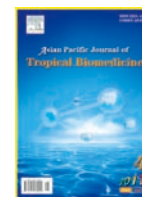




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(11)60055-5 © 2011 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Dengue fever treatment with *Carica papaya* leaves extractsNisar Ahmad^{1,2*}, Hina Fazal^{1,3}, Muhammad Ayaz⁴, Bilal Haider Abbasi¹, Ijaz Mohammad⁵, Lubna Fazal⁵¹Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan²Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar 25000, Pakistan³Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Peshawar 25000, Pakistan⁴Department of Environmental Sciences, University of Peshawar, Peshawar 25000, Pakistan⁵Postgraduate Medical Institute (PGMI), Lady Reading Hospital, Peshawar 25000, Pakistan

ARTICLE INFO

Article history:

Received 28 February 2011

Received in revised form 20 March 2011

Accepted 6 April 2011

Available online 30 April 2011

Keywords:

Dengue fever

Carica papaya

Leaves extracts

Platelets

Potential activity

ABSTRACT

The main objective of the current study is to investigate the potential of *Carica papaya* leaves extracts against Dengue fever in 45 year old patient bitten by carrier mosquitoes. For the treatment of Dengue fever the extract was prepared in water. 25 mL of aqueous extract of *C. papaya* leaves was administered to patient infected with Dengue fever twice daily *i.e.* morning and evening for five consecutive days. Before the extract administration the blood samples from patient were analyzed. Platelets count (PLT), White Blood Cells (WBC) and Neutrophils (NEUT) decreased from $176 \times 10^3/\mu\text{L}$, $8.10 \times 10^3/\mu\text{L}$, 84.0% to $55 \times 10^3/\mu\text{L}$, $3.7 \times 10^3/\mu\text{L}$ and 46.0%. Subsequently, the blood samples were rechecked after the administration of leaves extract. It was observed that the PLT count increased from $55 \times 10^3/\mu\text{L}$ to $168 \times 10^3/\mu\text{L}$, WBC from $3.7 \times 10^3/\mu\text{L}$ to $7.7 \times 10^3/\mu\text{L}$ and NEUT from 46.0% to 78.3%. From the patient feelings and blood reports it showed that *Carica papaya* leaves aqueous extract exhibited potential activity against Dengue fever. Furthermore, the different parts of this valuable specie can be further used as a strong natural candidate against viral diseases.

1. Introduction

Dengue viruses, mosquito-borne members of the Flaviviridae family, are the causative agents of dengue fever[1]. Dengue is the most important emerging viral disease of humans that in recent decades has become a major international public health concern. Dengue is found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas[2]. It is estimated that there are between 50 and 100 million cases of dengue fever (DF) and about 500 000 cases of dengue haemorrhagic fever (DHF) each year which require hospitalization[3]. Dengue fever is spread through the bite of an infected *Aedes aegypti* mosquito. The mosquito gets the virus by biting an infected person[4]. The first symptom of the disease appears in about 5–7 days after the infected mosquito bites a healthy person. It is possible to become infected by dengue multiple times because the virus has four different serotypes. Although each infection confers lifelong immunity to that particular

serotype, a subsequent infection with a different serotype increases the risk of contracting the much deadlier form known as dengue hemorrhagic fever (DHF)[5]. The symptoms of dengue fever include high fever, rash, and a severe headache (dengue triad). Additional symptoms include severe joint and muscular pain (breakbone fever), nausea, vomiting, and eye pain. Although dengue fever itself is rarely fatal, it can be an extraordinarily painful and disabling illness and may become epidemic in a population following the introduction of a new serotype. Dengue fever is usually a self-limited illness, and only supportive care is required. Acetaminophen may be used to treat patients with symptomatic fever. Aspirin, Brufen nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics and corticosteroids should be avoided as these do not help but cause gastritis and/or bleeding. In children, Reye's syndrome (encephalopathy) may be a serious complication. Patients with known or suspected dengue fever should have their platelet count and Hematocrit measured daily from the third day of illness until 1–2 days after defervescence. No vaccine is available for the prevention of dengue infection. Immunogenic, safe tetravalent vaccines have been developed and are undergoing clinical trials. The only way to prevent dengue virus acquisition is to avoid being bitten by a vector mosquito[1–4]. Fresh plant materials of

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Carica papaya (*C. papaya*) were collected from University of Peshawar. Their botanical identities were determined and authenticated by Dr. Lajber Khan, Head, Medicinal Botanic Centre (MBC), PCSIR Laboratories Complex, Peshawar KPK, Pakistan. The plant material was washed with water, cut into pieces and grinded with blender. 25 mL of aqueous extract of *C. papaya* leaves was administered to patient infected with Dengue fever. The same doze was given to the patient twice daily *i.e.* morning and evening for five consecutive days. By 72 hours, effectiveness of the water extract was investigated biochemically from blood samples. Serum was separated by centrifugation at 3 000 rpm for 15 minutes. The separated serum profile was used for the estimation of biochemical parameters like Platelets count (PLT), White Blood Cells (WBC), Red Blood Cells (RBC) and Neutrophils (NEUT), *etc.*

2. Case report

A male patient aged 45 years old working as truck driver in a cement factory, was brought to the emergency department with sever fever of 104 ° F. The symptom included breathing problem, sever vomiting, red skin, shivering, sever body pain and high blood pressure. He reported that he had been driving truck and transporting cement to various regions of KPK, Pakistan. During his journey he used to stay at different locations for food, at that time he was bitten by mosquito carrying Dengue virus. After 24 hr of which he started feeling sever pain in whole body with fever and teary eyes. These symptoms increased time to time over the passing hours, which were followed by vomiting. In the same days three to four patients surrounding the mentioned area (Figure 1) died of Dengue fever. Subsequently the patient was admitted in emergency unit in Abbottabad Medical Complex. On the basis of screening blood test examination he was declared to be a victim of Dengue fever.

The serological studies revealed an abrupt decline in the patient platelets counts. The patient has been given different broad spectrum antibiotics and anti malarial drugs but there were no signs of improvement, and his condition became

worse time by time. He had been given different food items but unfortunately vomiting did not stopped. At the same time our team was working on the biological activities of *C. papaya*. For the patient's treatment, the leaves of the plant (Figure 2) were collected and thoroughly washed with water. The leaves were grinded in fruit juicer with water. As the taste of *C. papaya* leaves extract was very bitter, some amount of sucrose was added for easy administration. About 25 mL of leaves extract was administrated orally, twice daily for five consecutive days. Before extract administration, the patient blood was evaluated for total platelets counts, after 24 hours each time. After extract administration the patient blood was rechecked for platelets counts again for five consecutive days. After which the patient starts improvement.

Before administration of leaves extracts, the patient blood test have been taken. In patient target area there was no specific lab to identify Dengue virus in patient blood. Therefore, from the tests results it was observed that PLT, WBC and NEUT decreased from normal level. After the infection the patient immediately felt fatigue and fever, these symptoms aggravated over the night. In the same month of October 2010, most patients of Dengue fever died, so from the patient symptoms and blood reports physicians recommended hospitalization. From the first blood report it was observed that PLT count was $176 \times 10^3 / \mu\text{L}$, WBC $8.1 \times 10^3 / \mu\text{L}$ and NEUT 84.0%, respectively. In the second report PLT count was $122 \times 10^3 / \mu\text{L}$, WBC $6.6 \times 10^3 / \mu\text{L}$ and NEUT 81.5%. The third day report showed the PLT counts, WBC and NEUT were $110 \times 10^3 / \mu\text{L}$, $4.4 \times 10^3 / \mu\text{L}$ and 71.8%, respectively. PLT, WBC and NEUT decreased very fast. The physicians told the family members of patient that if the PLT count were below $20 \times 10^3 / \mu\text{L}$ then he would begin bleeding and may die. Physicians in different hospital recommend PLTs injection isolated from donor blood. But in this case on the fourth and fifth day the PLT count decreased to $71 \times 10^3 / \mu\text{L}$ and $55 \times 10^3 / \mu\text{L}$, respectively, while the WBC and NEUT reached to $4.0 \times 10^3 / \mu\text{L}$, $3.7 \times 10^3 / \mu\text{L}$ and 60.0%, 46.0% respectively (Table 1). Different antibiotics and anti-malarial drugs were administrated orally and intravenously, but no successful results.

Table 1
Blood test reports before the administration of *C. papaya* leaves extract.

Parameters	1st day	2nd day	3rd day	4th day	5th day
WBC	$8.10 \times 10^3 / \mu\text{L}$	$6.60 \times 10^3 / \mu\text{L}$	$4.4 \times 10^3 / \mu\text{L}$	$4.00 \times 10^3 / \mu\text{L}$	$3.70 \times 10^3 / \mu\text{L}$
RBC	$5.28 \times 10^6 / \mu\text{L}$	$4.96 \times 10^6 / \mu\text{L}$	$4.94 \times 10^6 / \mu\text{L}$	$5.23 \times 10^6 / \mu\text{L}$	$5.00 \times 10^6 / \mu\text{L}$
HGB	14.4 s/dL	13.5 s/dL	14.3s/dL	16.2s/dL	15.2s/dL
HCT	45.6%	43.0%	42.9%	47%	42%
MCV	86.4 fL	86.7 fL	87.0 fL	90 fL	90 fL
MCH	27.3 Ps	27.2 Ps	28.9 Ps	31.1 Ps	27.1 Ps
MCHC	31.6 s/dL	31.4 s/dL	33.3 s/dL	34.1 s/dL	32.1 s/dL
PLT	$176 \times 10^3 / \mu\text{L}$	$122 \times 10^3 / \mu\text{L}$	$110 \times 10^3 / \mu\text{L}$	$71 \times 10^3 / \mu\text{L}$	$55 \times 10^3 / \mu\text{L}$
LYM%	10.7%	13.8%	20.5%	36%	17%
MXD%	5.3%	4.7%	7.7%	–	–
NEUT%	84.0%	81.5%	71.8%	60%	46%
LYM #	$0.9 \times 10^3 / \mu\text{L}$	$0.9 \times 10^3 / \mu\text{L}$	$0.9 \times 10^3 / \mu\text{L}$	–	–
MXD #	$0.4 \times 10^3 / \mu\text{L}$	$0.3 \times 10^3 / \mu\text{L}$	$0.3 \times 10^3 / \mu\text{L}$	–	–
NEUT #	$6.8 \times 10^3 / \mu\text{L}$	$5.4 \times 10^3 / \mu\text{L}$	$3.2 \times 10^3 / \mu\text{L}$	–	–
RDW	43.4 fL	44.5 fL	48.5 fL	–	–
PDW	9.9 fL	11.2 fL	15.7 fL	–	–
P-LCR	14.1%	20.2%	–	–	–
MPV	8.4 fL	9.3 fL	8.7 fL	–	–

Table 2
Blood test reports after the administration of *C. papaya* leaves extract.

Parameters	1st day	2nd day	3rd day	4th day	5th day
WBC	$3.80 \times 10^3 / \mu\text{L}$	$4.40 \times 10^3 / \mu\text{L}$	$5.30 \times 10^3 / \mu\text{L}$	$5.90 \times 10^3 / \mu\text{L}$	$7.70 \times 10^3 / \mu\text{L}$
RBC	$4.71 \times 10^6 / \mu\text{L}$	$5.33 \times 10^6 / \mu\text{L}$	$4.99 \times 10^6 / \mu\text{L}$	$5.21 \times 10^6 / \mu\text{L}$	$5.26 \times 10^6 / \mu\text{L}$
HGB	15.2 s/dL	16.8 s/dL	14.5 s/dL	16.0 s/dL	15.2 s/dL
HCT	42.1%	48.2%	42.9%	46%	46.6%
MCV	89.0 fL	90.0 fL	86.0 fL	84.6 fL	86.6 fL
MCH	32.6 Ps	31.5 Ps	28.9 Ps	29.1 Ps	27.2 Ps
MCHC	36.5 s/dL	34.8 s/dL	34.3 s/dL	34.1 s/dL	35.1 s/dL
PLT	$73 \times 10^3 / \mu\text{L}$	$120 \times 10^3 / \mu\text{L}$	$137 \times 10^3 / \mu\text{L}$	$159 \times 10^3 / \mu\text{L}$	$168 \times 10^3 / \mu\text{L}$
LYM%	40%	42.0%	20.5%	31%	19%
MXD%	5.3%	4.4%	6.7%	–	–
NEUT%	56.0%	64.2%	71.1%	73.0%	78.3%
LYM #	$0.70 \times 10^3 / \mu\text{L}$	$0.90 \times 10^3 / \mu\text{L}$	$0.80 \times 10^3 / \mu\text{L}$	–	–
MXD #	$0.27 \times 10^3 / \mu\text{L}$	$0.24 \times 10^3 / \mu\text{L}$	$0.31 \times 10^3 / \mu\text{L}$	–	–
NEUT #	$5.80 \times 10^3 / \mu\text{L}$	$6.20 \times 10^3 / \mu\text{L}$	$3.20 \times 10^3 / \mu\text{L}$	–	–
RDW	45.4 fL	43.6 fL	46.1 fL	–	–
PDW	6.7 fL	7.2 fL	13.7 fL	–	–
P-LCR	11.6%	19.2%	–	–	–
MPV	3.9 fL	8.5 fL	14.1 fL	–	–



Figure 1. The target area of Dengue fever.

The *C. papaya* leaves extract in water was given to the patient twice daily. The patient started vomiting as eating, so physicians recommended fruits and fruit juices. 25 mL of extract was given to the patient in the morning and evening. After two consecutive days the blood samples were checked for different parameters. In the first blood report it was observed that the PLT count, WBC and NEUT increased to $73 \times 10^3 / \mu\text{L}$, $3.8 \times 10^3 / \mu\text{L}$ and 56.0%, respectively. With similar dose of extracts, on the next day the blood report indicated that the PLT count reach to $120 \times 10^3 / \mu\text{L}$ while WBC and NEUT reach to $4.4 \times 10^3 / \mu\text{L}$ and 64.2%, respectively. On the third day, it was observed that PLT count ($137 \times 10^3 / \mu\text{L}$), WBC ($5.3 \times 10^3 / \mu\text{L}$) and NEUT (71.1%) increased. In the fourth blood report (PLT: $159 \times 10^3 / \mu\text{L}$, WBC: $5.9 \times 10^3 / \mu\text{L}$ and NEUT: 73.0%) and fifth report (PLT: $168 \times 10^3 / \mu\text{L}$, WBC: $7.7 \times 10^3 / \mu\text{L}$ and NEUT: 78.3%) the PLT, WBC and NEUT reach to their normal levels (Table 2).

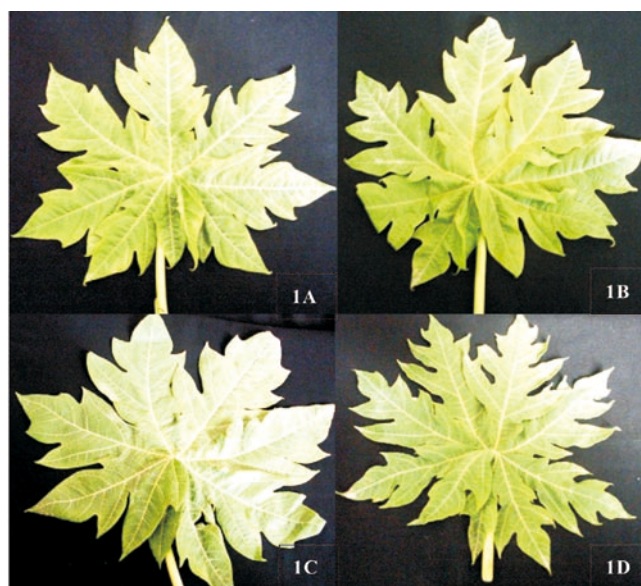


Figure 2. Different age leaves of *C. papaya* used for aqueous extraction.

3. Discussion

The main objective of the current study is to investigate the potential of *C. papaya* leaves extracts against Dengue fever. The secondary metabolite of plants origin makes up a vast repository of compounds with a wide range of biological activities. There have been many reports of higher plant extracts possessing relatively good potential to inhibit viruses (Van Den Berghe, 1978). Many plants extracts including *Spilanthes calva*, *Sterculia guttata*, *Balanites aegyptiaca*, *Vitex negundo*, *Solanum xanthocarpum*, *Artemisia annua*, *Fagonia indica*, *Nerium indicum*, *Trigonella foenum*, in different solvents have been reported to exhibit activity against *Aedes aegypti* L., a vector of dengue fever[6–12].

C. papaya contains two important biologically active compounds *vis*: chymopapain and papain which are widely used for digestive disorders[13]. It showed that papaya-derived papain, caricain, chymopapain, and glycine endopeptidase can improve acidic pH conditions and pepsin degradation. Other active compounds of *C. papaya* are lipase, or CPL, a hydrolase, which is tightly bonded to the water-insoluble fraction of crude papain and is thus considered as a “naturally immobilized” biocatalyst[14]. According to the folk medicine, papaya latex can cure dyspepsia and also applicable for external burns and scalds. Seeds and fruits are excellent antihelminthic and anti-amoebic[15]. Dried and pulverized leaves are sold for making tea, also the leaf decoction is administered as a purgative for horses and used for the treatment of genito-urinary system. Unripe and semi-ripe papaya fruits are ingested or applied on the uterus to cause abortion. However, the consumption of unripe and semi-ripe papaya fruits could be unsafe during pregnancy, but consumption of ripe fruits during pregnancy causes no risk[16].

It is reported that the plant extracts of *Quercus lusitanica* have maximum activity against DENV-2 Replication of Dengue virus[17]. It is also reported that the methyle gallate of plant origin interacts with herpes simplex virus proteins and alters the adsorption and penetration of the virion[18]. It is observed that *Gastrodia elata* B1 has effect on the multiplication cycle of Dengue virus serotype 2[19]. It is reported that two compounds 1-β-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole (ETAR) and 1-β-D-ribofuranosyl-4-ethynyl[1,3]imidazole (IM18), significantly reduced replication of dengue virus serotype 2 (DENV-2) in cultured Vero cells and a novel trypsin Kazal-type inhibitor from *Aedes aegypti* has thrombin coagulant inhibitory activity[20].

The latex, ripe fruits, unripe fruits, seeds, seeds juice, root, leaves, flower and stem bark of *C. papaya* are used as antimicrobial, anthelmintic, antimalarial, antifungal, anti-amoebic, hepatoprotective, male and female antifertility, immunomodulatory and against histaminergic[21]. This case report shows the activity of *C. papaya* leaves extracts against Dengue fever.

The Dengue attacks start in late summer and end in early winter in Pakistan. Most patients infected are in the age of 30–45 years old. The Dengue fever repeats every year and causes several deaths. The rise of PLT count in the present case from $55 \times 10^3/\mu\text{L}$ to $168 \times 10^3/\mu\text{L}$ indicates the activity of *C. papaya* leaves extract. However, this is a preliminary work and more works on isolating the active compounds from this valuable specie are needed which may help in control of such infectious diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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