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Effect of a Suspension of *Carica papaya* Leaf Extract on the Thrombocytopenia Induced by Carboplatin in Mice

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Abstract: A low platelet count characterises thrombocytopenia. Thrombocytopenic disorders (dengue, chikungunya, malaria, etc.) are on the rise in cities. This is now present in over 125 countries. Low platelet counts are caused primarily by decreased platelet generation and increased platelet degradation. It has been shown in several research that papaya leaf extract may increase platelet count. The major objective of the proposed research was to examine the haematology and histopathology of different organs for any adverse effects of *Carica papaya* leaf extract on raising platelet count in induced thrombocytopenic experimental animals. To test whether a rise in platelet count was present, papaya leaves were macerated in a mixture of n-hexane, acetone, ethanol, and methanol in distilled water for 8 h using a Soxhlet device. Toxicologists may benefit from experimental animal haematological and histopathological studies.

Keywords: *Carica papaya* extracts, Organic solvents, Platelet count, Thrombocytopenia, Carboplatin

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Introduction

Platelet counts that are lower than the 2.5th lower percentile of the normal platelet count distribution are used to characterise the condition known as thrombocytopenia. U.S. data from the National Health and Nutrition Examination Survey III (NHANES III) back the long-held estimate of

150 x 109/L as the bare minimum of health (Chen *et al*., 2004). However, platelet counts between 100 and 150 x 109/L may not always indicate illness if they remain steady for more than 6 months (Stasi *et al*., 2012), suggesting that a cutoff value of 100 x 109/L may be more appropriate for detecting a pathologic condition (Rodeghiero *et al*., 2009). When platelet counts are above 200 x 109/L, clinical symptoms are typically limited to simple bruising; however, when platelet counts fall below 100 x 109/L, the chance of spontaneous mucocutaneous bleeding increases significantly (Sheikh *et al*., 2014). The major causes of thrombocytopenia are insufficient platelet production and excessive breakdown. Platelet production (PNH) is decreased in BM failure, megaloblastic anaemia, leukaemia, myeloma, myelofibrosis, solid tumour infiltration, aplastic anaemia, and paroxysmal nocturnal hemoglobinuria. In autoimmune ITP, SLE, and viral illnesses such as EBV, dengue, HIV, DIC, TTP, and hypersplenism, there is excessive damage. Thrombocytopenia is the most common cause of poor primary hemostasis. This might result in significant bleeding. Significant quantitative or qualitative platelet dysfunction causes mucocutaneous haemorrhage (Tahir *et al*., 2014). The most common causes of thrombocytopenia include bone marrow failure, reduced platelet survival and splenic platelet sequestration, leptospirosis, malaria, and dengue, all of which are associated with platelet destruction and bone marrow suppression, and sepsis. A combination of the aforementioned factors might result in thrombocytopenia. Multiple causes, including primary immune thrombocytopenia (ITP) and hepatitis C virus infection, may contribute to the development of thrombocytopenia in diverse kinds of thrombocytopenia (Chakravarty *et al*., 2013).

Carica papaya L. belongs to the plant family Caricaceae. The chemical components found in papaya leaf have been demonstrated to have antioxidant, anti-hypertensive, wound healing, hepatoprotective, anti-inflammatory, antibacterial, anti-amoebic, and anti-helminthic activities. Recent phytochemical analyses of plants show that they have a long folkloric history of use in the treatment of thrombocytopenia. Disease phyto-therapy research is a contemporary trend in the treatment of tropical illnesses and hereditary abnormalities like sickle cell anaemia, with the objective of developing cheaper, alternative medications that the general people may use right away (Imaga *et al*., 2009). It was studied how quickly platelet counts recovered in dengue-infected children. The boys were given a teaspoon of powdered papaya leaf paste every four hours. Platelet counts increased considerably after 12 hours of initiating medication, reaching 100,000 in one case and 250,000 in the other within two days. Patients were given fresh juice produced from *C. papaya* leaves 15 min after breakfast for three days. The study revealed a significant increase in platelet counts after 40 h when compared to values 8 h after the intervention began (Soobitha *et al*., 2013). When an experimental animal was given a suspension of powdered crude *Carica papaya* leaves in palm oil, the treatment group's thrombocyte counts rose regularly. The impact of papaya leaf formulations was larger and statistically significant, suggesting that they might be used to treat thrombocytopenic purpura (Kathiresan *et al*., 2009). Several clinical and pre-clinical research have shown that raising platelets has a favourable benefit trend. There was a large rise on the fourth day of admission. However, large-scale randomized clinical trials have been recommended to further demonstrate its importance in dengue management. According to Zunjar *et al*. (2016), *Carica papaya* leaves decoction possesses exceptionally potent anti-thrombocytopenic properties. According to the findings, the anti-thrombocytopenic effect of *Carica papaya* leaves is due to alkaloids. Furthermore, no clear toxicity or negative effect was seen in animals. Gamulle *et al*. (2012) discovered that mature leaf of *Carica papaya* concentration (MLCC) might be used to produce a plant-based treatment for thrombocytopenia. It was also discovered that MLCC made from mature *Carica papaya* leaves can be taken orally, is non-

toxic, and effectively increases platelet, WBC, and RBC counts in both normal (non-thrombocytopenic) and thrombocytopenic rats. The thrombocytopenic parameter of papaya leaf extract is studied using crude water extract, followed to some extent by methanolic and ethanolic extracts. The current study investigated at how different papaya leaf extracts performed in polar and non-polar solvents.

Materials and Methods

Sample preparation:

The authenticated, disease-free sapling of papaya plant of breed "Taiwan 786" was purchased from Ram Biotech Limited, Nashirabad, Maharashtra, India. These saplings were given organic manure at fifteen days intervals. Fifteen-day-old leaves were shade-dried, powdered and used in an extraction process. Powdered plant parts (leaves) weighing 25 g were soaked in 250 ml of ethanol, methanol, n-hexane, and acetone, respectively, for 6 h at the boiling point in a Soxhlet device. To minimize the volume, extracts were concentrated at room temperature. Same was macerated in batches of 250 ml of distilled water for 6 h at intervals. After 6 h, we filtered the extracts using Whatman No. 1 filter paper and concentrated the filtrates at room temperature to decrease the volume.

Experimental Design:

Phase-I:

Standardization of carboplatin dosage:

The carboplatin dose was standardised to assess the drug's toxicity and lethality. A single intraperitoneal dose of carboplatin produced thrombocytopenia in experimental Sprague Dawley rats (Fresenius Kabi Oncology Ltd). 12 Sprague Dawley rats of either sex were administered carboplatin doses of 50 mg/kg body weight, 100 mg/kg body weight, and 150 mg/kg body weight. The dosage of carboplatin of 50 mg/kg body weight was determined since it was shown to be acceptable for inducing thrombocytopenia with no or very little adverse effects and was non-fatal, while the other two doses were found to be deadly.

Phase-II:

Standardization of extract doze: A group of 6 animals (Sprague Dawley rats) of either sex was tested to determine the doze of extract for 5 different solvents in 2 different concentrations of 100 mg/kg body weight and 200 mg/kg body weight, as well as a set of standard drugs prednisolone. These findings were reached despite no increase in platelet count. As a result, the new preclinical experiment was designed with a double dose of 400 mg/kg body weight. This doze produced results consistent with the hypothesis, indicating an increase in platelet count.

Phase-III:

Animal trials:

A total of 48 Sprague Dawley rats were divided into eight groups of six (three males and three females) each. After establishing thrombocytopenia in all animals, on the sixth day, the control group (group 0) was given 0.3 ml distilled water, whereas the experimental groups were given 400 mg/kg body weight of extracts orally (groups 1 to 6) (Group 1: hexane extract, Group 2: acetone extract, Group 3: ethanol extract, Group 4: methanol extract, Group 5: distilled water extract, Group 6: standard). From day 6 through day 27, this was repeated every other day. On days 6, 15, and 25, blood was obtained retro-orbitally to assess platelet count increase.

Platelet Counting:

The induction of thrombocytopenia was confirmed by assessing platelet counts using the conventional techniques.

Histological studies: At the conclusion of the trial, animals were slaughtered for histopathological examinations of the liver, lungs, kidney, spleen, heart, and intestine to assess the extract's impact on organs and toxicity using conventional techniques.

Results and Discussion

Animal studies were performed on Sprague Dawley rats to assess the increase in platelet count as a consequence of extract therapy after inducing thrombocytopenia with carboplatin. Retero-orbital blood samples were taken from rats in each group on day 0, the sixth, fifteenth, and twenty-fifth days of the experiment. The platelet count in control, n-hexane, acetone, ethanol, methanol, distilled water extract and standard were 8.83±1.03, 8.99± 0.97, 8.90±1.04, 9.18±1.39, 9.40±1.29, 8.90±1.04, 9.68±1.45 x 10^5/cmm, respectively, before induction of thrombo-cytopenia in rats (day 0). The drug carboplatin has been used to cause thrombocytopenia. It is a Cis-platin derivative. It is a second-generation platinum medication that has shown clinical efficacy in the treatment of malignant tumours. It was selected because it has less adverse effects such as toxicity, nausea, and vomiting (Rang *et al*., 2007). Carboplatin induces a fast drop in circulating platelet number (Ulich *et al*., 1995), and it affects mature multi-lineage hemopoietic cells but not stem cells (Chabner *et al*., 2011).

On day 6, after carboplatin-induced thrombo-cytopenia, the platelet count was reduced by 71.46%, 74.22%, 73.53%, 77.89%, 79.03%, 75.51%, and 75.10% in the control, n-hexane, acetone, ethanol, methanol, and distilled water extract, and standard, respectively. From day 6 onwards, on every other day until the completion of the trial, extract and standard were administered according to the predetermined doze and schedule. The platelet count increased by 18.19%, 42.49%, 42.26%, 49.92%, 60.10%, 70.97%, and 139.94% on the 15th day in the control, n-hexane, acetone, ethanol, methanol, and distilled water extract, and standard groups, respectively. Platelet count increased by 126.52%, 166.36%, 178.94%, 181.20%, 190.36%, 216.96%, and 303.66% on the 25th day in the control, n-hexane, acetone, ethanol, methanol, and distilled water extract, and standard groups, respectively. The percentage increase in platelet count and the amount of platelets detected are shown in Table 1 and Figure 1.

The present study revealed that difference between male and female animal platelet count was not significant.

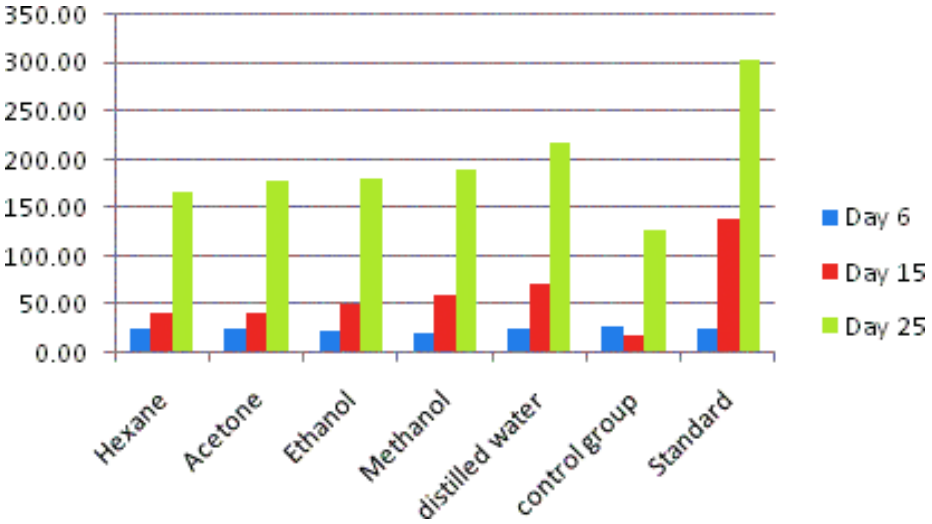
Even after 27 days of treatment, histopathological examination of the liver, lungs, kidney, intestine, heart, and spleen of the control and treated groups revealed no morphological differences or toxicity. This suggests that *Carica papaya* leaf extracts are safe and have no toxic or side effects. The representative histopathological images of the various organs are shown in Figure 2.

Following a carboplatin injection (50 mg/kg body weight), platelet count was decreased. On the seventh day, carboplatin had the highest effect, followed by an increase in platelet count. *Carica papaya* leaf extracts did not cause a decline in platelet count throughout the experiment period. Platelet count was higher on days 6, 15, and 25 in all experimental groups compared to day 1 in the same group (Fig. 1). According to the data, the platelet count significantly increased above baseline. When platelet count in experimental groups was compared to the standard (Prednisolone) and control (Natural recovery), it was observed that the standard group's platelet count increased quicker and at a greater level than the experimental groups, while the control group's platelet count was lower. The gender difference in platelet count was not significant in any of the groups. One animal study indicated that drinking papaya leaf juice increased platelet count in 5 healthy mice within hours, suggesting an increase in platelets from bone marrow. Gammulle *et al*. (2012) reported at how papaya leaf juice affected hydroxyl urea-induced thrombocytopenia in mice. When 7.2 ml/kg of juice was given for three days in a row, it increased platelet count by 76.5% above the control.

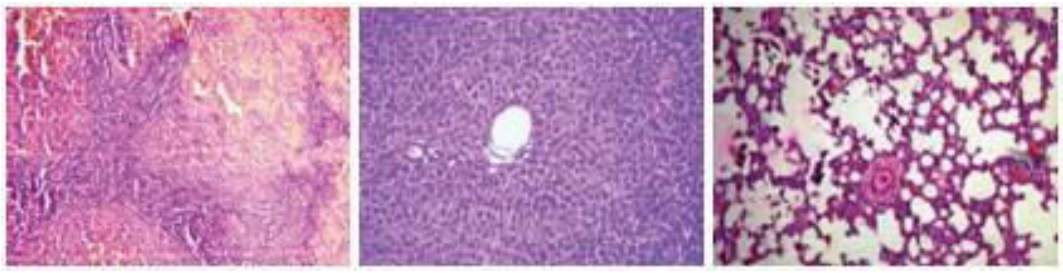
Tahir *et al*. (2014) reported at the platelet count differences between male and female Carica papaya leaf juice. The difference between male

Table 1: Platelet Count × 10^5/ cmm Mean ± SD observed in various extracts treated rat groups

Group name	Day 0	Day 6	Day 15	Day 25
Hexane	8.99 ±0.97	2.31±0.63	3.30±0.54	6.17.5±0.73
Acetone	8.90±1.04	2.35±0.84	3.35±0.78	6.57±0.64
Ethanol	9.18±1.39	2.03±1.12	3.04±0.95	5.70±0.52
Methanol	9.40±1.29	1.97±0.86	3.15±0.96	6.00±0.68
Distilled water	8.90±1.04	2.18±0.89	3.73±0.84	6.91±0.85
Control	8.83±1.03	2.52±0.75	2.97±0.84	5.70±0.28
Standard	9.68±1.45	2.41±0.62	5.78±1.12	9.73±1.30



Kidney **Heart** **Intestine**



Spleen **Liver** **Lung**

Fig. 2: Histopathological images of the variousorgans.

and female plants was not statistically significant, but a higher dose (10 ml/kg) evoked a much greater response than a low dose (5 ml/kg). This is consistent with our findings. Many active ingredients in papaya leaf juice may be responsible for increasing blood cell counts in a variety of ways. Papain, chymopapain, alkaloids, flavonoids, flavonols, tannins, and benzyl glucosinolate are all active chemicals found in papaya leaves. These substances improve and/or augment the ability of megakaryocytes to produce enough platelets to maintain an adequate platelet count in mammals, especially during chemotherapy. Leaf proteolytic enzymes, such as papain and chymopapain, are biologically active substances. Protein breakdown by these enzymes may increase platelet count because caspase (protease) activation regulates proplatelet production. Flavonols and flavonoids (including kaempferol, quercetin, myricetin, and fisetin) are commonly used in pharmaceutical formulations. They have been shown to have an anabolic effect (Songlin *et al*., 2009), which could explain why they stimulate blood cell production. Tannins are large polyphenolic compounds that contain sufficient hydroxyls and other groups (including carboxyl) to form strong complexes with proteins and other macromolecules. Tannins' complex-forming properties may contribute to their beneficial effects on platelet count in the blood because retraction events release individual pro-platelets (Patel *et al*., 2005). Using the protocols provided by Godkar and Godkar (2006), animals were sacrificed at the end of the experiment for histopathological examinations of the liver, lungs, kidney, spleen, heart, and intestine to assess the effect of the extract on organs and its toxicity. All extracts demonstrated no harmful effects on vital organs such as the liver, lungs, kidney, spleen, heart, and intestine, according to histopathological investigations. As a consequence, these extracts are safe to use orally in order to boost platelet count.

Conclusion

The initial dip in platelet count produced by carboplatin was averted by papaya leaf extract, and the platelet count eventually rose without any hazardous side effects. The effect of extracts on other haematological parameters was insignificant, and extracts had no effect on male or female rats. It is clear from this that *Carica papaya* extracts contain potential component(s) responsible for increasing platelet count; thus, additional research into the isolation, purification, and characterization of such component(s) is urgently required. However, this is a preliminary study, and more research is needed to isolate and identify the active bio-components of *Carica papaya* leaves in order to develop a suitable platelet-producing formulation.

References

Chabner BA, Bertino J, Cleary J, Ortiz T, Lane A, Supko JG and Ryan D. (2011) Chemotherapy of neoplastic diseases. In: The pharmacological basis of therapeutics, 12th Edition, McGraw – Hill Companies, China, pp. 1665-1770.

Chakravarty S, Reddy BR, Sudhakar SR, Saxena S, Das T, Meghah V, Swamy CV, Kumar A and Idris MM. (2013) Chronic unpredictable stress (CUS)-induced anxiety and related mood disorders in a zebrafish model: altered brain proteome profile implicates mitochondrial dysfunction. PloS ONE 8(5): e63302.

Chen J, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, Whelton PK and He J. (2004) The metabolic syndrome and chronic kidney disease in US adults. Annals Internal Med. 140(3):167-174.

Gammulle A, Ratnasooriya W, Jayakody JR, Fernando C, Kanatiwela C and Udagama PV. (2012) Thrombocytosis and anti-inflammatory properties and toxicological evaluation of *Carica papaya* mature leaf concentrate in a murine model. Int J Med Plants Res. 1(2): 21-30.

Godkar PB and Godkar DP. (2006) Textbook of medical laboratory technology. Bhalani Publishing House.

Imaga NO, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO, Oigbochie V, Kehinde MO and Bamiro SB. (2009) Antisickling property of Carica papaya leaf extract. African J Biochem Res.3(4):102-106.

Kathiresan S, Surash R, Sharif M, Mas Rosemal N and Walther H. (2009) Thrombocyte counts in Sprague-Dawley rats after the administration of papaya leaf suspension. Wien Klin Wochenschr 121(3): 19-22.

Patel SR, Hartwig JH and Italiano JE. (2005) The biosynthesis of platelet from megakaryocyte

proplatelet. J Clin Invest. 115: 3348-3354.

Rang HP, Dale MM, Ritter JM and Flower RJ. (2007) Rang and Dale's Pharmacology, Churchill Livingstone, Edinburgh.

Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, Bussel JB, Cines DB, Chong BH, Cooper N and Godeau B. (2009) Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood 113(11): 2386-2393.

Sadhasivam K, Ramanathan S, Mansor SM, Haris MH and Wernsdorfer WH. (2009) Thrombocte counts in spraguedawley rats after the administration of papaya leaf suspension. Wien Klin Wochenschr 121: 19-22.

Sheikh N, Papadakis M, Ghani S, Zaidi A, Gati S, Adami P, Carré F, Schnell F, Avila P, Wilson M and McKenna W. (2014) Comparison of ECG criteria for the detection of cardiac abnormalities in elite black and white athletes. Circulation 129(16): 1637-1649.

Songlin P, Ge Z, Yixin H, Xinluan W, Pingchung L, Kwoksui L and Ling Q. (2009) Epimedium-derived flavonoids promote osteoblastogenesis and suppress adipogenesis in bone marrow stromal cells while exerting an anabolic effect on osteoporotic bone. Bone 45(3): 534- 544.

Soobitha S, Choon TC and Cheong KC. (2013) *Carica papaya* leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue hemorrhagic fever. Evidence-Based Complem Altern Med. 10: 1155.

Stasi R. (2012) How to approach thrombocytopenia. ASH Education Program Book 1: 191-197.

Tahir N, Zaheer Z, Kausar S and Chiragh S. (2014) Prevention of fall in platelet count by *Carica papaya* leaf juice in carboplatin induced thrombocytopaenia in mice. Biomedica 30(1): 21-25.

Ulich TR, del Castillo J, Yin S, Swift S, Padilla D and Senaldi G. (1995) Megakaryocyte growth and development factor ameliorates carboplatin– induced thrombocytopenia in spraguedawley rats. Blood 86: 971-976.

Zunjar V, Dash RP, Jivrajani M, Trivedi B and Nivsarkar M. (2016) Antithrombocytopenic activity of carpaine and alkaloidal extract of *Carica papaya* Linn. leaves in busulfan induced thrombocytopenic Wistar rats. J Ethnopharmacol. 181: 20-25.