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"POTENTIAL BENEFITS OF PAPAYA LEAVES AND GRAPE FRUIT JUICES FOR MANAGEMENT OF THROMBOCYTOPENIA"

[1]Bhargavi Yanamadala*, [2]Sushma Attuluri, [3]Dr. Aishwarya Dinakaran, [4]Dr. Aliya Parveen, [5]Tahmina Sultana, [6]Sahithi K.

[1]Krupanidhi College of Pharmacy, Carmelaram Road, Off Sarjapur Road, Chikka Bellandur, Carmelaram Post, Varthur Hobli, Bengaluru, Karnataka 560035, India.
[2]Assistant Professor, Department of Pharmacology, Nizam Institute of Pharmacy, Deshmukhi (V), Pochampally (M), Behind Mount Opera, Yadadri Bhuvanagiri (Dist)-508284, Telangana, India.
[3]Assistant Professor, Department of Pharma. D, Nizam Institute of Pharmacy, Deshmukhi (V), Pochampally (M), Behind Mount Opera, Yadadri Bhuvanagiri (Dist)-508284, Telangana, India.
[4]Assistant Professor, Department of Pharma. D, Nizam Institute of Pharmacy, Deshmukhi (V), Pochampally (M), Behind Mount Opera, Yadadri Bhuvanagiri (Dist)-508284, Telangana, India.
[5]Assistant Professor, Department of Pharmacology, Nizam Institute of Pharmacy, Deshmukhi (V), Pochampally (M), Behind Mount Opera, Yadadri Bhuvanagiri (Dist)-508284, Telangana, India.
[6]BDS, Malla Reddy Institute Of Dental Sciences, Suraram X Roads, Jeedimetla, Quthbullapur, Hyderabad, Telangana 500055, India.

***Corresponding Author: Bhargavi Yanamadala**
Krupanidhi College of Pharmacy, Carmelaram Road, Off Sarjapur Road, Chikka Bellandur, Carmelaram Post, Varthur Hobli, Bengaluru, Karnataka 560035, India. **Mail ID:** bhargavisajja25@gmail.com, mohdsaleempharma@gmail.com

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ABSTRACT

Background and Objective: *Carica papaya* leaves exhibits wide range of biological functions such as antifungal, antimalarial, anti-inflammatory, anticancer, antisickling etc and purple grapes demonstrates anticancer, antihyperglycemic and wound healing activities. The present study was focussed on potential benefits of *carica papaya* leaves and purple grape juice on thrombocytopenia. The study also investigates the combined effect of *carica papaya* leaves and purple grape juice and its formulation in treating thrombocytopenia. **Methods:** The Sprague dawley rats of either sex weighing between 150-200 Gms were used. Animals received the test compound or the vehicle (controls) by oral route for 10 days. After 10 days, blood samples were collected from the treated rats under ether anaesthesia and platelets were counted using haemocytometer. Heparin was administered subcutaneously and blood samples were collected at 60 min and 24 hrs after the administration of heparin to estimate the platelet count. The percentage change in the thrombocytes count was determined in all groups. Body weight was checked every 5 days and was tabulated. **Results:** Homogenate of *carica papaya* leaves and purple grapes were found to be effective in increasing platelet count at both the doses (200 mg/kg and 500 mg/kg p.o.). Moreover, combination of papaya leaves (200 mg/kg) and purple grape (500 mg/kg) was significantly more effective in terms of increasing platelet count when compared to normal control. Similarly, formulation continue to preserve the efficacy of the therapeutic benefits of combination of papaya leaves and purple grape in ratio of 1:2.5 for ameliorating thrombocytopenia as well as maintaining body weight. **Interpretation and Conclusion:** Homogenate of *carica papaya* leaves and purple grapes were found to be effective in elevating platelet count at both low (200 mg/kg) and high doses (500 mg/kg) when given orally. Combination of the *carica papaya* leaves low dose and purple grapes high dose attenuated the progression of thrombocytopenia by increasing platelets count. Formulation of combination of papaya leaves and purple grapes was found equally effective as combination. Hence formulation of combination may be potentially useful in treating thrombocytopenia.

KEYWORDS: Thrombocytopenia, Platelets, *Carica papaya* leaves, Purple grapes, Heparin.

INTRODUCTION [1-17]

Thrombocytopenia

A normal human platelet count ranges from 1,50,000 to 4,50,000 platelets per microlitre of blood. The term thrombocytopenia describes a decrease in platelet count below 50,000-1,00,000 cells/µl. Often, low platelet levels do not lead to clinical problems; rather, they are picked up on a routine complete blood count. Ocassionally, there may be bruising in the forearms, petechia, nosebleeds and bleeding gums. A person with thrombocytopenia may also complain of malaise, fatigue and general weakness. In acquired thrombocytopenia, the

patient's history may include the use of one of the several offending drugs. Adults may have large, blood-filled bullae in the mouth.

There are three major types of thrombocytopenia.

1. Drug induced thrombocytopenia (various medications can lead to thrombocytopenia)
a. -immune (certain medications cause your body to make antibodies that destroys platelets.)
b. -non immune (certain drugs prevent your bone marrow from making platelets)
2. Idiopathic thrombocytopenic purpura (immune systems destroys the platelets that our body produces)
3. Thrombotic thrombocytopenic purpura (low platelet count and clot in tiny blood vessels)

Herbs have been an integral part of society since the beginning of human civilization and are valued for their culinary and medicinal properties. With the development of patented medicines in the early part of the 20th century, herbal medicine lost ground to the new synthetic medicines touted by scientists and physicians to be more effective and reliable. Nevertheless, herbal remedies are still popular globally.

Platelets (PLTs) are essentially tiny cells that are part of the anatomy of blood cells and play a significant role in the clotting of blood. PLTs are the first responders when the brain receives a signal that there is some blood loss as a result of some kind of injury or trauma. They utilize the help of proteins, minerals and vitamins to help stop the bleeding and clot the injury. The average lifespan of a single platelet is considered to be around eight to twelve days, after which a new batch of platelets is produced and takes over. Some individuals may have a lower than normal count of platelets while others may have a higher count. PLTs play a key role in hemostasis, clot stability and retraction as well as in vascular repair and anti-microbial host defense. Upon vessel wall damage, PLTs undergo a highly regulated set including adhesion, spreading, aggregation, release reactions as well as exposure of procoagulant surfaces to rapidly form a hemostatic plug that occludes the site of damage. When PLT function is impaired, the bleeding risk increases, but (hyperreactive) PLTs are also involved in many pathophysiological events like thrombosis, vessel constriction, atherogenesis, tumor growth and metastasis, inflammation including atherosclerosis and the subsequent formation of arterial thrombi resulting in stroke and myocardial infarction.[3] The body has a mechanism to bring the blood cells to normal. Healthy diets containing flavonoid could help us in maintaining normal homeostasis.

Carica papaya L. belongs to the plant family *Caricaceae*. It is being cultivated widely for consumption as fresh fruit, dried and crystallised fruit as well as for use in drinks, jams and candies.[7] Green fruit, the leaves and flowers may also be used as a cooked vegetable. Nakasone and Paull have shown that papaya is a good source of calcium and an excellent source of vitamins A and *C. Papaya* also has several industrial uses. Biochemically, its leaves and fruit are complex, containing several proteins and alkaloids with important pharmaceutical and industrial applications. Commonly, *Carica papaya* is used as food or as medication in folk medicine. Considerable work has been carried out on plant parts such as fruit, seed and root, indicating the presence of biologically active compounds. The quantity of the compounds differs in fruit, latex, leaves and roots and varies with the extraction method, age of the plant part, the cultivation and the gender of the tree. In Malaysia, many people take papaya leave juice to increase platelet count when they get dengue fever. It's a fact that, to date, there's no known medical cure for dengue fever since it's caused by a virus transmitted through the bite of the Aedes mosquito. However, it seems drinking papaya leaf extract helps to bring up the platelet count.

Purple grape (*Vitis vinifera* L) is not only very tasty and refreshing, but it also has healing properties. It acts as a natural body tonic, slows aging, prevents dementia and degenerative diseases, cleans the blood, removes the fat from the body, strengthens the heart and etc. Purple grapes contain lots of fruit sugar (18-20%), providing a high energy value, so 1 kg of grapes can replace 25 to 30% of daily energy needs. It contains plenty of vitamin C and smaller amounts of vitamin B1, B2, B6 and carotene. Also grape is rich in minerals - potassium, calcium, iron, phosphorus, magnesium and boron. Purple grapes contain phenolic substances that provide color and flavor, resveratrol, anthocyans and antioxidants (catechins, quercetin) that provide a healing effect. It has been demonstrated that 10 ml/kg of Purple Grape juice significantly inhibited in vivo platelet activity and experimental coronary thrombosis. Grapes and grape juice contain many of the same biologically active phenolic compounds such as catechines, quercetin, kaemferol and anthocyanin that are found in red wine.[14] As stated above, papaya leaves and purple grape juices have been used in folklore for ameliorating large number of ailments. Traditionally, one of the common remedial actions was for combating fall in platelet count. Even though modern method of treatment is successful in alleviating the pains and discomforts of human being, there is still dearth of discovery for effective management of thrombocytopenia. Corroborating with this, we have designed this study to fight against fall in platelet count in experimental models of animals with the help of nature's gift of herbs and fruits. Hence the present study is designed to explore the role of papaya leaves and purple grape juices separately as well as in combination on platelet count during collagen induced thrombocytopenia in rats.

OBJECTIVES

The objective of the present research will be to explore the role of *papaya leaves* and purple *grape fruit* juices on platelet count in animals with thrombocytopenia.

Specific Objectives

Level 1

1) To collect and authenticate the papaya leaves and purple grape fruit.
2) To standardize the method of preparation of papaya leaves and purple grape fruit.
3) To standardize the thrombocytopenic dose of heparin
4) To established the effective dose of papaya leaves and purple grape fruit under normal conditions for platelet count.
5) To ascertain the different concentrations of papaya leaves and purple grape fruit individually as well as in combination for influencing the platelet count.

Level 2

1) To study the extent of platelet enhancement by papaya leaves and purple grape fruit individually in thrombocytopenic animals.
2) To know the platelet count when papaya leaves and purple grape fruit are used in combination during thrombocytopenia.

Level 3

1) To prepare suitable formulation of mixture containing effective concentrations of papaya leaves and purple grape fruit juices.

METHODOLOGY [18-41]

Experimental Animals

Male Sprague dawley Rats weighing 150-180 g were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental protocol (KCP/IAEC-34/2011-12).

Heparin

Heparin injection IP (Batch No: UK 143) is purchased from Gland Pharma Limited, D.P.Pally, Dundigai Post, Hydrabad-500 043, India.

Apparatus and Chemical used

1. Analytical balance
   Schimadzu, Japan
2. Haemocytometer
   Schimadzu, Japan
3. Centrifuge
   Remo
4. Phase Microscope
   Schimadzu, Japan
5. Heparin
   Gland Pharma Limited
6. Sucrose S D
   Fine Chemicals
7. Sodium Benzoate S D
   fine Chemicals
8. Ammonium Oxalate S D
   fine Chemicals
9. EDTA
   Nice Chemicals Pvt Ltd
10. Micropippette
    Schimadzu, Japan

Preparation of *Carica papaya* homogenate

Carica papaya leaves was procured from the nearby areas and thoroughly washed with water. 5g of leaves were grinded in blender with 20 ml of distilled water.[78] Dose of *Carica* papaya (200 mg/kg and 500 mg/kg) was selected based on OECD guidelines and was administered within 30 min of preparation.

Preparation of purple grape fruit homogenate

Purple grape fruits were procured from the nearby areas and thoroughly washed with water**.** Homogenate was prepared by grinding 5g grapes in 20 ml of distilled water and Dose of *Purple grape* (200 mg/kg and 500 mg/kg) was selected based on OECD guidelines and was administered within 30 min of preparation.

Acute toxicity studies

The acute toxicity study was carried out according to the limit test described by the OECD guidelines. Test dose of 2g/kg, 3g/kg and 5g/kg were given to mice. Hence, $1/25^{th}$ and $1/10^{th}$ of the safe dose corresponding to 200 mg/kg and 500 mg/kg orally was selected as high and low dose respectively.

Phytochemical estimations of the Almond

Bitter almond was subjected to qualitative analysis to investigate the presence of various phytochemical constituents such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, amino acids, saponins, tannins and flavonoids.

Test for Alkaloids

1. *Hager's Test*: Extract was treated with Hager's reagent (saturated picric acid solution). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
2. *Mayer's Test*: Extract was treated with Mayer's reagent (potassium mercuric iodide solution). Formation of a cream coloured precipitate indicates the presence of alkaloids.
3. *Dragendroff's Test*: Extract was treated with dragendroff's reagent (potassium bismuth iodide solution). Formation of orange brown precipitate indicates the presence of alkaloids.
4. *Wagner's Tes*t: Extract was treated with Wagner's reagent (iodine potassium solution). Formation of

reddish brown precipitate indicates the presence of alkaloids.

Test for carbohydrates

1. *Molisch Test*: Extract was treated with molisch reagent (α-naphthol in 95% ethanol) and few drops of sulphuric acid were added through the side of the test tube. Appearance of violet ring at the junction indicates the presence of carbohydrates.
2. *Fehling's Test*: A small portion of the extract was treated with felhing's reagent A (Copper Sulphate in water) and felhing's reagent B (sodium potassium tartarate) and heated in a water bath. Formation of red colour precipitate indicated the presence of reducing sugars.
3. *Barfoed's Test*: Extract was treated with barfoed's reagent (copper acetate in water and glacial acetic acid), and heated in a water bath. Red coloured precipitate indicates the presence of sugars.
4. *Benedictt's Test*: Extract was treated with benedictt's reagent (copper sulphate + sodium citrate + sodium carbonate in water), and heated for 10 minutes. Red coloured precipitate indicates the presence of sugars.

Test for Proteins and amino acids

1. *Millon's Test*: Extract was treated with millons's reagent (mercuric nitrate in nitric Acid). Red colour indicates the presence of proteins.
2. *Biuret Test:* Extract was treated with sodium hydroxide. Copper sulphate solution was added drop wise. Violet colour is produced which indicates the presence of proteins.
3. *Ninhyrin Test*: Extract was treated with ninhydrin reagent and ammonia and heated. Violet colour indicates the presence of proteins.

Test for Steroids, Triterpenoids and Cardiac Glycosides

1. *Salkowski Test*: To the solution of extract in chloroform, a few drops of sulphuric acid were added and the moisture was shaken and allowed to stand for some time. Red colour is produced in the chloroform layer, which indicates the presence of steroids.
2. *Liebermann-Burchard Test*: Small portion of extract was dissolved in chloroform. To this 1ml acetic anhydride and then 2ml concentrated sulphuric acid was added through the side of the test tube. A reddish violet colour at the junction of two liquids indicates the presence of steroids, triterpenoids and cardiac glycosides.
3. *Baljet Test*: To the solution of extract in water a few drops of sodium picrate reagent was added. Formation of yellow colour indicates the presence of cardiac glycosides.
4. *Keller Killani's test*: A portion of extract was treated with 1ml of glacial acetic acid and a few drops of ferric chloride were added to it. To this mixture 2ml concentrated sulphuric acid, carefully through the sides of the test tube. A reddish brown colour is formed at the junction of two layers and bluish green colour in the upper layer which indicates the presence of deoxy sugars in the carbohydrates.

Test for Tannins

1. *Ferric Chloride Test*: Extract was treated with ferric chloride solution. Formation of blue colour indicates the presence of tannins.
2. *Lead Acetate Test*: Extract was treated with lead acetate solution; yellow precipitate indicates the formation of tannins.

Test for Saponins

1. *Froth test*: Diluted 1ml of the extract with distilled water to 20ml and shaken in a graduated cylinder for 15min. One-centimeter layer of foam indicates the presence of saponins.
2. *Haemolysis test*: 2ml of 1% Nacl was mixed with 2ml of extract,to this 5 drops of blood was added. Haemolysis indicates the presence of saponin.

Test for Flavonoids

1. *Ferric Chloride Test*: Extract was treated with few drops of ferric chloride solution. Formation of blackish blue colour indicates the presence of flavonoids.
2. *Lead Acetate Test*: Extract was treated with lead acetate solution; yellow precipitate indicates the formation of flavonoids.

Induction of thrombocytopenia

Subcutaneous injection of low molecular weight heparin at the dose of 2000 IU/kg[84] was given to rats daily for 10 days which leads to activation of platelets causing thrombocytopenia in rats.

Experimental protocol

The dose of heparin for the study was selected based on the concentration of heparin sufficient enough to induce thrombocytopenia.

Multiple level studies was carried out to ascertain the role of papaya leaves and purple grape fruit as platelet enhancer.

Level 1 (8 weeks)

Animals will be divided into four groups (n=10)
Group I: Heparin administration
Group II: Papaya leaves juice administration (low dose)
Group III: Papaya leaves juice administration (high dose)
Group IV: Purple grape juice administration (low dose)
Group V: Purple grape juice administration (high dose)
Group VI: Papaya leaves and purple grape fruit juices in combination (MOST EFFECTIVE DOSE).

Blood samples were collected from tail vein under ether anaesthesia, prior as well as on 10^{th} day after drug administration from all animals.

Level 2 (8 weeks)
Animals will be divided into three groups (n=10)
Group I: Papaya leaves juice (low dose) + heparin administration
Group II: Papaya leaves juice (high dose) + heparin administration
Group III: Purple grape juice (low dose) + heparin administration
Group IV: Purple grape juice (high dose) + heparin administration
Group V: Papaya leaves and purple grape fruit juices in combination (MOST EFFECTIVE DOSE) + heparin administration.

Blood samples were collected from tail vein under ether anaesthesia, prior as well as on 10^{th} and 11^{th} day after drug administration from all animals.

Level 3 (6 weeks)
Animals were divided into three groups (n=10)
Group I: Prepared formulation of papaya leaves and purple grape fruit + heparin administration
Group II: Excipients of the formulation + heparin administration

Blood samples were collected from tail vein under ether anaesthesia, prior as well as on 10^{th} day after drug administration from all animals.

Platelet counting
Preparation of 1% ammonium oxalate
1.0 g of ammonium oxalate was weighed and dissolved in 100ml of distilled water. It was kept in refrigerator and was used in 4 days of preparation.

Procedure for platelet counting
Blood was collected from tail of rat under ether anaesthesia and mixed in EDTA. 20µl of blood was mixed in 0.98ml of 1% ammonium oxalate making a dilution of 1:100. It is then swirled and then allowed to stand for 10 minutes, so that RBC are destroyed. After 10 minutes, it is again swirled to miw the contents. Few drops are kept on haemocytometer. A clean haemocytometer coverslip is placed on clean haemocytometer. A filled haemocytometer is placed in covered patridish with moist cotton ball for 10 mins. This provides a moist chamber to prevent evaporation of the solution in the counting chamber while allowing time for platelets to settle so they may be more accurately counted. To perform the plt count, the haemocytometer was removed from the moist chamber and placed on microscope stage. (40x) objective was used to count plt.

Calculating the platelet count

$$\text{Cells/µl} = \frac{\text{average} \times \text{D(mm)} \times \text{DF}}{\text{A(mm)}^2}$$

D- depth factor(10)
DF- dilution factor(100)
A- area(1 mm^2)
Avg- average plts in 25 chambers

Procedure
The Sprague dawley rats of either sex weighing between 150-200 gms were used. Animals received the test compound or the vehicle (controls) by oral route for 10 days. After 10 days, blood was collected from tail of the rats under ether anaesthesia and platelets were counted using haemocytometer. Then test compound or vehicle (control) was continued. After the end of the absorption time (60 mins), the thrombocytopenia-inducing substances heparin was injected slowly by subcutaneous route for 10 days. Blood was collected and plts were counted. The percentage of thrombocytes (or leukocytes) was determined in vehicle control and dosage groups at the different times following injection of the inducer relative to the initial value of control or dosage group, respectively. Calculated percent values of controls were taken as 100%. Percent inhibition of thrombocytopenia (or leucocytopenia) was calculated in dosage groups relative to controls. Body weight was checked every 5 days and was tabulated.

Formulation
Ingredients
25 g purple grapes
10 g *Carica papaya* leaves
66 g sucrose
10 mg sodium benzoate

Procedure of formulation
Papaya leaves were weighed and blended to powder form using a blender. Weighed quantity of purple grapes were added and again blended to get a fine paste. 66 g of sucrose was taken and 40ml of distilled water was added in it. It was boiled till syrupy consistency was achieved. Solution was cooled at room temperature. Paste of herb was added. Weighed quantity of sodium benzoate was added. Make up the volume to 100ml. Store in a cool place.

Statistical analysis
Statistical significance is evaluated by means of the unpaired Student's *t*-test when comparisons were made between two groups. Further group analysis were carried out by using one-way analysis of variance (ANOVA) followed by Tuckey's comparison test. The values will be expressed as mean ± SEM and $p < 0.05$ will be considered significant.

RESULTS

Table 1: Phytochemical investigation of aqueous extract of Carica papaya leaves and Purple grapes.

Sl no.	Tests	Inference	Result for Carica papaya leaves	Result for Purple grapes
1.	**Test for alkaloids**			
1a	Hager's test	Yellow colour	+	_
1b	Mayer's test	Cream precipitate	+	_
1c	Dragendroff's test	Orange precipitate	+	_
1d	Wagner's test	Red-brown precipitate	+	_
2.	**Test for carbohydrates**			
2a	Molisch's test	Violet colour	+	+
2b	Fehling's test	Brick-red colour	+	+
2c	Barfoed's test	Red colour	+	+
2d	Benedict's test	Red colour	+	+
3.	**Test for steroids, triterpenoids and glycosides**			
3a	Liebermann-Burchard test	Reddish-violet colour	+	+
3b	Salkowski test	Red colour	+	+
3c	Baljet's test	Orange colour	+	+
3d	Keller-Killani test	Red colour	+	+
4	**Test for saponins**			
4a	Froth test	1 cm. Foam	+	+
4b	Haemolytic test	No precipitate	+	+
5.	**Test for tannins**			
5a	Ferric chloride test	Blue colour	+	+
5b	Lead acetate test	Yellow colour	+	+
6.	**Test for proteins and amino acids**			
6a	Millon's test	Red colour	+	+
6b	Biuret test	Violet colour	+	+
6c	Ninhydrin test	Violet colour	+	+
7.	**Test for flavanoids**			
7a	Ferric chloride test	Blackish red colour	+	+
7b	Lead acetate test	Yellow colour	+	+

+ Sign indicates presence of phytochemical constituent and – sign indicates absence.

Table 1: STANDARDISATION OF HEPARIN INDUCED THROMBOCYTOPENIA IN RATS

Sl no.	Weight (gms)	Plt count (10^5cells/µl) before heparin	Hepa rin (ml)	Plt count(10^5cells/µl) at 60 mins	Percentage change in plt count	Plt count (10^5cells/µl) at 24 hrs	Percentage change in plt count
1	**198**	**3.6**	**0.078**	**2.6**	**-27.8%**	**2.5**	**-30.6%**
2	**182**	**3.6**	**0.072**	**2.6**	**-27.8%**	**2.6**	**-27.8%**
3	**180**	**3.8**	**0.070**	**3.1**	**-18.5%**	**3.2**	**-13.8%**
4	**190**	**3.6**	**0.075**	**3.0**	**-17.7%**	**3.2**	**-12.2%**
5	**200**	**4.1**	**0.080**	**3.4**	**-17.1%**	**3.3**	**-20.6%**
6	**204**	**3.9**	**0.082**	**3.0**	**-23.1%**	**3.1**	**-20.6%**
7	**192**	**3.5**	**0.076**	**2.8**	**-20.0%**	**2.8**	**-20.0%**
8	**210**	**3.8**	**0.084**	**2.9**	**-23.7%**	**3.1**	**-19.5%**
9	**208**	**4.0**	**0.083**	**3.1**	**-22.5%**	**3.0**	**-25.0%**
10	**188**	**3.6**	**0.075**	**3.1**	**-13.9%**	**2.9**	**-19.5%**

+ sign indicate rise in platelet count, whereas, vice versa for – sign.

Table 2: Effect of purple grape juice (low dose) on platelet count in heparin induced thrombocytopenia in rats

Sl no.	Weight (gms)	Plt count (10^5 cells/µl) before PGLD	PGLD (ml)	Plt count (10^5 cells/µl) on 10^{th} day	Percentage change in plt count	Heparin (ml)	Plt count (10^5 cells/µl) at 60 mins	Percentage change in plt count	Plt count (10^5 cells/µl) at 24 hrs	Percentage change in plt count
1	199	3.9	0.159	3.8	-02.6	0.079	3.8	0.00	3.0	-21.1
2	198	3.8	0.158	4.0	+05.2	0.078	3.2	-20.0	3.1	-22.5
3	192	3.2	0.153	3.3	+03.1	0.077	2.8	-15.2	3.0	-09.1
4	199	4.4	0.159	4.2	-04.6	0.078	3.6	-14.3	3.2	-23.9
5	195	4.3	0.156	4.5	+04.6	0.078	4.0	-11.2	3.9	-13.4
6	191	3.2	0.152	3.4	+06.2	0.076	2.7	-20.6	2.7	-20.6
7	210	4.1	0.162	3.6	-12.2	0.084	3.7	+02.7	3.6	0.00
8	199	3.6	0.159	3.9	+08.3	0.079	3.6	-07.7	3.6	-07.7
9	193	3.9	0.160	4.0	+02.5	0.077	3.2	-20.0	3.4	-15.0
10	205	4.1	0.161	4.1	0.00	0.082	3.8	-07.4	3.3	-19.6

+ sign indicate rise in platelet count, whereas, vice versa for – sign. PGLD-purple grape low dose.

Table 3: Effect of purple grape juice (high dose) on platelet count in heparin induced thrombocytopenia in rats

Sl no.	Weight (gms)	Plt count (10^5 cells/µl) before PGHD	PGHD (ml)	Plt count (10^5 cells/µl) 0n 10^{th} day	Percentage change in plt count	Heparin (ml)	Plt count (10^5 cells/µl) at 60 mins	Percentage change in plt count	Plt count (10^5 cells/µl) at 24 hrs	Percentage change in plt count
1	193	3.0	0.38	3.8	+26.6%	0.077	2.9	-23.7%	2.8	-22.4
2	197	3.4	0.39	4.0	+17.6%	0.078	3.8	-05.0%	3.5	-12.5
3	185	3.2	0.37	3.7	+15.6%	0.074	3.1	-03.2%	3.0	-19.0
4	191	4.1	0.38	4.6	+13.5%	0.076	3.8	-17.4%	3.6	-21.8
5	184	3.8	0.36	3.6	-05.3%	0.074	3.6	0.00%	3.5	-02.8
6	200	4.1	0.40	4.5	+09.7%	0.080	4.0	-11.2%	4.0	-11.2
7	216	3.7	0.45	4.0	+08.1%	0.086	3.6	-10.0%	3.4	-15.0
8	207	3.9	0.42	3.6	-07.7%	0.083	3.8	+05.5%	3.6	0.00
9	197	3.1	0.39	3.9	+25.8%	0.078	3.9	0.00%	2.9	-23.7
10	185	3.0	0.37	3.9	+30.0%	0.074	3.1	-20.0%	3.4	-12.9

+ sign indicate rise in platelet count, whereas, vice versa for – sign.

Table 4: Effect of papaya leaves juice (low dose) on platelet count in heparin induced thrombocytopenia in rats

Sl no.	Weight (gms)	Plt count before PLLD	PLLD (ml)	Plt count on 10^{th} day	Percentage change in plt count	Heparin (ml)	Plt count at 60 mins	Percentage change in plt count	Plt count at 24 hrs	Percentage change in plt count
1	210	4.4	0.168	4.9	+11.3	0.084	4.0	-18.4	4.1	-12.4
2	197	4.2	0.150	4.3	+02.3	0.078	3.8	-11.7	3.8	-11.7
3	193	3.6	0.153	4.0	+11.1	0.077	3.6	-10.0	3.7	-07.5
4	205	4.2	0.160	5.1	+21.4	0.082	3.2	-37.3	3.2	-37.2
5	189	3.1	0.145	3.6	+16.1	0.075	4.0	+11.1	3.8	+05.5
6	205	4.1	0.160	3.9	-04.9	0.082	3.9	0.00	4.0	+02.5
7	185	3.0	0.140	4.0	+33.3	0.074	3.8	-05.5	3.6	-10.0
8	190	2.9	0.152	4.1	+05.1	0.075	3.8	-07.4	3.8	0.00
9	193	3.2	0.153	3.6	+12.5	0.077	3.6	0.00	3.8	+05.5

+ sign indicate rise in platelet count, whereas, vice versa for – sign.
PLLD – papaya leaves low dose.

Table 5. Effect of papaya leaves juice (high dose) on platelet count in heparin induced thrombocytopenia in rats

Sl no.	Weight (gms)	Plt count (10^5 cells/µl) before PLHD	PLHD (ml)	Plt count (10^5 cells/µl) on 10^{th} day	Percentage change in plt count	Heparin (ml)	Plt count (10^5 cells/µl) at 60 mins	Percentage change in plt count	Plt count (10^5 cells/µl) at 24 hrs	Percentage change in plt count
1	198	4.0	0.39	4.5	+12.5	0.078	4.0	-11.2	3.8	-15.6
2	200	4.3	0.40	4.2	-02.4	0.080	4.1	-02.4	4.0	-04.8
3	192	4.1	0.38	4.3	+04.8	0.077	3.8	-11.4	3.8	-14.0
4	195	3.6	0.39	3.9	+08.3	0.078	3.2	-18.0	3.2	-12.9
5	190	3.7	0.38	5.0	+35.1	0.075	4.2	-16.0	4.2	-20.0
6	200	3.9	0.40	4.2	+07.6	0.080	3.8	-09.6	3.6	-14.3

+ sign indicate rise in platelet count, whereas, vice versa for – sign.
PLHD – papaya leaves high dose.

Table 6: Effect of combination of papaya leaves juice (low dose) and purple grape juice (high dose) on platelet count in heparin induced thrombocytopenia in rats.

Slno.	Weight (gms)	Plt count (10^5 cells/µl) before treatment	PLLD +PGHD (ml)	Plt count (10^5 cells/µl) on 10^{th} day	Percentage change in plt count	Heparin (ml)	Plt count (10^5 cells/µl) at 60 mins	Percentage change in plt count	Plt count (10^5 cells/µl) at 24 hrs	Percentage change in plt count
1	205	3.8	0.16 + 0.41	4.2	+10.5%	0.082	3.6	-15.3%	3.7	-17.7%
2	204	4.1	0.16 + 0.41	4.0	-2.5%	0.082	3.9	-3.5%	3.9	-3.5%
3	197	3.6	0.15 + 0.39	3.7	+8.3%	0.078	3.4	-13.9%	3.0	-24.1%
4	180	3.2	0.13 + 0.35	4.0	+25%	0.072	3.2	-20.0%	3.3	-17.5%
5	184	3.9	0.14 + 0.36	4.4	+12.8%	0.073	3.9	-12.4%	3.8	-13.7%
6	193	3.7	0.15 + 0.38	3.9	+5.4%	0.077	3.6	-7.7%	3.6	-7.7%
7	210	3.9	0.17 + 0.42	3.7	-5.4%	0.084	3.7	0.0%	3.8	+2.7%
8	204	4.0	0.16 + 0.41	4.2	+5.0%	0.081	3.8	-9.6%	3.7	-12.0%
9	195	3.8	0.15 + 0.39	4.3	+13.1%	0.078	4.0	-7.0%	4.2	-2.4%
10	197	3.9	0.15 + 0.39	4.1	+5.1%	0.078	3.7	-9.8%	3.7	-9.8%

+ sign indicate rise in platelet count, whereas, vice versa for – sign. PLLD - papaya leaves low dose PGHD - purple grape high dose.

Table 7: Effect of excipients of the formulation on plt count in heparin induced thrombocytopenia in rats.

Sl no.	Weight (gms)	Plt count (10^5 cells/µl) before treatment	Excipeints (ml)	Plt count (10^5 cells/µl) on 10^{th} day	Percentage change in plt count	Heparin (ml)	Plt count (10^5 cells/µl) at 60 mins	Percentage change in plt count	Plt count (10^5 cells/µl) at 24 hrs	Percentage change in plt count
1	210	4.3	1.15	4.1	-04.7	0.084	3.6	-12.2	3.6	-11.1
2	185	4.2	0.92	4.2	0.00	0.073	3.5	-16.7	3.5	-16.7
3	190	3.8	0.94	3.9	+02.6	0.076	3.0	-23.1	3.1	-20.6
4	194	3.8	0.97	3.7	-02.7	0.078	4.1	+10.8	4.2	+13.5
5	195	4.1	0.97	4.0	-02.5	0.078	3.2	-22.0	3.2	-22.0
6	200	3.6	1.00	3.6	0.00	0.080	3.0	-16.7	2.9	-19.5
7	204	3.7	1.09	3.9	+05.4	0.081	3.1	-20.6	3.2	-17.0
8	188	3.1	0.93	3.4	+09.6	0.074	2.8	-17.7	3.0	-11.8
9	190	3.5	0.94	3.5	0.00	0.075	2.8	-20.0	2.7	-22.9
10	200	3.6	1.00	3.4	-05.6	0.080	3.0	-11.8	3.6	-05.9

. + sign indicate rise in platelet count, whereas, vice versa for – sign.

Table 8: Effect of formulation on plt count in heparin induced thrombocytopenia in rats.

Sl no.	Weight (gms)	Plt count (10^5 cells/µl) before formulation	Formulation (ml)	Plt count (10^5 cells/µl) on 10^{th} day	Percentage change in plt count	Heparin (ml)	Plt count (10^5 cells/µl) at 60 mins	Percentage change in plt count	Plt count (10^5 cells/µl) at 24 hrs	Percentage change in plt count
1	195	3.6	0.97	4.1	+13.8%	0.078	3.7	-9.8%	3.7	-9.8%
2	198	4.2	0.99	4.4	+4.7%	0.079	4.6	+4.5%	4.1	-6.9%
3	180	3.8	0.90	4.3	+13.1%	0.072	3.7	-14.0%	3.7	-14.0%
4	200	3.7	1.00	4.2	+13.5%	0.080	4.0	-4.8%	4.0	-4.8%
5	185	3.6	0.92	4.0	+11.1%	0.074	3.5	-12.5%	3.4	-15.0%
6	204	3.4	1.02	3.9	+14.7%	0.081	3.8	-2.6%	3.9	0.00%
7	190	4.1	0.95	4.1	0.0%	0.076	3.6	-12.2%	3.7	-9.8%
8	185	4.1	0.92	4.4	+7.3%	0.074	3.9	-11.4%	3.9	-11.4%
9	195	3.6	0.95	3.9	+8.3%	0.078	3.6	-7.7%	3.7	-5.2%
10	210	3.2	1.05	4.2	+31.2%	0.084	3.7	-11.9%	3.9	-7.2%

+ sign indicate rise in platelet count, whereas, vice versa for – sign.

Table 9: Effect of Papaya leave juice, purple grape juice and their combination in formulation on Platelet count in heparin induced thrombocytopenia in rats

Sl no.	Treatment	Plt count (10^5 cells/µl) before treatment	Plt count (10^5 cells/µl) after treatment (percentage change)	Plt count (10^5 cells/µl) 60 mins post heparin administration (percentage change)	Plt count (10^5 cells/µl) 24 hrs post heparin administration (percentage change)
1	Normal control	3.75±0.06368	3.75±0.06368 (0.00%)	2.88±0.06799 (-23.20%)	2.92±0.07717 (-22.02%)
2	Papaya leaves (low dose)	3.86±0.1024	4.10±0.1986 (+07.04%)***	3.64±0.1144 (-11.32%)***	3.64±0.1132 (-11.32%)***
3	Papaya leaves (high dose)	3.64±0.1811	4.18±0.08724 (+12.83%)***	3.76±0.1333 (-10.15%)***	3.71±0.08333 (-11.25%)***
4	Purple grape (low dose)	3.73±0.1310	3.88±0.1162 (+4.02%)***	3.42±0.1400 (-11.96%)***	3.28±0.1632 (-15.2%)***
5	Purple grape (high dose)	3.52±0.1356	3.96±0.1087 (+11.25%)***	3.56±0.1222 (-10.21%)***	3.47±0.1147 (-11.72%)***

+ sign indicate rise in platelet count, whereas, vice versa for – sign.

Sl no.	Treatment	Plt count (10^5 cells/µl) before treatment	Plt count (10^5 cells/µl) after treatment (percentage change)	Plt count (10^5 cells/µl) 60 mins post heparin administration (percentage change)	Plt count (10^5 cells/µl) 24 hrs post heparin administration (percentage change)
6	Combination (PLLD+PGHD)	3.95±0.09098	4.53±0.09978 (+14.68%)***ac	3.83±0.09195 (-8.89%)***a	3.85±0.1204 (-8.33%)***a
7	Excipient (SUCROSE+SB)	3.77±0.1136	3.78±0.06799 (+0.21%)	3.00±0.1585 (-23.31%)	2.97±0.1886 (-25.3%)
8	Formulation	3.80±0.08433	4.37±0.09638 (+15.00%)***ac	3.94±0.08969 (-8.60%)***ac	3.94±0.05207 (-8.60%)***ac

Results are Mean±SEM of 10 rats in each group. One-way ANOVA followed by Tuckey's comparison test for multiple comparisons was applied for comparing the Parameters with NC and other groups. The difference was considered to be significant when *** P<0.0001, ** P<0.001, * P<0.05 when compared to Normal control group, [a]P<0.05, when compared to PLLD(papaya leaves low dose, [b]P<0.05 when compared to PLHD(papaya leaves high dose), [c]p<0.05 when compared to PGLD(purple grape low dose), [d]p<0.05 when compared to PGHD(purple grape high dose).

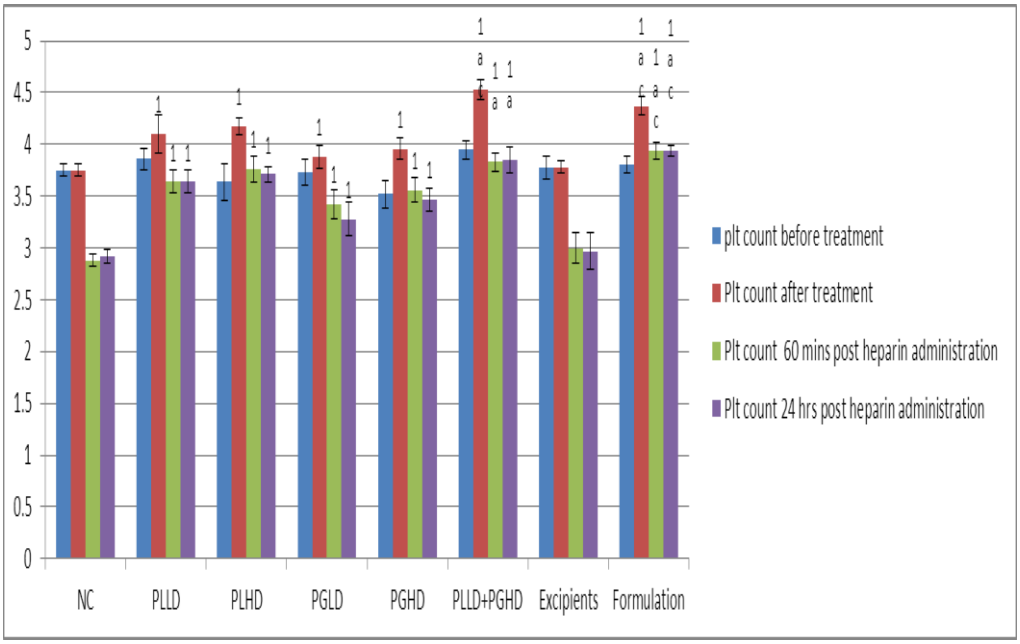
EXPERIMENTAL MODELS

Effect of *Carica papaya* leaves and *Purple grape* juice on plt count in normal rats

Table 9 shows that papaya leaves juice and purple grape juice produced a significant increase in plt count pre heparin induced thrombocytopenia in rats. Both high dose of papaya leaves juice and purple grape juice(500mg/kg, p.o.) and low dose of papaya leaves juice and purple grape juice (200mg/kg, p.o.) were effective in increasing plt count when compared to NC. Combination and formulation of papaya leaves and purple grape in a ratio of 1:2.5 were more effective compared to monotherapy.

Effect of *Carica papaya* leaves and *Purple grape* juice on plt count in thrombocytopenic rats

Table 9 also shows that Papaya leaves juice and purple grape juice produced a less decrease in plt count post heparin induced thrombocytopenia in rats when compared to normal control. Both high dose of papaya leaves juice and purple grape juice(500mg/kg, p.o.) and low dose of papaya leaves juice and purple grape juice (200mg/kg, p.o.) were effective in maintaining plt count in heparin induced thrombocytopenia in rats when compared to NC. Combination and formulation of papaya leaves and purple grape in a ratio of 1:2.5 were more effective compared to monotherapy.



Graph for plt count was done before and after test drug treatment and also pre and post heparin induced thrombocytopenia in rats. One-way ANOVA followed by Tuckey's test for multiple comparisons was applied for comparing the plt counts among different groups. The difference was considered to be significant when [1]P<0.001, [2]P<0.01, [3]P<0.05 when compared to Normal control group, [a]P<0.05, when compared to PLLD(papaya leaves low dose, [b]P<0.05 when compared to PLHD(papaya leaves high dose), [c]p<0.05 when compared to PGLD(purple grape low dose), [d]p<0.05 when compared to PGHD(purple grape high dose).

Table 10: Effect of Papaya leave juice, purple grape juice and their combination in formulation on body weight.

Days	0th day	5th day	10th day	15th day	20th day	25th day
NC	195.2±3.3	194.3±2.8	195.6±3.1	193.6±2.6	190.1±2.9	186.3±2.4
PLLD	197.7±2.8	204.6±1.6	205.9±1.9	205.1±2.1	203.4±2.2	199.2±1.9
PLHD	196.0±1.3	206.8±0.7	209.1±1.2	210.9±1.5	209.3±0.8	206.4±0.9
PGLD	198.1±1.8	204.2±2.6	206.3±2.4	207.6±2.4	205.7±1.8	201.3±1.9
PGHD	195.5±3.2	209.0±1.8	211.9±2.8*	213.3±2.4*	211.4±1.6*	207.9±2.1*
Combination (PLLD+PGHD)	196.9±2.9	211.7±3.1*	215.4±2.6*	215.8±2.8*	214.3±3.1*	209.7±2.6*
Excipient (SUCROSE+SB)	194.2±2.9	194.8±1.9	196.2±1.6	195.4±1.4	189.6±1.2	182.4±1.1
Formulation	195.6±2.4	209.9±2.3	212.8±2.1*	214.0±2.1*	213.6±1.8*	209.2±1.6*

Results are Mean±SEM of 10 rats in each group. One-way ANOVA followed by Dunnet comparison test for multiple comparisons was applied for comparing the Parameter with NC. The difference was considered to be significant when *** P<0.0001, ** P<0.001, * P<0.05 when compared to Normal control group.

Effect of *Carica papaya* leaves and *Purple grape* juice on body weight in rats

The body weight at different periods of various groups of animals during the study period are given in the table no. 10, which shows that the mean body weight of rats at day 0, which was recorded before induction of heparin and which were recorded during and at the end of the treatment protocol. The mean body weight of normal rats was gained during the study period. The body weights of thrombocytopenic control on groups were found to be reduced throughout the treatment period. During the same period of treatment the mean body weight of animals treated with both high dose of papaya leaves juice and purple grape juice(500mg/kg, p.o.) and low dose of papaya leaves juice and purple grape juice (200mg/kg,p.o.) was maintained when compared to NC and heparin treatment. Combination and formulation of papaya leaves and purple grape in a ratio of 1:2.5 were more effective compared to single doses.

DISCUSSION

Thrombocytopenia refers to an abnormally low number of platelets (blood cells produced in the bone marrow, that help form blood clots and stop bleeding). A reduction in the number of platelets may result in bleeding, especially from the smaller vessels.[89] In our present study, heparin was used to induce thrombocytopenia as heparin has a high affinity for platelet factor 4 (PF4), a positively charged tetrameric protein found in the alpha granules of platelets and on the surface of cells such as endothelial cells and platelets. When heparin binds to PF4 a complex is formed that undergoes a conformational change and exposes new epitopes that act as immunogens. HIT occurs as a result of the binding of antibodies, typically immunoglobulin (Ig) G, to the heparin-PF4 complex. These anti-heparin-PF4 complex antibodies activate platelets via Fcγ IIa receptors and cause the release of prothrombotic microparticles, platelet consumption, and thrombocytopenia. Platelet activation also leads to the release of PF4 from the granules, thereby perpetuating the cycle of complex formation and platelet activation. The released microparticles increase the production of thrombin, which is responsible for the thrombotic events. The antigen-antibody complex also interacts with monocytes and leads to production of tissue factor and endothelial damage, both of which favor the development of thrombosis. It has also been suggested that PF4 itself could neutralize the anticoagulant effects of heparin and promote a prothrombotic state. Thus, all of these processes end paradoxically in the development of thrombosis due to heparin use.

The use of herbs and other natural products as remedies has gained popularity, proved by scientific evidence.[97] Present study was focused on treating thrombocytopenia by using carica papaya leaves and purple grape fruit in rats. Carica papaya homogenate was found to increase plt count. It is possible that Carica papaya leaves possesses active constituent(s) containing erythropoietin-like substance(s) or contain active biological principle(s) stimulating erythropoietin synthesis or release either by inducing hypoxia or directly acting on the kidneys to cause erythropoietin secretion. Also, the dose-related increase in the platelet and total leukocyte counts could be due to release of the glycoproteins, thrombopoietin and leukopoietin, in a similar way as impaired oxygenation of the kidney induces erythropoietin release. However, these hypotheses still require validation.

Purple grape fruit homogenate was also found to be effective in thrombocytopenia to increase plt count. This effect is due to capability of purple grape juice to enhance platelet release of NO. Platelets and megakaryocytes are known to contain constitutive NOS (cNOS) and stimulated platelets release NO. Platelet release of NO has been found to have modest effects on aggregation as well as more profound effects on platelet recruitment to a growing thrombus. Thus, enhanced release of platelet-derived NO may have contributed to inhibition of aggregation detected after Purple grape juice oral supplementation. How Purple grape juice enhances platelet release of NO is unknown. Combination and formulation was found to be significantly more effective in increasing plt count pre heparin treatment and treating thrombocytopenia may be due its synergistic effect. There was considerable recovery in the weights of animals when treated with single herb when compared to nomal control group but combination and formulation of carica papaya leaves and purple grape juice were more effective when compared to single herb.

CONCLUSION

In conclusion, the present results suggested that carica papaya leaves juice and purple grape juice were effective in treating heparin induced thrombocytopenia in their low and high doses (200mg/kg amd 500mg/kg p.o respectively). Further studies showed that carica papaya leaves and purple grape juice also helped in maintaining body weight compared to normal control. Combination and formulation of carica papaya leaves low dose and papaya leaves high dose were found to be more effective both in treating thrombocytopenia and maintaining body weight than monotherapy.

SUMMARY

Thrombocytopenia may lead to severe complications like internal bleeding, nose and mouth bleeding. If there is bleeding into the brain or digestive tract it can be potentially life-threatening[83]. The present research was undertaken to evaluate potential of carica papaya leaves and purple grape fruit to treat thrombocytopenia in experimental animals. Two doses of carica papaya leaves and purple grape fruit (200mg/kg and 500mg/kg) were selected based on oral acute toxicity studies in mice following OECD guidelines. The homogenates of carica papaya leaves and purple grape juice were prepared. Heparin was used to induce thrombocytopenia as heparin activates platelets and platelet aggregation. The

subcutaneous administration of 2000 IU/kg of heparin daily for 10 days leads to thrombocytopenia in Sprague Dawley rats. This study was divided into 3 levels:

In first level, all animals were pre-treated with vehicle or test drugs for 10 days. On 11th day blood samples were collected and plts were counted. From 11th day, treatment was continued along with heparin. A rise in the plt count was seen pre heparin administration and after induction of thrombocytopenia, percentage decrease in plt count was minimal. The effectiveness of test drugs was in following order:

Papaya leaves juice (high dose) > purple grape juice (high dose) > Papaya leaves juice (low dose) > purple grape juice (low dose).

In second level, combination of papaya leaves (low dose) and purple grape (high dose) was given to animals for 10 days and same procedure as above was continued. The combination was found to be more effective than monotherapy.

In third level, animals were divided into 2 groups. The formulation was made taking papaya leaves juice and purple grape juice in ratio of 1:2.5. In one group, formulation and in other excepients of formulation was given for 10 days. And same procedure as above was continued. The excepients of formulation didn't show any rise of plt count and even after induction of thrombocytopenia, percentage decrease in plt count was almost same as vehicle group. The formulation showed steap increase in plt count and after induction of thrombocytopenia, percentage decrease in plate count was minimal. The body weight at different periods of various groups of animals during the study period was checked, which shows that the mean body weight of normal rats was gained during the study period. The body weights of thrombocytopenic control on groups were found to be reduced throughout the treatment period. During the same period of treatment the mean body weight of animals treated with both high dose of papaya leaves juice and purple grape juice(500mg/kg, p.o.) and low dose of papaya leaves juice and purple grape juice (200mg/kg,p.o.) was maintained when compared to NC and heparin treatment. The combination and formulation of papaya leaves and purple grape in a ratio of 1:2.5 were more effective compared to monotherapy.

REFERENCES

1. Aravind G. Bhowmik D. Duraivel S. Harish G. Traditional and medicinal uses of carica papaya. Journal of medicinal plant studies.2013. volume1.issue1.2320-3862.
2. S Hettige. Salutary effects of carica papaya leaf extract in dengue fever patients-a pilot study. Sri Lankan Family Physician.2008.29.17-19.
3. Management of dengue infection in adults. Clinical practice guidelines. MOH/P/PAK/209.10 (GU).
4. Battula P.caripill an advance treatment for dengue. International journal of pharmacy and pharmaceutical research. May 2016; 6(2): 2349-7203.
5. Sundus A. Siddique O. Ibrahi Faisal M. Effects of papaya leaves on thrombocyte counts in dengue-a case report. 369-366.
6. Halim Z S. Waduqe N R. Abevsinghe S.Doescarica papaya leaf extract increase the platelet count? An experimental study in a murine model. Asian Pacific Journal Of Tropical Biomedicine. 2013 sep.; 3(9): 720-724.
7. A C Gowda. P N Kasture. K H Nagabhusha. A Pilot Study to evaluate the effectiveness of carica papaya leaf extract in increasing platelet count in cases in cases of dengue with thrombocytopenia. Indian Medical Gazatte. March 2005; 109-116.
8. Yunita F. Hanani E. Kristiano J. The effect of carica papaya L. leaves extract capsules in dengue fever patient. Int. J. Med. Arom. Plants. December 2012; 2(4): 573-578.
9. Kasture N P.K H Nagabhushan. Kumar A. A multicentre double blind, placebocontrolled. randomized, prospective study to evaluate the efficacy & safety of carica papaya leaf extract, as empirical therapy for thrombocytopenia associated with dengue fever. Journal of the association of physicians of India. june 2016; 64: 15-20. www.wjpr.net Vol 6, Issue 7, 2017. 1681 Thomas et al. World Journal of Pharmaceutical Research
10. Pradeep B E.A review on the potential therapeutic profile of carica papaya leaf extract in the management of dengue fever.WHO. 2009; 10-11.
11. Subenthiran S.Choon T C. Thayan R. Abdullah N R.Carica papaya leaves juice significantly accelerates the rate of increase in patients with dengue fever and dengue haemorrhagic fever.
12. C V M NareshKumar. V Taranath.Y Siva Prasad.D V R Sai Gopal. Therapeutic potential of carica papaya leaf extract in treatment of dengue patient. International journal of applied biology & pharmaceutical technology volume6.july-sept 2015.ISSN 0976-4550.
13. Anjum V, Ansari SH, Naquvi KJ, Arora P, Ahmad A. Development of quality standards of Carica papaya Linn. Leaves, Sch Res Lib 2013; 5(2):370-376.
14. Ayurvedic pharmacopoeia of India, Govt of India, I.
15. Parle M, Gurditta. Basketful benefits of Papaya, Int Res J Pharm 2011; 2(7):6-12.
16. Arvind G, Debjit B, Duraivel S, Harish G. Traditionaland Medicinaluses of Carica papaya, J Med Car Pap 2013; 1(1):2320-3862.
17. Parle M, Gurditta. Basketful benefits of Papaya, Int Res J Pharm 2011; 2(7):6-12.
18. Jean Bruneton. Carica papaya, In: Pharmacognosy, phytochemistry of medicinal plants, Tech Docu Fra 1999; 2:221-223.
19. The Wealth of India – A dictionary Indian raw materials and industrial products: Raw material

series, Ca-Ci, publications and information directorate, CSIR 1992; 3:276-293.
20. KM Nadkarni. Indian material medica, Pop Pra Pvt Ltd, Bombay 1954; 1:273-277.
21. The Wealth of India – A dictionary Indian raw materials and industrial products: Raw material series, Ca-Ci, publications and information directorate, CSIR 1992; 3:276-293.
22. Marotta F, Weksler M, Naito Y, Yoshida C, Yoshioka M. Nutraceutical supplementation, effect of a fermented papaya preparation on redox status and DNA damage in ~ 8 ~ International Journal of Herbal Medicine healthy elderly individuals and relationship with GSATM1 genotype, a randomized, placebo controlled, Cross - over study, Ann Ny Acad Sci 2006; 1067(1):400- 407. 11. Marotta F, Pavasuthipaisit K, Yoshida C, Albergati F, Marandolo P. Relationship between aging and susceptibility of erythrocytes to oxidative damage, in view of nutraceutical interventions, Rejuven Res 2006; 9(2):227-230.
23. Dominguez de Maria P, Sinisterra JV, Tsai SW, Alcantara AR. Carica papaya lipase, an emerging and versatile biocatalyst, Biotechnol Adv 2006; 24(5):493-499.
24. Krishna KL, Paridhavi M, Jagruti Patel A. Review on nutritional, medicinal and pharmacological properties of papaya (Carica papaya Linn), Nat prod Rad 2008; 7(4):364-373.
25. Arvind G, Bhowmik D, Duraivel S, Harish G. Traditional and medicinal uses of Carica papaya, J Med Car Pap 2013; 1(1):2320-3862.
26. Natarjan Sudharkar, Theivanai @ Vidhya R M. Potential medical properties of Carica papaya Linn, Int J Pharm Sci 2014; 6(2):168-173.
27. Arvind G, Bhowmik D, Duraivel S, Harish G. Traditional and medicinal uses of Carica papaya, J Med Car Pap 2013; 1(1):2320-3862.
28. Zunjar V. Pharmacognostic physiochemical and phytochemical studies Carica papaya Linn leaves, Pharmacog J 2011; 3:214-217.
29. Anjum V, SH Ansari, Kamran Naquvi J, Poonam Arora, Adil Ahmad. Development of quality standards of Carica papaya Linn leaves, Sch Res Lib 2013; 5(2):370-376.
30. Okunola Alabi A, Muyideen Haruna T, Chinedu Anokwuru P, Jegede T, Abia H. Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of Carica papaya on clinical and fungi isolates, Pel Res Lib 2012; 3(5):3107-3114.
31. Pigli RK, Runja C. Medicinal plants used in dengue treatment, Int J Chem Nat Sci 2014; 2(1):70-76.
32. Krishna Prabha V, Thomas F. Formulation, nutrient and microbial analysis of papaya leaves and guava incorporated RTS beverage, Int J Cur Microbiol Appl Sci 2014; 3(5):233-236.
33. Soobitha S, Tan CC, Kee CC, Thayan R, Mok BT. Carica papaya leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever, Evi Bas Compl Alter Med, 2013, 148-151.
34. Fenny Y, Endang H, Jusuf K. The effect of Carica papaya leaves extract capsules on platelets count and haematocrit level in dengue fever patient, Int J Med Arom Pla 2012; 2(4):573-578.
35. Patil S, Shetty S, Bhide R, Narayanan S. Evaluation of platelet augmentation activity of Carica papaya leaf aqueous extract in rats, J Pharmacog Phytochem 2013; 1:227-232.
36. Susiji W, Roshitha NW, Rajapakse Peramune Veddikkarage Jayanthe Rajapakse, Senanayake Abeysinghe Mudiyanselage Kularatne. Does Carica papaya leaf extract increase the platelet count? An experimental study in a murine model, Asian Pac J Trop Biomed 2013; 3(9):720-724.
37. Baskaran C, Ratha Bai V, Velu S, Kubendiran Kumaran. The efficacy of Carica papaya leaf extract on some bacterial and fungal strain by well diffusion method, Asian Pac J Trop Dis, 2012, 347-349.
38. R Sumathi. Phytochemical analysis and in-vitro antimicrobial activity of aqueous and solvent extracts of Carica papaya against clinical pathogens, Int J Adv Res Biol Sci 2014; 1(1):73-77.
39. Sheikh Fauziya, Krishnamurthy R. Papaya (Carica papaya): source material for Anticancer, CIB Tech J Pharm Sci 2013; 2(1):25-34.
40. Khaled Rashed, Meng-Ting Luo, Lin Tao Zhang, YongTang Zheng. Phytochemical screening of the polar extracts of Carica papaya Linn and the evaluation of her Anti-HIV-1 activity, J Appl Ind Sci 2013; 1(3):49-53.
41. Achini Gammulle, Ratnasooriya WD, Jayakody JRAC, Charmain Fernando, Charmini Kanatiwela. Thrombocytosis and Anti-inflammatory properties and toxicological evaluation of Carica papaya mature leaf concentrate in a murine model, Onl Int J Med Pla Res 2012; 1(2):21-30.