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How to Cope with Dengue in Developing Countries Like Pakistan?

1Filza Sohail, 2Gazi Mahabubul Alam, 3Rizwan Rasul Khan and 4Rahmah Naeem  
1GM College, Sargodha Road, Faisalabad, Pakistan  
2Institute of Education, International Islamic University Malaysia, Jalan Gombak 53100, Kuala Lumpur, Malaysia  
3Department of Internal Medicine, University Medical and Dental College, Faisalabad, Pakistan  
4Department of Dentistry, University Medical and Dental College, Faisalabad, Pakistan

Corresponding Author: Filza Sohail, GM College, Sargodha Road, Faisalabad, Pakistan

ABSTRACT

A viral disease like dengue lacking a specific form of treatment is a high menace to human health. Situation becomes worse in developing countries like Pakistan because of poor health care services and facilities. Using data from earlier works and analyzing them, this review aims to explore the disease epidemiology. Dengue Virus (DENV) destroys the immune system and causes health problems like headache, inflammation, bleeding, hypertension and mental disorders. Death also can be caused through dengue because of its adverse effects on liver which also may result in hepatitis. Dengue spread can be controlled through many ways like modulating the environment and devastating its vector. Biological control appears as potential approach to control its vector, especially the use of Wolbachia. Currently, no vaccines are available against this virus and antiviral drugs are also not significantly effective. Phytochemical studies revealed that apple, papaya and lemon are rich source of carotenoids, esters, flavonoids, phenolic acid, terpene and vitamins. Apple was found to have a number of antiviral compounds like phytoestrogens, procyanidins and rosmarinic acid. Although there are few reports of antiviral compound obtained from papaya but it has been reported to have evocative beneficial effects on immune system. The phytochemicals behave as strong anti-oxidant and anti-inflammatory agents which can help the body against dengue-induced inflammation and oxidation stresses. Several other features are also found in these reviewed phytochemicals that can protects the human body from the adverse effects of dengue infection. In nutshell, the consuming of papaya and apple should be incorporated in daily routine life especially during the season when this disease appears in its epidemic form.

Key words: Aedes aegypti, CD8+, CD4+, anti-oxidant, anti-inflammatory, platelet, hepatitis, secondary complications, Wolbachia

INTRODUCTION

During last ten years, the total number of dengue patients has been doubled (Bigongiari, 2010). WHO (World Health Organization) in 2010 also reported a graver concern is that about 100 million cases of dengue are not administrated by medical staff and 0.5-2.5% of these suffers with severe harmful consequences of virus (Deen et al., 2006). Dengue is a vector born virus and has four different types DENV-1, DENV-2, DENV-3 and DENV-4 called serotypes (Jahan, 2011). Its four serotypes originated approximately 1000 years ago and from past few hundred years, it starts infecting humans (Holmes and Twiddy, 2003). Dengue has large distribution due to its low

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entomological threshold level and it is mainly transmitted by the biting of infected *Aedes aegypti* and *Stegomyia albopicta* (Scott and Morrison, 2010; Cecilio et al., 2009). Many other dengue transmitting vectors are also known but several studies on *A. aegypti* describes its major significant role in transmitting the virus (Anderson and Rico-Hesse, 2006; El-Badry and Al-Ali, 2010; Focks et al., 2000; Harrington et al., 2005; Mohammed et al., 2008; Phongsamart et al., 2008). *A. aegypti* extensively favors the dengue dispersal due to its diverse distribution in phytotelmata and peridomestic environments like earthen pots, containers, tyres etc. (Adebote et al., 2008; Adebote et al., 2008; Bashar et al., 2005).

In Pakistan, Dengue may be observed throughout the year but high dengue inflectional period is from October to December (Khan et al., 2010; Tahir et al., 2010). According to a report on tertiary care hospital, in Pakistan large number of dengue infections is mostly detected during winter season (Wasay et al., 2008). Pakistan is one of the developing nations where approximately, one fourth of its population lives below the line of poverty (The World Bank). It is thus basic health care facilities are not readily available for most of the people resulting use of alternate medicines is a much common phenomenon (Qureshi et al., 2001; Khan et al., 2003; Karim et al., 2011; Sohail et al., 2011). Since last couple of years, dengue has emerged as an epidemic disease and use of papaya, apple and lemon extracts to treat dengue fever was found to be very common. But, there are not much scientific studies available to support the use of these extracts for the treatment of this disease. So literature was reviewed to find the potential beneficial activity of these plants against dengue on the basis of their reported phytochemicals. In addition a brief look at disease symptoms and approaches to control it has been provided under the lights of previous studies.

**EFFECTS ON HUMAN BODY**

Symptoms caused by dengue may be in overlap with other diseases. These symptoms are fever, headache, nausea, skin rash and ocular pain (Ali et al., 2011; Humayoun et al., 2010). Dengue patients suffer from gastrointestinal bleeding, headache and several neurological problems. It is also observed that 100% of the dengue patients have significantly lower values of platelets in their body while the lower number of neutrophils and leukocyte were also recorded in 58 and 88% of the patients, respectively (Hakim et al., 2011). Dengue also disturbs the liver by increasing the concentrations of alanine transaminase (ALT) and aspartate transaminase (AST), liver enzymes. There are chances of mild-severe hepatitis on infection with dengue, which increases the complications like gastrointestinal bleeding, hypertension, mental disorders and inflammation of gall bladder and ultimately leads to the death of patients (Almas et al., 2010; Parkash et al., 2010; Souza et al., 2008). Dengue also affects the muscle cells by causing inflammation, which may be due to the increased intracellular Ca²⁺ concentrations (Salgado et al., 2010). The development of disease symptoms directly correlates with the presence of IgM antigen of dengue (Tang et al., 2008). While the presence of viral RNA does not play any role in dengue caused symptoms, the disease severity could increase if patient again come into contact with different serotype of dengue virus (Sierra et al., 2010). This severity may be due to increased imbalance in immune system regulation. The dengue virus also harms its mosquito vector; it cause apoptosis in mosquito tissues during its life cycle (Shafee and AbuBakar, 2006a). The observed apoptosis was not the part of metamorphic programmed cell death as the apoptosis was directly proportional to the number of virus antigen positive cells.
HUMAN IMMUNE RESPONSES TO VIRUS

Today's vulnerable environmental and poor human health conditions make the global spread of dengue rapidly (Guzman and Istariz, 2010). Therefore, the understanding of significant features of human immune system in relation to viral attacks may help to reduce the severity of this disease. The human immune system is of two types called innate and adaptive immune system (Cooper and Herrin, 2010; Smith and Weyrich, 2010). The immunity against pathogen and injury is governed by both innate (e.g., through platelets) and induced (through a variety of lymphocytes e.g., T and B cells) immune systems. The T and B cells have antigen recognizing complex to identify the harming pathogens. On the other hand, platelets stabilize the disturbed homeostasis and translocate the information among immunity responsive T, B and neural cells (Varga-Szabo et al., 2008; Wannemacher et al., 2011). Monocytes (type of white blood cells) are the source of innate and adaptive immunity responses (Auffray et al., 2009; Serbina et al., 2008). As they take part in the development process of dendritic cells and macrophages, healing and clearance of pathogens. Moreover, these are also an important tool against DENV (Klomporn et al., 2010). Any pathogen like virus affects the various immune responses; it targets the antigen identification complex, apoptosis and hormonal immune responses (Tortorella et al., 2008). Virus causes the abnormality in the function of B cells, which may be due to some kinds of association developed between virus and B cells (Stamstaki et al., 2009). CD4+ (primary immune response) and CD8+ (secondary immune response) are the types of T cells and stop the invasion of virus (Jiang and Chess, 2004; Strowig et al., 2009) and number of these cells indicates the severity of disease (Ahasan et al., 2004; Kagone et al., 2011). The absence of these T cells in body can negatively increase viral-mediated diseases, as was observed in mouse infected with Epstein-Barr virus. The dengue virus receives more prevalent situation in mice depleted with CD8+ T cells while CD4+ T cells depletion didn't show any kind of effect (Yauh et al., 2009; Yauh et al., 2010). But the T and B cells are not the major inhibitors of virus particles instead macrophages play major role in the inhibition of virus (Blackley et al., 2007; Kou et al., 2008). Thus for a successful infection virus has to kill the macrophages but the T and B cells do have some antiviral effects.

Upon viral infection the CD8+ T cells enhanced the expression of Programmed Death 1 (PD1) responses with the help of CD4+ T cells (Barber et al., 2003; Petrovas et al., 2003). Virus triggers the expression of T regulatory cells (CD4+CD25+ T cells) and these cells suppress the proliferation of virus specific CD4+ T and CD8+ T immune cells (Boettler et al., 2005; Weiss et al., 2004). Thus CD4+CD25+ T cells are responsible for the pathogenic effects of virus in the body but its activity may be suppressed over time as observed in HCV recovered patients (Pearson et al., 2008). The platelet can destruct the virus by engulfing it (Torre and Pugliese, 2008; Youssefian et al., 2002). But if virus bound with platelet through specific ligands, it may alter the platelet's activity (Plaujac et al., 2010). The altered platelets activity can disseminate the virus within the body as Hantavirus causes the change in the platelets activity to favor its dissemination (Gavrillovskaya et al., 2010). Nitric oxide concentration plays a significant role in different body functions (Najati et al., 2008; Moazedi et al., 2010). Dengue virus elevates the nitric oxide (NO) levels, which inhibit the adhesion of platelets and cause bleeding (Mendes-Ribeiro et al., 2008). NO toxicity may be the main reason of dengue caused health problems (Chaturvedi and Nagar, 2009). Some dengue proteins e.g. Non-Structural Protein 1 (NS1) are homologue of coagulatory proteins (Lin et al., 2011). The resultant antibody produced against these viral proteins cross react with coagulatory proteins and damage the platelets. The homologue responsible portion of NS1 is the C-terminal and if C-terminal is removed, this viral protein would be unable in affecting the
platelets (Chen et al., 2009). The antibodies produced against NS1 also cause severe damages to liver, which may be due the elevated levels of AST and ALT enzymes (Lin et al., 2008a). The infection of dengue can be minimized by strengthening the immune responses.

As DENV does not affect the single cell type or organ of the body, it can be concluded that dengue has complex mechanisms of action. This may also be due to the reason that its infection in one type of cell can severely effects whole body health. For example, the T cells, which recognize any antigen of pathogen upon dengue-caused reduction, would be unable to recognize other antigens. Likewise, dengue caused reduction in platelets not only affects the coagulatory responses but also results in reduce signaling between other immune cell. Thus, there is not only the need to reduce the dengue infections but also to treat the other complications that originate as consequences of this disease.

CONTROL OF DENGUE

Viral diseases such as rabies, herpes, influenza, hepatitis and HIV have always remained as important discourse amongst health scientists in order to have better understanding of these diseases (Farooq et al., 2006; Oguntibeju et al., 2007; Ilboudo et al., 2009; Movahed and Shoa, 2010; Shabahang, 2010; Hassanzadeh et al., 2011). Studies based on the prevalence of these diseases helps to identify the geological distribution and factors responsible for their spread (Moghim et al., 2007; Ilboudo et al., 2007; Talai et al., 2007; Daryani et al., 2009; Sagna et al., 2010). The non-preventive measures for urbanization, globalization, modern air transport, water and waste management cause the rapid infestation of environment with dengue and its vector (Barboza et al., 2008; Gubler, 2010; Kyle and Harris, 2008; Ooi and Gubler, 2008; Ooi and Gubler, 2009). This huge spread of dengue can be minimized by modulating our environment like managing the cool air supply in the houses, use of insecticide treated materials and frequent distance from neighboring houses (Reiter et al., 2003; Vanlerberghe et al., 2011). There are two fundamental types of controls. They are (1) “before infection” and (2) “after infection” (Fig. 1). “Before infection control” is based on preventive measures to avoid disease e.g., vector control and vaccination. “After infection control” consists of efforts that are focused on suppression of virus attack and its related complications.

![Schematic Diagram](image)

Fig. 1: A schematic diagram describing the general layout of dengue disease management
REDUCTED DISSEMINATION OF VIRUS

The best way to control a virus is to control its vector; A. aegypti is the main vector of dengue virus and control of this vector will definitely result in the reduced spread of this disease. Thus, the spread of dengue can be controlled by elimination of its vector A. aegypti; many studies have been conducted in this regard (Morrison et al., 2008; Paulraj et al., 2011; Raghavendra et al., 2011). The control of A. aegypti is necessary as it also causes another important lethal disease, malaria (Yasinzai and Kakarsulemankhel, 2003). Synthetic insecticides are not much effective against A. aegypti population as many of its strains have obtained the resistance against the commonly used insecticide and insects are only susceptible to the insecticide at specific life stage (Ahmad et al., 2007; Ocampo et al., 2011). Moreover, the use of these synthetic insecticides is also harmful for environment and causes stern health issues (Al-Jahdali and Bisher, 2007). So environment friendly and effective method like biological control of A. aegypti appears as more suitable approach. There are many approaches to biologically control the virus’s vector e.g. Bacillus thuringiensis israelensis (Alam et al., 2008). Its ‘Cry’ proteins have potential to control A. aegypti but these are sensitive to heat; heat suppresses the proteins’ activity. The marine environment also offers biological control against this mosquito species in the form of sponges like Clathria gorgonoides, Calyspsiongus diffusa, Haliclonina pigmentifera, Sigmadocia carnosa etc. (Sujatha and Joseph, 2011). Among these C. gorgonoides and C. diffusa showed the significant larvicidal activity at V instar larvae stage. The marine algae also showed larvicidal activity against A. aegypti (Manilal et al., 2011). The potentially important larvicidal activity was found in Lobophora variegata. Another important biological control of A. aegypti is Wolbachia. It causes alternations in host reproductive organs and modulates the host genomic expression to confirm its establishment in the host body (Hussain et al., 2011; Saridaki and Bourtzis, 2010). The Wolbachia strains cause viral transmission reducing phenomenon in mosquito like shortened life-span and cytoplasmic incompatibility (McMeniman et al., 2009; Yeap et al., 2010). It also lessened the feeding success of mosquito on human body which may be due to the tissue damage caused by Wolbachia (Moreira et al., 2009a; Turley et al., 2009). Less feeding eventually result in less transmission of virus. The potential of Wolbachia to infect the mosquito depends upon the Wolbachia strain type; establishment of genetically different Wolbachia strains may cause more promising effects (Walker et al., 2011; Xi et al., 2005).

The spread of dengue can also be limited by the use of nanoparticles. The nanoparticles of silver upon exposure to UV radiations show the efficient ability to kill A. aegypti larvae, in a concentration dependant manner (Sap-Iam et al., 2010). The virus transmission can also be controlled, if the immune system of A. aegypti is strong enough to reject the virus infection (Sanchez-Vargas et al., 2009; X et al., 2008). Ahmed et al. (2008) observed that the application of Nigella sativa derived thymoquinone strengthen the immune responses of A. aegypti. In later studies it was observed that Wolbachia can provide strength to anti-dengue immune responses of A. aegypti (Bian et al., 2010; Premi et al., 2010; Moreira et al., 2009b). It enhances the expression of immune genes of A. aegypti against dengue infection and it strongly reduces the number of virus particles in the mosquito tissues. Among all the vector control methods, Wolbachia mediated biological control appears to be the prominent option.

VACCINES FOR DENGUE

Dengue has different mechanism of actions depending upon the type of cell line and in humans the immunity responses are highly dependent on their genetic polymorphism (Chaturvedi et al.,
Today's approach is to obtain anti-dengue vaccines but these vaccines are still under development due to their adverse effects and short-lived immunity responses (Guirakhoo et al., 2006; Kanesa-Thasan et al., 2001; Guzman et al., 2010; Murphy and Whitehead, 2011). The virus particles based vaccine can be used to develop the immunity against dengue (Mota et al., 2005; Shafee and AbuBakar, 2006b). Tambunan and Parikesit (2011) provide the in silico design of E DENV vaccines for dengue-2 and 3. E DENV are the virus proteins which facilitate the attachment of virus on the host cell. But the functionality of these vaccines also depends upon the genetic system of human being so there is need of more computational power to fully express the efficiency and establishment of these vaccines. The intramuscularly applied plasmid of DEN-2 non-structural protein 1 (NS1) in mice causes decrease in morbidity and ultimately leads to the increased chances of survival (Wu et al., 2003). The immunity in the newborn of this NS1-treated mouse was more pronounced. But the increasing demand of DNA derived vaccines has increased the need of large quantities of pure DNA plasmid and the extraction of DNA plasmid is an important and difficult procedure (Duarte et al., 2007; Li et al., 2008a). Moreira et al. (2007) found the PEG 400 (20/20% w/v) system as the best PEG/phosphate system for the extraction of dengue 2 plasmid DNA vaccines from the lysate cells of E. coli. It can extract 37% of DNA plasmid. Thus this can be concluded that the development of vaccines to cure dengue has many limitations and there is need of more research on their efficiency and production.

ANTIVIRAL AGENTS

In nature many compounds are present that can be employed against viral diseases (Barsanirnivassan et al., 2009; Montaz and Abdollahi, 2010; Vignesh et al., 2011). Squalamine, a compound obtained from dogfish shark and sea lamprey has the potent antiviral activity against many viruses including dengue (Zasloff et al., 2011). In vitro it has concentration dependent protective effects on human endothelial cells against dengue. Nearly thirty seven licensed antiviral drugs are present today, yet no reliable anti-dengue drug is present (De Clercq, 2004; Czeizel et al., 2006). But, dengue proliferation can be controlled by targeting the vulnerable sites of dengue life cycle using the small drug molecules (Wilder-Smith et al., 2010). According to Schul et al. (2007) the use of antiviral drugs even after acute dengue infection can significantly cause the reduction in virus particles and virus-caused disease. They further proposed that AG129 mouse model is an appropriate object to study the anti-dengue drugs. Another chemical compound NITD008, which is the analogue of adenosine, also showed the potential antiviral activity against many vector-borne viruses especially dengue (Yin et al., 2009). It shows the dengue virus titer and virus-caused disease reducing ability in both in vitro and in vivo studies. But the significance of any anti-dengue drug is needed to check in human trials. Moreover the antiviral drug should also be checked on the basis of its origin, cost, cytotoxicity, purification etc. (Sorisko et al., 2007). As many natural anti-dengue drugs derived from plants were rejected in past due to their difficult extraction and cytotoxic effects. Thus the development of anti-dengue drugs has many hurdles, but their development should be checked on critical basis.

PHYTOCHEMICAL STUDY OF APPLE, LEMON AND PAPAYA FOR THE TREATMENT OF DENGUE FEVER AND ITS RELATED COMPLICATIONS

The daily intake of 400-600 g of fruits and vegetables prevents from many diseases (Heber, 2004). The phytochemicals found in these plants have many therapeutic effects on human body (Garba et al., 2006; Musa et al., 2008; Yang et al., 2008a; Prakash and Gupta, 2009;
Table 1: Some reported phytochemicals of apple, lemon and papaya

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Phytochemicals name</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phytochemicals of Apple</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin</td>
<td>Ascorbic acid</td>
<td>Li et al. (2008b), Vanzani et al. (2005)</td>
</tr>
<tr>
<td>Phenolic acid</td>
<td>Caffeic acid, Chlorogenic acid, Coumaric acid and Derivatives, Gallic acid, Ursolic acid, Rosmarinic acid</td>
<td>Anzad et al. (2010), Boyer and Liu (2004), Ceymann et al. (2011), He and Liu (2007), He and Liu (2008), Hosain et al. (2009)</td>
</tr>
<tr>
<td><strong>Phytochemicals of Lemon</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Limettin, Quercetin and Vicenin-2</td>
<td>Akhila et al. (2009), Ghasemi et al. (2009), Gorgus et al. (2010), Ortuno et al. (2011), Ramful (2011)</td>
</tr>
<tr>
<td>Vitamin</td>
<td>Ascorbic acid</td>
<td>Burdurdu et al. (2006), Ramful (2011)</td>
</tr>
<tr>
<td>Phytoalexin</td>
<td>Scoparone</td>
<td>Ortuno et al. (2011)</td>
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<tr>
<td>Terpene</td>
<td>Limonene, β-Pinene, Nerol, Geraniol</td>
<td>Gattuso et al. (2007), Pandey et al. (2010), Vekiar et al. (2002)</td>
</tr>
<tr>
<td><strong>Phytochemicals of Papaya</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin</td>
<td>Ascorbic acid, Vitamin A, Vitamin B&amp; B12</td>
<td>Imaga et al. (2010b)</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Kaempferol, Quercetin, Myricetin and Rutin</td>
<td>Mian and Mohamed (2001), Rivera-Pastrana et al. (2010)</td>
</tr>
<tr>
<td>Phenolic acid</td>
<td>Ferulic acid, Chlorogenic acid, Caffeic acid, p-Hydroxybenzoic acid and Vanillic acid</td>
<td>Camini et al. (2007), Rivera-Pastrana et al. (2010)</td>
</tr>
<tr>
<td>Ester</td>
<td>Benzaldehyde, β-damascenone and benzyl isothiocyanate</td>
<td>Zhou et al. (2011)</td>
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<tr>
<td>Anthracene derivative</td>
<td>Anthraquinone</td>
<td>Lee et al. (2010)</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Lycoene and β-carotene</td>
<td>Ayoola et al. (2008), Imaga et al. (2010a)</td>
</tr>
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</table>

Hussein et al., 2010; Karthikeyan and Mirunalini, 2010; Asmawi et al., 2011). These are also responsible for the protective effects against inflammation, oxidation, neural-degenerations, etc. hence these chemicals inhibits the metabolic-dysfunctioning (Ekor et al., 2003; Oloyede et al., 2008; Nirmala et al., 2008; Potchoo et al., 2008; Wahab et al., 2009; Sivabal and Anuradha, 2010; Hasani-Ranjbar et al., 2010; Patil and Patil, 2011; Lawal et al., 2011). The papaya leaf extract, apple and lemon juice have been recommended by the folks to treat dengue fever but there is very little scientific literature available to support the use of these plant remedies. So in next section some of the important phytochemicals have been explored with reference to their potential therapeutic attributes (Table 1).

**PHYTOCHEMICAL SIGNIFICANCE OF APPLE**

Apple is from one of the major fruits of Northern areas of Pakistan and has many medicinal uses (Sher et al., 2010). The methanolic and aceton extracts of apple pomace have antioxidant and antiviral activity against some viruses (Suarez et al., 2010). One of apples' phytochemicals ‘phytoestrogens’ has the potential anti-dengue property, it is also found in other fruits and have strong antiviral actions (Torres-Sanchez et al., 2000; Martin et al., 2007; Ogbuewu et al., 2010).
Sialic acid is an essential requirement of eukaryotic cells and accounted for the normal development (Schwarzkopf et al., 2002; Balean and Sahin, 2006; Senthil et al., 2007; Pavana et al., 2008; Mathur et al., 2010). Dengue causes the oxidative stress in the body by oxidating the plasma proteins and lowering the sialic acid concentration (Rajendiran et al., 2008). Thus antioxidants can help in maintaining the protein structure disturbed by oxidative stress. Apple contains many strong antioxidants like catechin and phloridzin (Boyer and Liu, 2004; Gilani et al., 2003; Beg et al., 2008; Gupta et al., 2008; Falah et al., 2008; Lelono et al., 2009; Rivas-Arreola et al., 2010; Kaur and Saraf, 2011), which can reduce the oxidative stress. The strong antioxidant like reduced glutathione (GSH) has the capability to inhibit the dengue production in the body and hence reduce the virulence of dengue (Tian et al., 2010). The apple juice has the excellent antioxidant ability; also promote the expression of anti-oxidating glutathione enzyme genes (Kujawska et al., 2011; Soyalan et al., 2011). The peels of unripe apple have oligomeric procyanidins, which simulate the innate immunity responses in DENV-infected cells (Kimmel et al., 2011). Many other procyanidins e.g. B1, B2, C1 etc. are also found in apple and procyanidin B2 was also detected in the human serum after the intake of procyanidin-rich food (Sano et al., 2003; Shoji et al., 2003). Procyanidins may have antiviral activities e.g., procyanidin B1 inhibits the hepatitis C virus by suppressing its RNA synthesis (Li et al., 2010; Zhuang et al., 2009). In Huh-7 cells procyanidin B1 showed concentration dependant effect against virus. Depending on this information this can be said that the procyanidins found in apple might have some antiviral activity. Thus apples not only directly can reduce the dengue infections but it also possesses indirect beneficial effect on dengue affected persons.

Catechin, the most active antioxidant flavonoid is found in apples and results based on HPLC and GC/MS studies showed its concentration 1.01 mg/kg w/w of apples (Sim et al., 2010). According to Pignatelli et al. (2006) catechin only in combination with quercetin can cause the recruitment of platelets. This recruitment of platelets was resulted by the inhibition of PKC-dependent NADPH oxidase activation. Coumaric acid is another medicinally known compound found in apple. p-Coumaric acid (isomeric form of coumaric acid) has antioxidant, anti-coagulantary and hepatoprotective activity with high absorption in the rats’ gut (Choi et al., 1998; Lee et al., 2008; Luceri et al., 2007; Zhang et al., 2007). It can reduce the ethanol caused oxidation and can inhibit the ADP-induced platelets aggregation by enhancing the plasma antioxidant activity. In addition it also reduces the thromboxane B2 production, which occurs during ADP-induced aggregation but it doesn’t showed any effect on platelet count and mean platelet volume. Coumaric acid contents of apple may be beneficial in reducing the oxidation and inflammation problems caused by dengue infections.

A gas chromatography-mass spectrometry study on apple skin confirms the presence of an antioxidant compound called rosmarinic acid (Amzad et al., 2010; Koroch et al., 2010). Rosmarinic acid has a potential antiviral activity against Japanese Eencephalitis Virus and HIV-1 (Dubois et al., 2008; Swarup et al., 2007). It inhibits the replication of both of these viruses. It also possesses the antithrombotic effect in the wistar rats’ vena cava, which may be due to the inhibition of collagen induced platelets aggregation (Zou et al., 1998). Thus this can be said that apple is a rich source of phenolic and polyphenolic compounds which are of significant medicinal importance. Some of these compounds have antiviral effects, which may play role in controlling the dengue virus but further investigation is still required.
PHYTOCHEMICAL SIGNIFICANCE OF LEMON

Lemon is a common cultivated plant in Pakistan, where in some areas its fruits are used commonly to treat the teeth problems (Hussain and Ishtiaq, 2009; Hussain et al., 2010). But many other characteristics can also be ascribed to this plant due to the presence of a range of important phytochemicals (Table 1). Scoparone is a phytoalexin; an immunoregulatory compound of lemon, which induces the reduction in NO levels by suppressing the expression of iNOS genes (Kim et al., 2007; Ortuno et al., 2011; Yang et al., 2008b; Yang et al., 2009). Thus immunoregulatory activity of scoparone could be considered as positive role in treating the dengue caused immunological problems. The other biologically important antioxidant compound of lemon is Vicenin-2, a flavonoid, which may has the anti-inflammatory activity (Aquila et al., 2009; Barreca et al., 2010; Ramful et al., 2010). Another medicinally important compound, limonene is the natural terpene found in different plants including lemon, has low toxicity; it reduces the heart burn and gastroesophageal reflux in dosage dependant manner (Al-Howiring, 2003; Talei and Meshkatalsadat, 2007; Gattuso et al., 2007; Meshkatalsadat and Mirzaei, 2007; Yoon et al., 2009; Di Vaio et al., 2010; Sun, 2007). It cause an increase in the concentrations of cytosolic calcium and cAMP and proteins kinase activity, which may energize the antiviral and anti-inflammatory immune responses (Hirota et al., 2010; Park et al., 2010; Romeilah et al., 2010). The curative properties of lemon can also be enjoyed by utilizing its essential oils, which have the excellent antioxidant property (Campelo et al., 2011). The other compounds of lemon with antioxidant property are neral, neryl acetate and geraniol (Tansi and Nacar, 2000; Vekiari et al., 2002; Meftahizade et al., 2010; Kadri et al., 2011). Thus lemon has many phytochemicals which can support the immune responses against virus caused problems, especially through its anti-oxidant agents.

PHYTOCHEMICAL SIGNIFICANCE OF PAPAYA

Carica papaya is an important plant with significant medicinal properties e.g. anti-inflammatory, antimicrobial and wound management (Rahmet et al., 2002; Saeed and Tariq, 2008; Raji et al., 2006; Zakaria et al., 2006; Oladumoye and Osho, 2007; Idu and Onyibe, 2007; Oladimeji et al., 2007; Goyal et al., 2009; Ajila et al., 2010; Ansari et al., 2011; Osadolor et al., 2011). It is also an important source of many phytochemicals (Table 1). On administration of papaya in its powder form at the rate of 5 mg kg⁻¹ of body weight, it can significantly increase the platelet count of dengue infected patients (Sathasivam et al., 2009). In another research it is found that its leaf aqueous extracts are responsible for a significant increase in platelets count, white blood cells and neutrophils in dengue infected patient (Ahmad et al., 2011). The fermented preparations of this fruit have antioxidant activity; it increases the reduced glutathione concentration in red blood cells and decreases the reactive oxygen species (Fibach et al., 2010). The papaya extracts also shows the positive effects on other immune responsive cells like macrophages; its extracts have a positive effect on the macrophage antiviral properties (Lidbury and Mahalingam, 2000; Rimbach et al., 2000; Ishikawa and Miyazaki, 2005; Mahbub-E-Sohbani et al., 2011; Du et al., 2011). The macrophages upon viral infection are responsible to produce antiviral antibodies. So this can be said that papaya may have many healthy effects on dengue infected patients due to its positive regulation of macrophages and platelets.

The medicinal importance of papaya phytochemicals is elucidated here to make an awareness of its remedial uses against dengue caused problems. Anthraquinone an important photochemical of may plants also found in papaya either in free or in bound form and many studies reported that
various anthraquinones have antiviral property against different viruses (Semple et al., 2001; Li et al., 2007; Kumar et al., 2007; Hassan et al., 2007; Imaga et al., 2010a; Sonibare et al., 2011; Xiong et al., 2011). The anthraquinone derivatives also possesses the anti-coagulatory activity, its derivatives have the potency to reduce the thrombin, arachidonic acid, collagen and platelet-activating factor-induced platelet aggregation (Baqi et al., 2009; Gan et al., 2008). These derivatives have potential to activate the CD34+ dendritic cells, which is important for immunological responses (Van de Ven et al., 2011). The anthraquinone derivatives are also important in stimulating the proliferation of resting human peripheral blood mononuclear cells and lymphocyte (Cherng et al., 2008). Another therapeutic compound of papaya is myricetin, which possess the in vitro anti-inflammmatory activity and can reduce the acetic acid-induced capillary permeability (Miean and Mohamed, 2001; Wang et al., 2010). It also has the sedative activity against chemical (acetic acid and formalin) caused neural problems; it may also have the anti-platelet activity (Tong et al., 2009). So both of these compounds have anti-inflammatory effects and can modulate the immunological anti-dengue responses.

Another flavonoid ‘kaempferol’ present in papaya, which showed antimicrobial (Taechowisan et al., 2008) and strong anti-inflammmatory activity by reducing the NO levels (Hamalainen et al., 2007). It is responsible for dose dependent reduction in NO levels, which was governed by the reducing in iNOS proteins and mRNA expression. Papaya is an important source of some vitamins like vitamin A, B12 and Folic acid, which might have some contribution in its therapeutic properties (Iywade and Onigbinde, 2006; Wall, 2006; AL-Sowyan, 2009; Imaga et al., 2010b; Jiao et al., 2010). Vitamin B12 deficiency can cause abnormalities in central nervous system, which may increase the complications in dengue patients with already affected nervous system (Bordignon et al., 2008; Scalabrino, 2009; Yauch and Shresta, 2008). Thus the presence of vitamin B12 may has healthy impact on dengue infected patients. Deficiency of vitamin A may also contribute to immune defects and can increase the prevalence of several diseases (Saeed et al., 2005; Lin et al., 2008b; Sommer, 2008; Uboh et al., 2008; Uboh et al., 2009; Qiu et al., 2010; Iribhogbe et al., 2011). Dengue virus causes aplastic anemia which can be inhibited by the application of folic acid (Albuquerque et al., 2009; Ganji and Kafai, 2009). Iron-folic acid can decrease the rate of anemia especially in women; by the increasing the haemoglobin levels (Casey et al., 2010). It also protects the endothelial cells from the oxidative stress by increasing the expression of dihydrofolate reductase (Gao et al., 2009). Dihydrofolate reductase regulates the tetrahydrobiopterin and NO superoxide production to suppress the oxidative stress (Crabtree et al., 2011). Ferulic acid is the form of phenolic acid is an important subject of antioxidant activity and so can protect the body from many health problems including neural disorders (Kanski et al., 2002; Srinivasan et al., 2007). Its antioxidant property is basically due to its functional hydroxyl and phenoxy groups. Lycopene is another anti-oxidant compound of papaya. It has the potential antioxidant property and thus inhibits the liver from oxidative stress (Serem et al., 2008) and might be helpful to reduce the HCV related complications. Bignotto et al. (2009) studied the anti-inflammatory effects of lycopene in two rat models. It was observed that lycopene impose a strong anti-inflammatory activity at 25 and 50 mg kg⁻¹ concentrations in both paw oedema and ischaemia-reperfusion models of rat. Herzog et al. (2005) noted that lycopene administration causes a decrease in inflammation causing agents like interleukin-1β, CXC chemokines, etc. Thus lycopene is an efficient anti-inflammatory agent and it also showed preventive effect on chromosomal aberrations (Aslanturk and Celik, 2005). Another phenolic acid named ‘vanillic acid’ is also present in papaya and some other plants (Mehboob et al., 2000; Tajuddin et al., 2002; Shaukat et al., 2003;
Jazayeri et al., 2007; Zhou et al., 2011). Vanillic acid possesses a strong hepatoprotective activity, as it decreases the activity of transaminase enzyme and disorganized hepatic sinusoids (Itoh et al., 2009). In addition, it also protects the liver from immune-induced liver injuries by decreasing the concentration of inflammatory cytokines, interferon (IFN)-gamma and other liver infecting agents. This investigation of papaya properties shows that papaya has many therapeutic properties and it is a rich source of highly protective biological compounds, which can treat many health problems.

**SHARED PHYTOCHEMICALS AND THEIR SIGNIFICANCE**

The three studied fruits have some common curative phytochemicals like ascorbic acid (vitamin C) and quercetin (Akhila et al., 2009; Bari et al., 2006; Chasemi et al., 2009; Li et al., 2008b; Sultana and Anwar, 2008; Ramful et al., 2011; Wach et al., 2007). The curative antioxidant ascorbic acid is an important coagulatory nutrient, which may reduce the severity of oxidation and anticoagulation problems of dengue infection (Fromberg et al., 2011; Savini et al., 2007; Padayatty et al., 2003). It has other remedial properties, which may able to treat dengue caused problems. Like, it has hypotensive property and can reduce the vascular tension in Stroke-Prone Spontaneously Hypertensive Rats (SHRSP) (Chen et al., 2001; Sato et al., 2011a). It can strongly reduce the oxidative stress in mice liver caused by high iron diets but it did not play any role in maintaining the physiological processes under low iron concentrations (Premkumar et al., 2007). Ascorbic acid also have anti-inflammatory responses, it significantly reduce the amount of inflammation causing C-reactive protein (Black et al., 2004; Block et al., 2009; Du Clos and Mold, 2004). Thus ascorbic acid in the presence of physiologically enough iron may be an important candidate of antioxidant properties. Quercetin is a strong antioxidant candidate (Cibin et al., 2006; Jun et al., 2007) and can modulate the memory impairments (Sternberg et al., 2008; Naseri et al., 2008; Ebrahimzadeh et al., 2009; Bahri-Sahloul et al., 2009; Tota et al., 2010). It also acts as an anti-inflammatory agent and protects the body from kinase activity of platelet, which cause coagulation (Bischoff, 2008; Navarro-Nunez et al., 2010; Van der Meijden and Heemskerk, 2010). It doesn’t has direct effect on coagulation causing stimulus (like thrombin) instead it interfere with the signaling pathway and stops the platelets aggregation (Nunez et al., 2009).

Caffeic acid and chlorogenic acid are the two phenolic acids shared by the papaya and apple (Bouyed et al., 2007; Boyer and Liu, 2004; Chinnici et al., 2004; Canini et al., 2007; He and Liu, 2008; Rivera-Pastrana et al., 2010). Caffeic acid has the antiviral property, its application before the infection can effectively reduce the replication of herpes simplex virus type 1 (Ikeda et al., 2011). The caffeic acid esters have noteworthy antioxidant properties, it possess negative effect on the collagen-induced platelet aggregation (Bakasso et al., 2008; Hsiao et al., 2007; Jayaprakasam et al., 2006). Its ester causes the direct inhibition of collagen binding to the platelet by binding to the platelets’ collagen receptor. Hence it reduces the collagen induced platelet aggregation, which may help to cope with heart problems of coagulation. Caffeic acid is also produced in intestine by the hydrolyzation of chlorogenic acid; chlorogenic acid is an antioxidant polyphenol (Sato et al., 2011b). But caffeic acid has more pronounced antioxidant effects than chlorogenic acid. The chlorogenic acid plays role in anti-inflammatory reactions by inhibiting the neutrophil locomotion and adhesion (Hebeda et al., 2011).

All studied fruits are good source of important phytochemicals, which possesses different biological properties. The phenolic compounds of these studied fruits have chief antioxidant property, which is important for treating many health problems. The variety of phytochemicals
provides variety of benefits to health as the phytochemicals have positive effects on immune-responsive cells. They also provide benefits to memory impairments.

CONCLUSION
Dengue is a vector born disease with a complex mechanism of action, as it can directly or indirectly destroy the activity of many immune cells. Its adverse effects are due to highly suppressed immune responses through IgM antigen of dengue. Figure 1 shows a general layout scheme for the approaches to deal with dengue virus. Its vector control appears to be the best approach because antiviral drugs are not much successful for this disease. Vaccines for dengue could be another preventive measure but these vaccines are still in developmental phase. In addition, these antiviral drugs and vaccines are not much common in developing countries where this disease is transforming into an epidemic. After viral infection treatment totally concentrates either in the suppression of viral particles or reduction in the severity of related complications. Papaya and apple both comprised of phytochemicals that have potential antiviral activities but lemon was only found useful in dealing with secondary complication issues. All of these three plants have significant antioxidant activities which might be helpful in reducing the indirect oxidant effects of this virus on different human tissues. After dengue infection the best approach seems to be the improvement of patient’s immune response, especially the platelets count in body. Papaya and apple both have been reported for their ameliorating effects on immune system. On the basis of reviewed literature it can be concluded that both papaya and apple have their potential use in the treatment of dengue. There is future need of clinical studies to deeply investigate the phytochemistry of papaya and apple to identify their best possible use in the treatment of dengue fever. In a developing country like Pakistan with weak health care system the use of medicinal plant (such as apple and papaya) should be promoted as cheaper and easily available alternate medicine source to deal with epidemic diseases like dengue.

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