EVALUATION OF PLATELET AUGMENTATION ACTIVITY OF *CARICA PAPAYA* LEAF AQUEOUS EXTRACT IN RATS

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Abstract

Carica papaya leaves have been used traditionally to treat indigestion, as a vermifuge. *Carica papaya* leaves have also been shown to possess anti-tumor and immunomodulatory effects. The current study aims at determining the effect of *Carica papaya* leaves aqueous extract in increasing the platelet count in thrombocytopenic rat model. Aqueous extract of *Carica papaya* leaves at concentration of 400mg/kg and 800mg/kg were given to cyclophosphamide induced thrombocytopenic rats for a period of fifteen days. Blood was withdrawn at various time intervals to determine the platelet count. Also, the clotting time was determined on the 15th day of the study by capillary method. *Carica papaya* leaf extract was found to increase the platelet count and also to decrease the clotting time in rats. The study aims at determining the possible effects of papaya leaves in thrombocytopenia occurring in dengue fever.

Key words: *Carica papaya*, Dengue, Thrombocytopenia, Platelets.

1. Introduction

Dengue is a viral disease, spread by mosquitoes of the genus *Aedes,* primarily *Aedes aegypti*, which is today the most important arboviral disease worldwide in terms of morbidity, mortality and economic impact[1]. The causative agents of dengue fever are dengue viruses, which have four antigenically different serotypes viz; DEN 1 to DEN 4 and belong to the genus flavivirus of the flaviviradae family[1]. Other mosquito species of *Aedes* genus responsible for spreading dengue include *A. albopictus, A. polynesiensis and A. scutellaris*[2]. The symptoms of Dengue hemorrhagic fever, the most serious form of dengue fever, are hypotension, increased vascular permeability, thrombocytopenia and hemorrhagic manifestations. Thrombocytopenia is

rare blood disorder that affects the platelets of the blood. Characteristics include low platelet count (100000 cells per mm[3] of blood or less) and low platelet survival time. Other symptoms include a tendency to bleed excessively into mucous membranes, especially during menstruation[3]. Recently, *Carica papaya* leaves have been successfully employed in folk medicine for the treatment of dengue infections with haemorrhagic manifestations, using suspensions of powdered leaves in palm oil. *Carica papaya* L. belongs to the plant family Caricaceae. A lot of work has been carried out on plant parts like fruits, seeds and roots, indicating the presence of biologically active compounds[4]. Cyclophosphamide is a synthetic alkylating agent been used for its antineoplastic and immunosuppressive activities, and was introduced as an antitumour agent in 1958. Cyclophosphamide was used as toxicant in the current study because of its capacity to induce stable thrombocytopenia [5]. In the current study, the ability of *Carica papaya* leaf aqueous extract in increasing the platelet count in cyclophosphamide induced thrombocytopenic rat model was evaluated.

2. Materials and Methods:

Plant material

Carica papaya leaf aqueous extract was obtained by sonication the powdered leaf material in water at 37° C.

Animals

Albino Wistar rats (100-125g) of either sex were used for the study. They were housed under standard conditions of temperature and light and fed with standard diet and water *ad libitum*. Experimental protocol was approved by Institutional Animal Ethics Committee.

Platelet augmentation activity

The rats were divided into four groups of six animals each: Group I was treated with saline (10ml/kg p.o) for a period of fifteen days; Group II served as toxicant group and was given cyclophosphamide (50mg/kg s.c) for three consecutive days; Group III was treated with *Carica papaya* leaf aqueous extract(400 mg/kg p.o) for a period of fifteen days along with cyclophosphamide (50mg/kg s.c) for first three days; Group IV was treated with *Carica papaya* leaf aqueous extract(800 mg/kg p.o) for a period of fifteen days along with cyclophosphamide(50mg/kg s.c) for first three days.

Blood was withdrawn from retro-orbital plexus on the 1st, 4th, 7th and 11th day of study after subjecting the animals to light anesthesia using ether and platelet count was determined by using automated cell counter Coulter Act-Diff[6] (Patrick and Singh, 2011). On the 15th day,

the clotting time of blood was determined by capillary method.

3. Results:

Platelet count:

Carica papaya leaf aqueous extract at concentrations of 400mg/kg and 800mg/kg were found to significantly increase the platelet count in cyclophosphamide induced rat model.

Clotting time: The clotting time of the treatment group were found to be considerably lower than that of toxicant group.

Statistical Analysis:

Values are mean ± SEM. One-way ANOVA followed by Tukey-Kramer posttest was applied for statistical analysis and the level of significance was set at $p \leq 0.05$.

Table 1. Effect of *C. papaya* leaf aqueous extract on platelet count in rats

Treatment group and dose (mg/kg)	**Mean Platelet count**			
	Time interval in days			
	1	**4**	**7**	11
Normal Control	713000 ± 19183	746000 ± 12359	722667 ± 17701	719167 ± 22773
Toxicant control (cyclophosphamide) (50mg/kg)	705667 ± 70610	608833 ± 39661	555167 ± 33787	164500 ± 13304
Caric papay a a aqueous extra ct (400mg/kg)	745333 ± 36741	721500 ± 33272[c]	633000 ± 26865	556833 ± 30414 [a]
Caric papay a a aqueous extra ct (800mg/kg)	894833 ± 26368 [c]	837333 ± 19249 [a]	1084000 ± 29871 [a]	831000 ± 16118 [a]

P values: [a] < 0.001, [b] < 0.01, [c] < 0.05 when Experimental groups compared with Toxicant control

Table 2. Effect of *C. papaya* leaf aqueous extract on clotting time in rats

	Mean clotting time (sec.) on day 15			
Groups	**Normal Control**	**Toxicant control (cyclophosphamide) (50mg/kg)**	***Carica papaya* aqueous extract (400mg/kg)**	***Carica papaya* aqueous extract (800mg/kg)**
Time (in secs)	97.5 ± 2.725	158 ± 7.073	129 ± 3.124 [a]	107 ± 2.06 [a]

P values: [a] < 0.001, [b] < 0.01, [c] < 0.05 when Experimental groups compared with Toxicant control

4. Discussion:

Dengue virus, the main cause of dengue fever induces bone marrow suppression. Since bone marrow is the manufacturing center of blood cells, its suppression causes deficiency of blood cells leading to low platelet count. Anaemia and spontaneous severe bleeding are the other consequences of bone marrow suppression. Dengue virus can bind to human platelets in presence of virus specific antibody and cause immune mediated clearance of platelets[7].
Spontaneous aggregation of platelets to vascular endothelial cell pre-infected by virus induces aggregation, lysis and platelet destruction. Anti-platelet antibodies generated after dengue virus infection causes destruction of platelets. Moreover, dengue virus causes platelet reduction and vascular alteration which is the principal factor causing haemorrhagic problems[8].

Vinca-alkaloids have been proven effective against anti-platelet macrophages in patients suffering from Idiopathic Thrombocytopenic Purpura (ITP)[9]. The saponins in Panax notoginseng have been shown to reduce platlet adhesion and aggregation, prevent thrombosis and improve microcirculation[7]. *Carica papaya* leaves contain various phytoconstituents like saponins, tannins, cardiac glycosides and alkaloids. The alkaloids present include carpaine, pseudocarpaine and dehydrocarpaine I and II. These constituents can act on the bone marrow, prevent its destruction and enhance its ability to produce platelets. Moreover, it can also prevent platelet destruction in the blood and thereby increase the life of the platelet in circulation.

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