WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 01, 1496-1504. Research Article ISSN 2277– 7105

EVALUATION OF THROMBOCYTOPENIA AND ITS RESTORATION POTENTIAL BY USING THE ETHANOLIC LEAF EXTRACT OF *CARICA PAPAYA*.L

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Article Received on
24 October 2014,

Revised on 15 Nov 2014,
Accepted on 07 Dec 2014

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ABSTRACT

Thrombocytopenia happens when body makes too few platelets, or the platelets That have been trapped in the spleen, or they are destroyed. If your spleen is enlarged, that can trap platelets, and they won't move through your body. Usually, thrombocytopenia has no symptoms. But when you do have them, they can include Lowercase b in bleeding, most often from the gums or nose. Red, flat spots on your skin, about the size of a pinhead. Blotches and bruises. This study aims to evaluate the possibility of using the leaves of *Carica papaya* to increase the level of platelets in the cases of thrombocytopenia, to screen and quantify various metabolites present in leaves and to identify the compound involved in the synthesis and restoration of thrombocytes. It is envisaged that this study would help in a preliminary understanding of the role of papaya leaves in altering the thrombocytes counts and also to identify the specific phytochemical involved in the reaction. Comparing the two bacteria used for this study it appears that *Salmonella enteritidis* is much more potent in bringing down the thrombocyte levels when compared to *Escherichia coli*.

KEYWORDS: *Carica papaya*, Thrombocytes, Thrombocytopenia, *Escherichia coli*, *Salmonella enteritidis.*

INTRODUCTION

Papaya (*Carica papaya* L.) is a tropical plant that contains diverse biologically active compounds of industrial and nutraceutical interest. It is one of the healthiest and nutritious

fruits in the world. It is a short-lived, unbranched plant with a hollow green or deep-purple stem (30-40 cm in diameter), which can grow up to 1.8 - 3.0 m each year.

Consumption of papaya may cut the risk of certain cancers. Treating human tumour cell lines with papaya leaf extracts resulted in apoptosis (cancer cell death) and inhibition of the growth of tumours. [1] Additionally, papaya fruits have several other applications, such as the relief of nervous pains and elephantoid growth, decrease in blood pressure in mice via adrenoreceptor route. [2]

The active components in papaya leaf extract have ascorbic acid, flavonoids, papain, chymopapain, cystatin, cyanogenic glucosides, and glucosinolates which help to increase the total antioxidant power in the blood and reduce oxidative damage. [3]

The capacity of papaya leaf extract to reverse the lowering of blood cells, especially platelets, is gaining attention in recent times. Decrease in blood platelets, a phenomenon referred to as thrombocytopenia, occurs during microbial infections, predominantly in cases of dengue, malaria etc. Regarding medical treatment of thrombocytopenia, not many suitable drugs are known. [4] Dengue is one of the most important viral diseases that is emerging as a major health concern mainly in tropical and sub-tropical regions of the world. There are no vaccines in the market for prevention of dengue fever. The main effect of reduced platelets count is an increased risk of bleeding, but this rarely occurs until there are less than 60 x 10^9 to 100 x 10^9 platelets per liter of blood. The normal platelet count in the circulating blood of a healthy person is typically between 150 x 10^9 and 400 x 10^9 per liter of blood. [5] There are sporadic reports on the online and print media based on individual experience that fresh papaya leaf extract has the marvellous ability to increase the platelet count in a short period of time. [6]

This study was embarked on to give a scientific basis to the folklore observations wherein fresh papaya leaf extract has been administered as a remedy for thrombocytopenia. The study was carried out at the laboratory level using chickens as the test organisms and lowering in platelet count was induced by infection with pathogenic strains such as *Escherichia coli* and *Salmonella enteritidis***.**

MATERIALS AND METHODS

Extraction of plant materials

The plant leaves of *C.papaya*, were collected, washed well and air-dried at room temperature (26°C) for 2 weeks, after which it was ground to a uniform powder. The ethanol extracts were prepared by soaking 100 g each of the dry powdered plant materials in 1 L of ethanol at room temperature for 48 h. The extracts were filtered after 48 h, first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40°C. The percentage yield of extracts ranged from 7–19 % w/w. [7]

Isolation of alkaloids and flavonoids

The isolation of alkaloids and flavonoids from the matured plant leaf extract was carried out by standard methods. [8]

Culturing the pathogenic strains

Escherichia coli and *Salmonella enteritidis* were grown on Brain Heart Infusion (BHI) media and incubated overnight at 37^0C. Then the harvested cells from the bacterial culture were checked for their purity status by gram staining. The density of the cells were adjusted to 10^9 cfu/ml by counting with haemocytometer.

Grouping of chickens

Totally 10 chicks were used for the study and were segregated into four groups as detailed (Table 1). To verify whether the administration of the pathogen or the plant extract had any effect on the physical parameters, the chicks were weighed and these reading were recorded as initial weight.

Table 1 - Seggregation of chicks for study

TRIAL I		TRIAL II & TRIAL III		
CONTROL 2 chicks - untreated	**POSITIVE CONTROL** 3 chicks - only leaf extract	**CONTROL** 2 chicks(Uninfected)	**POSITIVE CONTROL** 2 chicks (only leaf extract) - 4g/kg body weight	**NEGATIVE CONTROL** 2 chicks (only pathogen)
NEGATIVE CONTROL 2 chicks - only bacterium	**TEST GROUP** 3 chicks - bacterium + leaf extract	**TEST GROUP - I** 2 chicks (pathogen + leaf extract)	**TEST GROUP - II** 2 chicks (pathogen + alkaloids) - 4g/kg body weight	**TEST GROUP - III** 2 chicks (pathogen + flavonoids) -4g/kg body weight

Initially in trial - I they were grouped into four groups inorder to check the ability of the bacterium in reducing the platelets. In trial II and trial III the chicks were grouped into six and the effectiveness of leaf extract were examined.

Induction of thrombocytopenia

To the negative, control chicks and to the test group 0.2 ml of culture (containing 10^9 cfu/ml) was administered and left undisturbed for 24 hours. Chicks belonging to the normal control group were left undisturbed and were not administered either with the pathogen or the plant extract.

Withdrawal of blood and counting of platelets

After 24 hours, the chickens were weighed. Then the blood was drawn from all the chicks and their platelets were counted. From the group I chicks blood was drawn in order to determine the normal platelet levels in the chick system.

Administration of plant extract

The negative control chicks were left undisturbed while the chicks from the test group were administered with *C. papaya* leaf extract and incubated for another 24 hours. The leaf extract had no effect on the chicks. All the chicks were left undisturbed for 24 hours.

RESULTS AND DISCUSSION

Withdrawal of blood and platelet count

After 24 hours, the weight of the chicks was measured and it was found that there was no change in the weight. Then the blood was withdrawn from all the groups of the chicks and their platelets were counted, in order to ensure whether any changes had occurred (Table 2).

Table : 2 Effect of leaf extract on platelet count in chicks

TREATMENT GROUP	TRIAL I				TRIAL II				TRIAL III	
	TIME INTERVAL (IN DAYS)		WEIGHT OF CHICKS (Kg)		TIME INTERVAL (IN DAYS)		WEIGHT OF CHICKS (Kg)		TIME INTERVAL (IN DAYS)	WEIGHT OF CHICKS (Kg)
	24 HOURS (Cells/ ml)	48 HOURS (Cells/ ml)	24 HOURS	48 HOURS	24 HOURS (cells/ ml)	48 HOURS (cells/ ml)	24 HOURS	48 HOURS	24 HOURS	24 HOURS
CONTROL (GROUP 1)	39	40	15	17	47	41	53	57	43	53
NEGATIVE CONTROL (GROUP II)	27	24	14	13	27	19	57	60	20	57
POSITIVE CONTROL (GROUP III)	31	44	18	16	45	39	51	55	42	42
TEST (GROUP IV)	34	36	16	15	25	37	43	46	38	43
ALKALOIDS	-	-	-	-	28	22	43	45	28	28
FLAVONOIDS	-	-	-	-	29	27	48	51	32	32

In trial I the effectiveness of the bacterium was checked after 48hours while in Trial II their effectiveness was checked after 72 hours which indicated very low platelet count. In trial III the chicks were infected and treated within 24 hours.

The control group chicks had 41 numbers of platelets. In positive group, the platelet level was 95 % than control. Chicks belonging to the negative group showed decreased platelet levels by 46 % over control. Then the control, positive and negative group chicks were left undisturbed because the positive group chicks were administered only with the leaf extract while the negative group is provided with the bacterium. The chicks belonging to the test group were treated with the leaf extract. Similarly, the test group II was treated with alkaloids and group III with flavonoids. The chicks were left undisturbed for another 24 hours. The blood drawn from the negative control group and test group was found to possess very low platelet count. It is understandable because these chicks were administered with the pathogens, which indicates that *Escherichia coli* and *Salmonella enteritidis* induced thrombocytopenia in chicks.

The 48 hour sample

After 48 hours, the chicks were weighed and there were no major changes observed. Then the blood was collected from all the chicks and counted. There was a platelet count of 95% in the positive group. There were furthermore decrease in the negative group in platelet by 46 % and there were no changes observed in the control group. It was observed that the test group I had platelets of 55 % which is similar to the control group and the test group II had 66 % increase in platelets count. In order to confirm the results animal trial experiments was carried out twice and similar results were observed.

Isolation of alkaloids and flavonoids

From the matured leaves the alkaloid and flavonoids were isolated as the matured leaves were found to possess high amounts. About 8.0 g of fresh leaves were taken and yield of isolated flavonoid was found to be 1.8g. Similarly 8.0 g of fresh leaf extract was used for alkaloid isolation and the yield was 2.1 g. Then the isolated compounds were characterized by thin layer chromatography (Figure 1). Their R_f values were calculated. The retention factor of alkaloids was 0.5 and flavonoids was 0.7.

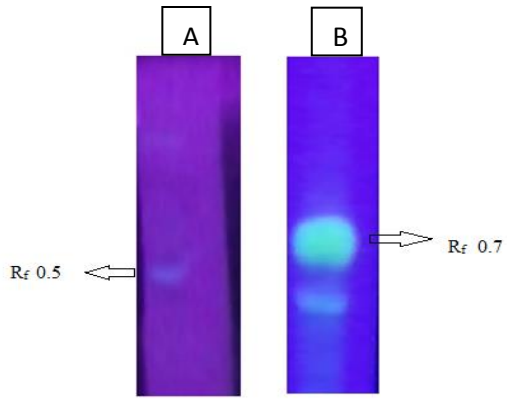

Fig. 1 - Thin layer chromatography

The isolated flavonoids and alkaloids were characterized using thin layer chromatography. The band indicated that the two compounds were present in appreciable quantity. A. Alkaloids (R_f 0.5) B. Flavonoids (R_f 0.7).

In an effort to identify the compounds from papaya leaves involved in increasing platelet counts, the entire phytochemical spectrum of the young and mature leaf of papaya was mapped. [9] The biochemicals present in significant proportion were then isolated and its effect in reversing thrombocytopenia was also evaluated. This served to identify the active constituent and also to understand its efficacy.

It is envisaged that this study would help in a preliminary understanding of the role of papaya leaves in altering the thrombocytes counts and also to identify the specific phytochemical involved in the reaction. It could serve as the starting point for a focussed effort on drug development for major health condition.

CONCLUSION

Comparing the two bacteria used for this study it appears that *Salmonella enteritidis* is much more potent in bringing down the thrombocyte levels when compared to *Escherichia coli*. *C.papaya* leaf extract was however able to restore the platelet levels to 89% to 93% of the values in control in both the cases. The isolated alkaloids could restore platelet levels by 66% to 74%. It therefore appears that administration of an isolated compound of a particular secondary metabolite is sufficient along with the other nutrient of the leaf extract. Similar works was carried out in the dengue infected patients. Since no progress with antibiotics medications, finally the patient was administered with 25mL of *C.papaya* leaf extract twice a day. Interestingly, their platelet count, white blood counts were dramatically increased within

5 days of administration. Without any viral medicines the patients were completely rectified from dengue virus. [10] *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. [11] From a total of 5 patients of dengue were selected and their blood test reports on the platelets counts were also examined before and after consuming papaya leaf juice, as per the medical report of patients the number of platelets had increased within the prescribed time (24 hrs) of drinking leaf juice of papaya in all 5 patients of dengue. The increase in number of platelets varied from patients to patients that ranged from 8000 to 11000. [12]

ACKNOWLEDGEMENT

I take this opportunity to express my profound gratitude and deep regards to the Management of Karpagam University for providing all facilities**.**

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