extract tablets (1100 mg/tablet) were given three times every day for 5 days. There was a significant increase in the platelet count ($p< 0.01$), which indicates the positive effect of *Carica Papaya* leaf extract in the thrombocytopenia patients [14]. Another study on the same year showed the efficacy and safety of *Carica Papaya* leaf extract. The data between control and group treated with *Carica Papaya* leaf extract are collected from PubMed, Cochrane Clinical Trial Registry and Google Scholar. A significant difference ($P < 0.00001$) was observed in a number of days of hospitalization between two groups. Enough data to show adverse effects of the extract are not available [15].

One more study was done to compare the platelet count of dengue patients with papaya leaf extract administration and without administration. Dengue patients were randomly divided into control group and study group. Papaya leaf extract capsule of 500 mg was administered once daily for consecutive five days to the test group. Blood cell counts, liver function test, renal function test for both groups were performed daily. Up to 48 hours, there was no significant change in platelet count between the control and study group. After 3rd day platelet count of study group was significantly high ($p<0.01$). It was continued till 7th day. Average hospitalization period of study group was 3.65±0.97 when the control group was 5.42±0.98 days (p value < 0.01). Average platelet transfusion requirement in study group was 0.685 units per patient and in control group it was 1.19 units per patient (p value <0.01) [16]. In another study, *Carica papaya* leaves extract was administrated orally for 5 days to a 23 years old man and the thrombocyte count was determined before and after administration. The thrombocyte count was found to increase from 28000/micro liter to 138000/micro liter [17]. In the same way, 45-year-old patient was administrated with aqueous extract of *Carica papaya* leaves twice daily for 5 days. Before and after administration of leave extract the platelets (PLT), White Blood Cells (WBC) and Neutrophils (NEUT) count were determined. Platelet count increased from $55\times10^3/\mu L$ to $168\times10^3/\mu L$, WBC from $3.7\times10^3/\mu L$ to $7.7\times10^3/\mu L$ and NEUT from 46.0% to 78.3% [18]. Based on the above-mentioned studies, it was found that the total platelet count was significantly increased in dengue virus infected patients.

To compare the normal dengue treatment and papaya leaf administration, a random population of 228 patients with Dengue Fever (DF) and dengue hemorrhagic fever (DHF) were selected and to half of the population *Carica papaya* leaves extract was administrated for 3 consecutive days. The other half of the population was receiving regular dengue treatment. For 48 hours, the total blood count was monitored and expression of Arachidonate 12-lipoxygenase (ALOX 12) and Platelet-Activating Factor Receptor (PTAFR) genes were analyzed. ALOX 12 and PTAFR genes were involved in production and activation of platelets. A significant increase ($P < 0.001$) in mean platelet count was observed. Expression of ALOX 12 (FC = 15.00) and PTAFR (FC = 13.42) genes were significantly high in test group than the control group [19].

III. *Carica papaya* Latex

Carica papaya latex extract was prepared using with different solvents such as chloroform, methanol and aqueously. These crude extracts were added to the larvae of *Aedes aegypti* and *Culex quinquefasciatus* which are the vectors of dengue virus transmission. After 24 hours, mortality of the larvae was assessed. Chloroform extract was showing a significant larvicidal activity on IInd and IIIrd instar larvae than other extracts. Silver nanoparticles were synthesized using aqueous latex extract and the larvicidal activity was found as 100% [20].

IV. *Carica papaya* Seed

Aqueous extracts (1%, w/v) of the seed tegument and cotyledon of *C. papaya prepared and* larvicidal activity were assessed individually and also in combination. 17 µg mL^{-1} tegument extract and 27 µg mL^{-1} cotyledon extract showed synergistic effect to give 100% larval mortality against *Aedes aegypti* larvae. When tegument extract was heated at 100 °C for 10 min, the enzyme tegupain was degraded and loss in larvicidal activity was observed. K_m, K_{cat} and K_{cat}/K_m of the enzyme tegupain was calculated as 58.8 µM, 28020 s^{-1} and 5×10^8 M^{-1} s^{-1}, respectively using thesubstrate Z-Phe-Arg-pNan. Enzyme inhibition was observed with cystatin and E64. Enzyme activity was doubled with 2 mM dithiothreitol (DTT), at 37 °C, pH 5.0 and there was no modification in enzyme activity when chelating agent EDTA was added to the extract [21].

V. *Carica papaya* Unripen Fruit Pulp

Peripheral blood leukocytes (PBL) and stem cells from human exfoliated deciduous teeth (SHED) showed an increased ($p < .05$) IL-6 production when it was treated with unripe papaya pulp juice (UPJ). SCF production in SHED culture was also significantly increased ($p < .05$) [22]. Molecular docking of kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one) and chymopain (disodium; 4,5-dihydroxybenzene-1,3-disulfonate), natural flavonols present in *Carica papaya* and gossypetin (3, 5, 7, 8, 3', 4'-hexahydroxyflavone), a natural flavonoid available in *Hibiscus sabdariffa* were done against non-structural and envelop proteins present in CHIKV and DENV. This study resulted in identification of lead components present in *Carica papaya* which can bind with the putative drug targets with minimum binding energy (kcal/mol) [23].

VI. Conclusion

During the primary infection of dengue virus, immunity is developed against the particular serotypes. But, when the secondary infection occurs, severe hemorrhagic fever, fluid leakage from blood vessels, sometimes from nose, gums and under the skin can cause death. In these cases, prevention of dengue infection is much better than treating it. Because of the presence of different bioactive compounds in *Carica papaya* seed and latex extract with unique properties, they can kill larvae of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes which are the vectors of dengue virus. Based on studies, it was inferred that the bioactive compounds *Carica papaya* leaf extract have specific role in increasing the platelet count in dengue virus infected patients. But the exact dosage of leaf extract to treat dengue patients is not very clear and activity of bioactive compounds present in ripened papaya is also not yet reported. So, further studies are required to confirm the dosage level, formulation and side effects of leaf extract juice.

Table 1: Botanical classification of *Carica papaya* [5]

Domain	Flowering plant
Kingdom	Plantae
Sub Kingdom	Tracheobionta
Class	Magnoliopsida
Subclass	Dilleniidae
Superdivision	Spermatophyta
Phyllum	Streptophyta
Order	Brassicales
Family	Caricaceae
Genus	Carica
Botanical Name	*Carica papaya* Linn

Figure 1: *Carica papaya* Tree

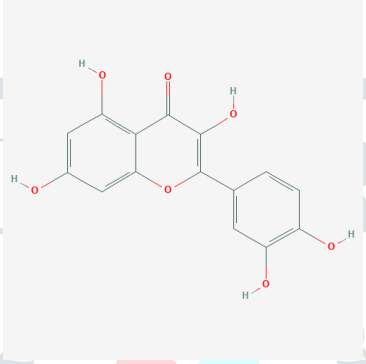


Figure 2: Quercetin [9]

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