



EFFECT OF FRESH PAPAYA LEAF EXTRACT ON VASCULAR SMOOTH MUSCLE UPASANA KACHROO

Department of Physiology, CHRISTIAN MEDICAL COLLEGE

Abstract : *Carica papaya* is a common tree or shrub cultivated across various tropical countries including India mainly for its ripe or unripe fruit although multiple parts of the plant have been in use of late. Papaya leaves have been used in traditional medicine for the treatment of Dengue fever. The use of *C. papaya* leaf juice had been shown to improve the platelet count in patients suffering from dengue fever. The increase in platelet count may be due to direct effect on bone marrow or due to release of sequestered splenic platelets as a result of its contraction brought about by the juice. In his study, we assessed whether *C. papaya* fresh leaf aqueous extract (FLP-AE) has any direct effect on vascular smooth muscle using an isolated goat artery preparation in the form of a spiral arterial strip. This strip was suspended in an organ bath containing bicarbonate Ringer at 37 degree C and aerated with carbogen. The change in tone was recorded by an isometric force transducer connected to a data acquisition system (PowerLab). A resting tension of 2 g was applied and tissue allowed to equilibrate for 30 minutes following which viability was assessed by using high potassium bicarbonate Ringer. Intervention in the form of FLP-AE 140 mg in 20 ml bicarbonate Ringer was given (n-6). To rule out the effect of any ions (like potassium) present in the aqueous extract, chloroform extract was also used in two experiments. The results showed that FLP-AE in the dose of 140 mg in 20ml, did not cause significant change in vascular tone as compared to values obtained when tissue was perfused with bicarbonate ringer (P- 0.188, n- 6). The experiments performed with the chloroform extract also corroborated well with the results obtained from the aqueous extract. We therefore conclude that fresh papaya leaf extract does not cause change in contractility of vascular smooth muscle and hence the increase noted in platelet count following consumption of fresh papaya leaf juice may not be due to splenic contractions.

Keyword : Papaya, Dengue, Vascular smooth muscle, Arterial Strip

Introduction: Belonging to the family Caricaceae, *Carica papaya* (papaya) is a fast growing, erect, unbranched tree or shrub which is indigenous to Central America. It is now widely cultivated in many tropical countries namely Bangladesh, India, Sri Lanka, Indonesia, West Indies, Malaysia etc. for its fruit either in its raw or ripe form (1). There are numerous products of commercial value obtained from various plant parts and these remain easily available. There has been long standing use of *C.*

papaya leaves for treatment purposes in folk medicine (2). Recent literature indicated that this plant has potential anti- inflammatory, wound healing, immune modulatory as well as antioxidant properties (4). Recent reports also seem to suggest that the consumption of *C. papaya* leaf juice or crude extract is beneficial in the treatment of Dengue fever. An arthropod borne virus from the Flaviviridae family named Dengue virus causes this illness. The vector that transmits this disease is the *Aedes aegypti* mosquito. Till date, 4 distinct but antigenically related serotypes have been identified (1, 2, 3 and 4). Infection may be in the more common form of a mild fever known as Dengue Fever or it may present as a more severe form known as Dengue hemorrhagic fever and/or Dengue shock syndrome (DHF/DSS). Non-specific symptoms like head ache, retro orbital pain, body ache, nausea, joint pain, weakness and rash characterize most infections (6, 7). Virus incubation period varies between 3 and 14 days after an individual has been bitten. After onset of early symptoms described above, the ensuing fever usually records temperatures between 39-40°C and lasts for a period of about 5-7 days. If the virus gains entry into the peripheral blood stream and treatment is not issued timely, damage to blood vessels and lymph nodes may result in DHF with bleeds originating from the nose, gums, underneath the skin. Deterioration may lead to breathing difficulty and development of DSS which may prove to be fatal (8, 9). Treatment of patients suffering from dengue fever is predominantly supportive with periodic monitoring of hydration, platelet count and hematocrit. Of the studies conducted as individual cases or large scale studies involving multiple subjects, the oral consumption of *C. papaya* leaf juice/extract was seen to have a positive effect on patient outcome in terms of improvement in platelet count and increase in WBC number (4, 7, 10). This effect of the leaf extract was seen to be quite useful as viremia induced thrombocytopenia can put patients in the path of potential harm.

A study done on rats to look at the acute, sub-acute and chronic toxicity of *C. papaya* leaf juice showed that it is safe for oral consumption (5). Although the exact mechanism of action by which *C. papaya* leaf extract causes increase in platelet count is not known, many hypotheses for the same have been put forward. The active principle present in the leaf may directly influence hematopoiesis especially the production

of platelets over other cell types (4). Another hypothesis is that the extract may exert its action by causing splenic contraction thereby releasing a large number of platelets into circulation (11). To test the above hypothesis, this study attempts to observe the effect that fresh leaf extract of *C. papaya* will have on vascular smooth muscle tone

Materials and Methods:

Goat legs were obtained from the abattoir and transported in ice cold ringer. Legs from one animal were used for individual experiments and a total of six individual experiments were performed. Limb artery segment was isolated and made into a spiral strip. The strip was then fixed in an organ bath containing bicarbonate ringer solution at 37°C aerated with carbogen. Other end of the spiral strip was connected to an isometric force transducer and data acquired in real time by means of PowerLab data acquisition system to measure and analyze changes in isometric tension of muscle. After applying a preload of 2g, the strip was allowed to equilibrate for 30 minutes. To demonstrate viability of tissue, high potassium ringer was used and change in contractility was recorded. This was followed by 15 minutes of wash with bicarbonate ringer for tissue to return to baseline tension. The strip was then perfused with fresh papaya leaf aqueous extract (FLP-AE) 140 mg in 20 ml of bicarbonate ringer for 15 minutes and recordings made. Final wash with bicarbonate ringer was given and recording of force was continued for 15 minutes. To end the experiment, high potassium ringer was used again to demonstrate that tissue was still viable and functioning. The recorded data was analyzed using Wilcoxon signed rank test (SPSS version 17.) and a P value of 0.05 was considered as significant. Composition of solutions used: pH- 7.4 titrated using 1M NaOH Preparation

CHEMICAL	BICARBONATE RINGER (mM)	HIGH POTASSIUM RINGER (mM)
NaCl	115	35
KCl	3	80
MgCl ₂	2	2
CaCl ₂	2	2
NaHCO ₃	25	25
Glucose	10	10

Preparation of papaya leaf juice extracts Fresh papaya leaf- aqueous extract (FLP-AE) 25 grams of fresh leaves were weighed. After removing the veins, they were ground with 100 ml of distilled water. The resultant juice was centrifuged at 2500 rpm for 10 minutes. The supernatant was filtered and put into 5 ml aliquots for lyophilization. 140 mg of lyophilized extract was used for each experiment.

Fresh papaya leaf- chloroform extract (FLP-CE) 25 grams of leaves were weighed. After removing the veins, they were soaked in 250 ml of chloroform for 48 hours. The resultant liquid was filtered. Filtrate was air dried for a few hours and then kept in the incubator to dry. The dried up extract was scraped off and refrigerated until further use.

Results: FLP-AE in the dose of 140mg in 20 ml bicarbonate ringer did not show any significant change in vascular tone from the baseline value in any of the experiments conducted (P - 0.188, n-6; Table 1, Fig 1, Fig 3). Furthermore, when FLP-CE in the dose of 140 mg (in 5% ethanolbicarbonate ringer) was used to look for the effect of the extract devoid of any ions like potassium, no significant change from baseline value was seen when compared with tissue perfused with only the vehicle (5% ethanol-bicarbonate ringer solution) (Fig 2)

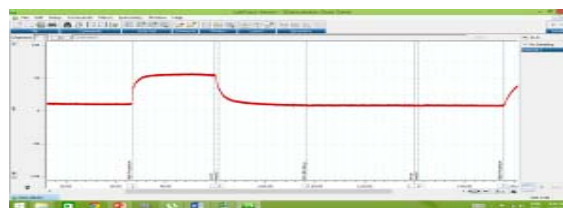


Fig. 1 shows representative tracing of the recording of isometric tension in spiral artery strip made through PowerLab. (Includes equilibration, perfusion with high potassium ringer, washes, perfusion with FLP-AE 140 mg)

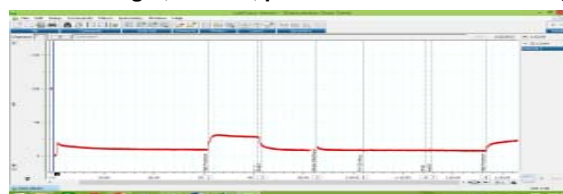


Fig. 2 shows representative tracing of the recording of isometric tension in spiral artery strip made through PowerLab while using FLP-AE 140 mg in 5% ethanol-bicarbonate ringer as vehicle.

Baseline (mN)	High Potassium (mN)	FLP-AE (mN)
10.8	22.9	10.5
9.7	46	9.43
9.73	24	10
6.6	30	6.4
7.8	54	7.6
9.6	38	6.37

Table 1. shows the values of isometric tension exhibited by spiral artery strip when perfused with Bicarbonate ringer (baseline), high potassium ringer and FLP-AE 140 mg. (n-6)

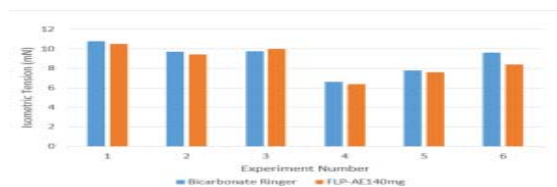


Fig. 3 shows the values of isometric tension exhibited by spiral artery strip when perfused with Bicarbonate ringer (baseline) and FLP-AE 140 mg. (n- 6)

Discussion: Our hypothesis at the beginning of the study was that fresh papaya leaf extract may cause contraction of vascular smooth muscle by virtue of which the sequestered platelets in the spleen are released into the circulation, thereby showing its effect in patients suffering from dengue where platelet count improves upon repeated consumption of this extract. Since in the experiments performed at our lab, FLP-AE in the dose of 140 mg did not produce any significant change in tension from the baseline value in any of the experiments performed (P- 0.188), it may be inferred that this phytochemical has a different mechanism of action by which the platelet number is increased. This observation was reaffirmed with the experiments performed with the chloroform extract (FLP-CE 140 mg) which was devoid of any ions like high potassium content usually seen in plant extracts (n-2). The improvement in platelet count may be a direct result of its action on the bone marrow promoting thromopoiesis. **Conclusion:** From our study

we conclude that fresh papaya leaf extract (at the dose of 140 mg) does not cause change in contractility of the vascular smooth muscle and hence the increase in platelet count following consumption of extract may not be due to splenic contractions. It would still be prudent to continue experimentation with this plant and understand its mechanism of action so that patients suffering from clinical conditions where improvement in platelet count is required would benefit from it.

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