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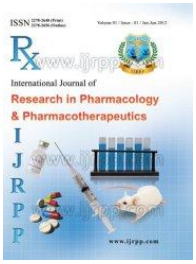

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A review of newer therapy in dengue fever

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ABSTRACT

Dengue is a viral disease that at present affects an enormous number of people in over 125 countries and is conscientious for a sizable number of deaths. There is no antiviral chemotherapy or vaccine for dengue virus and management of the disease is done on supportive measures, so various treatments are being investigated. Studies have indicated that the juice of the leaves of the *Carica papaya* plant from the family Caricaceae could help to increase the platelet levels in these patients. This review explains some of the published studies on this topic. Although many of the studies and case reports published in literature lack adequate information, some of the studies do raise the possibility that this treatment could be an important option in the future. Further large scale studies could establish the usefulness or ineffectiveness of this natural product in the treatment of dengue.

Key words: Dengue fever, Newer therapy, *Carica papaya.*

INTRODUCTION

Dengue is a sensitive viral disease with latent mortal complications. Dengue fever was primarily referred as "water poison" related to flying insects in a Chinese medical encyclopedia. In recent decades, Dengue is the most significant emerging viral disease in humans. It turns out to be a most important global public health concern. Dengue is prominent in tropical and subtropical regions around the world, mostly in urban and semi urban area. Dengue viruses (DV) belong to the family Flaviviridae of which four serotypes of the virus referred to as DV-1, DV-2, DV-3 and DV-4. DV is made of positive stranded encapsulated RNA virus and is composed of three structural protein genes, which instruct the nucleocapsid or core protein, a membrane-associated protein, an enveloped glycoprotein and seven non-structural proteins.

Dengue Fever - A Brief Overview

Dengue fever (DF) is spread through the bite of an infected *Aedes aegypti* mosquito. The mosquito gets the virus by stinging an infected human being. Subsequent to the infected mosquito bites a healthy person, the primary symptom of the infection appears

in about 57 days. It is apparent to become infected by dengue several times for the reason that the virus has four different serotypes. Even though each infection grants lifelong immunity to that particular serotype, a consequent infection with a unlike serotype raises the threat of constricting the much deadlier type known as dengue hemorrhagic fever (DHF).

The WHO 2009 classification splits DF into two groups: uncomplicated and severe, though the 1997 WHO classification is still widely used. The key distinguishing symptoms of dengue illness are continuous high fever lasting 2-7 days, haemorrhagic tendency as revealed by a positive tourniquet test, thrombocytopoenia and evidence of plasma leakage manifested by haemoconcentration. An incubation episode changeable as of 3 to 14 days is followed by a febrile illness consisting of sudden-onset fever, rash, myalgia, headache, and arthralgia. The common feature of the illness is Thrombocytopenia. Hemorrhagic manifestations are developed in a patient such as bleeding of the gastrointestinal tract and gums, epistaxis and petechiae. Post the incubation period, the illness commences rapidly and, in patients by means of moderate to severe disease, is followed by three stages febrile, critical and recovery. Because of its vibrant character, the rigorousness of the infection will merely be evident around defervescence i.e., during the conversion of the febrile to the afebrile stage, which frequently matches with the start of the critical stage.

Contemporary information in relation to the physiopathology of DHF proposes extension of the immune reaction due to the incidence of heterotypic antibodies adjacent to a serotype of the dengue virus by the moment of new infection, as a justification for the higher occurrence of DHF in repeat dengue infections. Young children in particular may be less able than adults to compensate for capillary leakage and are consequently at greater risk of dengue shock[5]. Treatment for dengue is usually symptomatic. Risk factors decide the severity of the disease and include age, secondary infection, ethnicity and perhaps chronic diseases (diabetes mellitus, sickle cell anemia and bronchial asthma). Several cases involve fluid management and platelet transfusions. The major problem of dengue treatment is that there are no efficient antiviral agents existing to treat dengue complications. Nevertheless symptomatic treatment works in nearly all mild cases, some cases, and advances to complications very fast and this often make it difficult to save the life of the patient.

Epidemiology of Dengue

Remarkable growth has been present around the world in the incidence of dengue over the last two decades. Larger than 2.5 billion populace – above 40% of the world's population – are currently at risk from dengue. WHO presently estimates that, there might be half a million DHF and 50–100 million dengue infections globally each year. At present prevailing in above 125 countries and diversified studies predicted that it infects almost 50-270 million people each year, ensuing in a considerable number of deaths[5]. Travelers from non-endemic places to the dengue-affected places are exposed to the chance of infection. This makes it a worldwide public health concern, affecting folks from countries still where the infection is not common. The epidemiology of dengue fevers in the Indian subcontinent has been very multifaceted and has significantly altered over nearly past six decades in provisions of prevalent strains, affected geographical locations and severity of disease.

Currently Existing Treatment alternatives

Treatment is guided by etiology and disease severity. The normal treatment options for management of Dengue include symptomatic treatment with fluid management. No specific treatment is available. On the other hand, there are Indian studies which have added in terms of superior management of DHF. A quick response to platelet and fresh frozen plasma (FFP) transfusion is reported in a study[1]. Anti-D has been used in children with DHF and severe refractory thrombocytopenia[2]. In an investigational study, pre-feeding mice with the trivalent chromium picolinate (CrP) in drinking water could abolish the adverse effects of DV infection on most of the hematological parameters[3]. Hippophae rhamnoides (Seabuckthorn, SBT) leaf extract has been shown to have a significant anti-dengue activity[4]. Acetaminophen may be used to treat patients with symptomatic fever. Aspirin, Brufen nonsteroidal antiinflammatory drugs (NSAIDs), antibiotics and corticosteroids be supposed to be avoided as these do not help but cause gastritis and/or bleeding.

Mechanism of Thromocytopenia in Dengue

Dengue hemorrhagic fever is characterized by a thrombocyte count of <100,000 cells/mm^3. Two mechanisms have been suggested that could be responsible for dengue induced thrombocytopenia impaired thrombopoiesis and peripheral platelet destruction. In support of the theory of impaired thrombopoiesis, studies have suggested reduced megakaryopoiesis at the onset of infection, which is normal at the time of clinical recovery. This effect could be due to a direct effect of the virus on the megakaryocytes, or an effect on the stromal cells, which are responsible for the release of cytokines and control of megakaryopoiesis. Studies have also indicated altered proliferative capacity, inhibition of differentiation and megakaryocytic progenitor apoptosis as possible mechanisms of thrombocytopenia. The other main mechanism proposed for thrombocytopenia is the increased peripheral platelet destruction by the DENV. This could be due to an autoimmune reaction, where antibodies produced by the host against the DENV bring about activation and destruction of platelets. Platelets may also show an increased reaction with leucocytes and endothelial cells, leading to their destruction. Platelet dysfunction due to abnormal activation and inhibition of platelet aggregation in dengue patients may also be responsible for the destruction. Recent studies indicate a direct infection of the platelets by the DENV. Increased levels of mediators like tumor necrosis factorα and interleukin1β were associated with the thrombocytopenia[7].

Plants species conventional for the dengue fever treatment

Natural products have become the main source of test material in the development of antiviral drugs based on traditional medical practices[8]. Traditional medicines are based on knowledge, experience and practices based on indigenous cultural beliefs and knowledge, and are used to maintain health, prevent, treat and diagnose physical or mental illness. Traditional medicinal plants have been reported to have antiviral activity[9, 10], and some have been used to treat viral infections in animals and humans. To date, 31 different species have been found to have the potential to treat dengue; some of these have not yet been investigated scientifically. In the Philippines, Euphorbia hirta, known locally as (tawatawa), is used in folk medicine to cure dengue fever by people in rural areas[11].

Carica papaya plant use in Dengue Medicine

The papaya plant or *C. papaya* has been used since ancient times for the treatment of a number of disease conditions. Various beneficial effects of extracts from the leaves, fruit and seeds have been suggested through scientific studies. The chymopapain and papain extracts of the leaves are useful in the treatment of digestive disorders[12]. Carica papaya) leaves are presumed to be due to several active components such as papain, chymopapain, cysteine, L-tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates. These are antioxidants that reduce lipid peroxidation, exhibit antitumor activity and immune modulatory effects. The extracts from fruits and seeds have bactericidal properties. The fruit juice and leaf extract have been demonstrated to have a wide variety of properties including anticancer, antioxidative, antiinflammatory, antibacterial, nephroprotective, hepatoprotective, hypoglycemic and hypolipidemic effects, and antisickling effect on sickle cell disease. The ripe fruit has been used against ringworm, whereas the green fruit has been used to lower blood pressure, as an aphrodisiac and to induce abortion[13]. The leaf extract has also been shown to have larvicidal properties against the Aedes aegypti mosquito, the vector of the dengue fever.

Total phenolic and flavonoid content of papaya leaf extracts

Total phenolic content in the cold water extract of Papaya leaves at three different maturity stages was determined using the Folin-Ciocalteu method[15]. Six different cold water extracts from each maturity stage was diluted in distilled water and 20 µl from each concentration was incubated with 110 µl of Folin-Ciocalteu reagent and 70 µl of 10% sodium carbonate at room temperature for 30 min, followed by absorbance reading at 670 nm. Gallic acid in five different concentrations (12.5, 25.0, 50.0, 100, and 200 µg/ml) was used to construct the standard curve. Total phenolic content in leaf extracts were estimated as Gallic acid equivalent (GAE) per g of dry matter. Total flavonoid content was determined using the aluminum chloride method. Six different cold water extracts from each maturity stage was diluted in distilled water and 100 µl from each concentration was incubated with 100 µl of 2% aluminum chloride

(in methanol) at room temperature for 10 min, followed by absorbance reading at 365 nm using a SPECTRAmaxPluse384 Microplate reader (Molecular Devices, Inc., USA). Five different concentrations
(7.81, 15.62, 31.25, 62.5, and 125.0 µg/ml) of quercetin were used to construct the standard curve. Total flavonoidin leaf extracts were estimated as quercetin equivalent (QE) per g of dry matter. Flavonoids and other phenolic compounds are reported to act as effective scavengers of free radicals[14].

Potential Mechanism of Action of Papaya Extract in Dengue

The papaya plant possibly brings about its effect in dengue by treating the thrombocytopenia associated with the condition. A study has reported membrane stabilizing properties of C. papaya L. leaf extracts in in vitro studies. The study found that C. papaya L. leaf extracts inhibited heat-induced and hypotonicity-induced hemolysis of erythrocytes obtained from both healthy individuals and individuals with dengue infection; the effect was observed at the lower concentrations of the extracts. Thus, the extracts are likely to possess membrane-stabilizing properties and protect blood cells against stress-induced destruction. This property may be useful in patients with dengue infection where the leaf extracts could possibly prevent platelet lysis. The authors postulate that this effect could be due to the presence of flavonoids and other phenolic compounds in the papaya leaves.

Research studies regarding the effect of papaya leaves in animals

A study in mice found an increase in thrombocyte counts in mice administered 15 mg of powdered papaya leaves/kg body weight between 1 and 12 h following dosing[16]. Another study found that the C. Papaya leaf aqueous extract at concentrations of 400 mg/kg and 800 mg/kg significantly increased the platelet counts in cyclophosphamide induced thrombocytopenic rat model. It also reduced the clotting time in the treated rats[17].

An in-vitro study demonstrated a significant inhibition of hemolysis. The inhibition effect shown by crude extracts of the C. papaya L. leaves at comparatively lower concentrations (37.5µg/ml) was comparable with that of standard antihemolysis compounds such as aspirin and indomethacin. This experimental evidence indicates that C. papaya L. leaf extracts could have a potential therapeutic efficacy in disease processes causing destabilization of biological membranes[18]. Another in-vitro study explained that upregulated synthesis of thrombopoietic cytokines both in peripheral blood leukocytes and stem cells from human exfoliated deciduous teeth revealed the potential mechanism of unripe papaya to induce thrombopoietic cytokines synthesis in cells of hematopoietic and mesenchymal origin[20].

A bioinformatic study analysed the antidengue activities of the extracts from Carica papaya by using bioinformatics tools. The flavonoid quercetin with highest binding energy against NS2BNS3 protease which was evident by the formation of six hydrogen bonds with the amino acid residues at the binding site of the receptor. Our results suggest that the flavonoids from Carica papaya have significant antidengue activities[19].

Research studies regarding the effect of papaya leaves in humans

A pilot study was conducted in Sri Lanka on 12 patients suspected of suffering from dengue. The patients had a platelet count of <130,000/cu mm, but only six patients were serologically confirmed to be suffering from dengue. The patients received 2 doses of papaya leaf extract at intervals of 8 h. They also received standard symptomatic care for dengue. The study found an increase in platelet count and total white blood cell count in patients administered papaya leaf extract within 24 h of treatment with the extract[21].

Another pilot study was conducted to investigate the platelet increasing property of Carica papaya leaf extract (CPLE) in patients with dengue fever (DF). An open labeled randomized controlled trial was carried out at two centres of Bangalore metropolis on 30 subjects in patients with thrombocytopenia associated with dengue. The subjects were randomized into two groups, as control and intervention group. Both the groups were managed by the standard management guidelines for dengue except steroid administration. In addition to this, the intervention group received CPLE tablet three times daily for five days. All of them were followed daily with platelet monitoring. The results showed that CPLE had a significant increase in the platelet count over the therapy duration, in dengue fever patients,

reiterating that it accelerates the increase in platelet count compared to the control group. There were few adverse events related to GI disturbance like nausea and vomiting which were similar in both groups. Thus, this study concluded that the Carica papaya leaf extract (CPLE) does significantly increase the platelet count in patients with thrombocytopenia associated[22].

A case report from Pakistan described the effective treatment of dengue in a truck driver with papaya leaf extract. The patient received 25 ml of papaya leaf extract twice a day for 5 days. A steady increase in the platelet and white blood cell count was observed after 2 days of treatment. However, the results of the study have to be interpreted with caution, taking into consideration the vague and incorrect details mentioned in the report. For example, the report states that the driver was bitten by a "mosquito carrying Dengue virus", 24 h after which he started developing symptoms. These and similar other statements raise questions regarding the credibility of this report[23].

A study conducted in Pakistan in a 23 year male patient. Blood samples were tested for complete blood count before and after the administration of the Carica papaya leaf juice. Thrombocyte count had increased from 28000/micro liter to 138000/micro liter at the end of five days. This shows the importance of in Carica papaya in treatment of dengue.

From the variety of reports published in scientific literature, it is known that Carica papaya leaf extract does have beneficial properties in dengue. It has been shown to bring about a rapid increase in platelet count. This could be possibly attributed to its membrane stabilizing property. The flavonoids and other phenols present in the extract have been suggested to provide the beneficial effects. One study found that the leaves of papaya plant are rich in several minerals. The researchers suggested that these minerals may balance the mineral deficiency caused by the virus and strengthen the immune cells against it. Most of the cases were given a crude leaf extract prepared by grinding the papaya leaves. The amount of extract given also differed among the studies. Thus, the active principle needs to be identified and the dosage standardized to conduct clinical studies on it to prove its efficacy in dengue beyond doubt. It is also necessary to conduct pharmacokinetic studies to ensure that the active principle is absorbed from the digestive tract. In addition to its effect against the virus, the papaya plant also appears to be effective against the Aedes mosquito. Thus, if proved to be effective, this plant could control dengue at two levels, at the level of transmission as well at the host level.

Table No: 1 Plant species used in the treatment of dengue fever26

S. NO	PLANT SPECIES	ACTIVE COMPONENTS	ACTIVITY
1.	Andropogon Citratum	Essential oil and citronella oil	Mosquito repellent
2.	Andrographis Paniculata	Methanolic extract	Antiviral inhibitory effect
3.	Azadirachta Indica	Aqueous extract	Antiviral inhibitory effect
4.	Carica Papaya	Aqueous leaf extract	Increasing platelet counts
5.	Curcuma Longa	Ethyl acetate extract	Inhibiting topoisomerase I and topoisomerase II, which play important role in DNA replication
6.	Euphorbia Hirta	Water decoction of leaves	Internal haemorrhaging will stop and dengue fever will be cured after 24h
7.	Mimosa Scabrella	Seed extract	Activity against YFV and DENV-1 in vitro and in vivo
8.	Momordica Charantia	Methanolic extract	Inhibitory effect on DENV-1 by antiviral assay based on cytopathic effects.

9.	Murraya Koenigii	Ethyl acetate crude extracts of the whole plant	Mosquitocidal
10.	Piper Longum	Ethanolic extract	Antiviral effect
11.	Psidium Guajava	Leaf extract	Avoid bleeding in DHF, and increased platelet counts
12.	Quercus Lusitanica	crude methanol extracts	Inhibits the replication of virus

CONCLUSION

Papaya extracts no doubt offers a cheap and possibly effective treatment for dengue. Various clinical and preclinical studies conducted have demonstrated a positive effect in dengue cases with thrombocytopenia. This review work demonstrates That same positive, beneficial trend in increasing the platelets significantly. However, large scale randomized clinical trials are necessary to further establish its pivotal role in the management of dengue.

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