

## PREVENTION OF FALL IN PLATELET COUNT BY CARICA PAPAYA LEAF JUICE IN CARBOPLATIN INDUCED THROMBOCYTOPAENIA IN MICE

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### ABSTRACT

**Background:** There are no effective methods to treat thrombocytopenia once it occurs. Transfusions, growth factor injections and bone marrow transplant have their limitations. So there is increased need for research of drugs that could prevent and treat thrombocytopenia. The objective of the study to determine the effect of different doses of male and female papaya leaf juice on prevention of carboplatin induced thrombocytopenia in mice.

**Methods:** A total of 55 Swiss albino mice were randomly divided into five groups (C, M<sub>10</sub>, M<sub>5</sub>, F<sub>10</sub> and F<sub>5</sub>). Thrombocytopenia was induced in all groups by a single intraperitoneal injection of carboplatin. Male papaya leaf juice was given to prevent of thrombocytopenia to groups M<sub>10</sub> and M<sub>5</sub> and female papaya leaf juice was given to F<sub>10</sub> and F<sub>5</sub>. On days 0, 7, 14 and 21 blood samples were collected by cardiac puncture for platelet count. Significance of difference was calculated by one way ANOVA.

**Results:** After carboplatin injection, platelet count decreased. Papaya leaf juice prevented fall in platelet count throughout the study period with p-value < 0.001. Difference between male and female papaya leaf juice was not significant while higher dose (10 ml/kg) produced significantly higher responses as compared to low dose (5 ml/kg).

**Conclusion:** Papaya leaf juice prevents reversible thrombocytopenia induced by carboplatin in a dose dependent manner. There is no difference between male and female plants in this respect.

**Key words:** Thrombocytopenia, Carboplatin, Carica papaya.

### INTRODUCTION

Thrombocytopenia is defined as persistent decrease in the number of blood platelets that may be associated with haemorrhagic tenderness.<sup>1</sup> It is caused by reduced platelet production in the bone marrow (BM) or excessive peripheral destruction of platelets.<sup>2</sup> Impaired platelet production occurs in BM failure, megaloblastic anaemia, leukaemia, myeloma, myelofibrosis, solid tumor infiltration, aplastic anaemia and paroxysmal nocturnal haemoglobinuria (PNH). Excessive destruction occurs in autoimmune ITP, SLE, viral infections like EBV, dengue and HIV, DIC, TTP and in hypersplenism.<sup>3</sup>

Treatment of thrombocytopenia depends upon cause. Platelet count often improves when underlying cause is treated or causative medication stopped.<sup>4</sup> Platelet transfusions are indicated in cases of thrombocytopenia due to decreased platelet production or if active bleeding is present.<sup>5</sup> The mainstay of initial treatment of new onset primary ITP is a short course of corticosteroids with or without intravenous immunoglobulin or anti-D. For relapsed or persistent ITP corticosteroids are given along with anti-B cell antibody

rituximab / thromboprotein receptor agonists or Intravenous immunoglobulin / anti-D which have to be given serially as they increase platelet count only temporarily. Splenectomy has a durable response and may be considered for severe and refractory cases. Alternately high dose immunosuppression is also considered.<sup>4</sup>

Recently, the use of Chinese herbal medicines (CHM) to prevent and treat myelosuppression induced by chemotherapy has been studied in China. The effects of herbal decoctions (Yiqi, et al) on peripheral blood cells of cyclophosphamide treated mice were studied.<sup>6</sup> This study established an increase in the cell count on the 14<sup>th</sup> and 28<sup>th</sup> day.

*Carica papaya* belongs to the plant family caricaeae. Papaya leaf juice is safe and has many pharmacological properties.<sup>7</sup> Papaya leaf contains minerals, carbohydrates, dietary fiber, fat, protein, vitamins, and metals. Vitamins present in papaya leaf are thiamin (B<sub>1</sub>), riboflavin (B<sub>2</sub>), niacin (B<sub>3</sub>), vitamin B<sub>6</sub>, ascorbic acid (C) and especially B<sub>17</sub> (laetrile) which is said to be used for treatment of cancer. Minerals and metals present in leaf are calcium, iron, magnesium, potassium and sodium. A phytochemical analysis of carica papaya

leaves discovered the presence of papain, chymopapain, alkaloids, flavonols, flavonoids, saponins, tannins, cardiac glycoside, anthraquinones, reducing sugar, steroids, phenols, and cardenolides.<sup>8</sup>

In Far Eastern folk medicine papaya leaf juice is used in fever with haemorrhagic manifestation. In Pakistan during the dengue epidemic of 2011 it was given to some patients with remarkable improvement in platelet count within two days. Personal accounts of some patients taking papaya leaf juice for low platelet count for other reasons are also available but no controlled clinical studies are done so far. Kumar and Gupta reported two cases of proven dengue with low platelet count who improved remarkably with papaya leaf juice within 12 hours of initiating the treatment.<sup>9</sup> Another study reports use of *Carica papaya* leaf juice (CPLJ) in 60 dengue patients hospitalized as well as outdoor, it was observed that CPLJ brought speedy recovery with great beneficial effects.<sup>10</sup> Keeping these observations in mind, it seems interesting to investigate the effect of papaya leaf juice on platelet count in thrombocytopenic mice.

## MATERIALS AND METHODS

Healthy adult Swiss albino mice of either sex weighing 35 – 45 grams were obtained from the Veterinary Research Institute Lahore. The mice were kept in the animal house of PGMI, Lahore one week prior to the commencement of the study for observation and acclimatization. The mice were fed on rat chow and water. Animals of various groups were kept in separate cages. The room temperature was maintained at  $22 \pm 2^\circ\text{C}$ . Although calculated sample size was 11 in each group, initially 15 animals were taken to account for mortality during the study.

### Plant Material

Papaya plants were cultivated in the home garden after identification of species by Botany Department, University of the Punjab, Lahore.

### Preparation of Papaya Leaf Juice

Medium sized (9 – 10 inch) fresh papaya leaves of male and female tree were taken in month of November and December. They were washed with water to remove dust. The leaves were pounded and squeezed through muslin cloth to obtain the juice. To standardize preparation no water was added and moderate pressure was applied during squeezing so that about 10 ml juices was obtained from each leaf. Fresh juice was prepared daily.

### Induction of Myelosuppression in Mice:

Myelosuppression was induced in experimental mice by a single intraperitoneal injection of carboplatin (Pharmaplatin Inj. 150 mg / 15 ml by Pharmedic Company) in a dose of 125 mg/kg body weight at day 0.<sup>11</sup>

## Experimental Design

75 mice were divided randomly into five groups of 15 animals each. After inducing myelosuppression in all animals, the control group (group C) was given 0.3 ml distilled water and experimental groups were treated as follows: 10 ml/kg male papaya leaf juice (group M<sub>10</sub>), 5 ml/kg male papaya leaf juice (group M<sub>5</sub>), 10 ml/kg female papaya leaf juice (group F<sub>10</sub>), and 5 ml/kg female papaya leaf juice (group F<sub>5</sub>) as a single morning dose daily from day 0 – 21. Papaya leaf juice was administered orally with the help of a feeding tube.

## Blood Sample Collection

Blood sample of 0.5 – 1 ml was drawn by cardiac puncture<sup>12</sup> under light ether anesthesia on day 0 (before carboplatin injection), 7, 14 and 21. The sample was collected by using 3 ml disposable syringe, put in EDTA vacuum tube and run in a haematology analyser (NIHON KOHDEN) for cell counts.

## Statistical Analysis

All the data were evaluated statistically with SPSS version 17 software. Platelet count was represented as mean  $\pm$  SD and compared with one way ANOVA among groups. For *post hoc* analysis Tukey's test was performed. For comparison of mean values between times at follow-up paired t-test was used. P-value  $\leq 0.05$  was considered significant.

## RESULTS

While taking blood sample by cardiac puncture, 2 – 4 mice in each group died so eleven mice in each group were included for statistical analysis.

At day 0 the platelet count ranged between  $632 \pm 53 \times 10^9/\text{L}$  for group F<sub>5</sub> and  $704 \pm 109 \times 10^9/\text{L}$  for group M<sub>10</sub>. After administration of carboplatin, the platelet count reduced to very low level ( $78 \pm 23 \times 10^9/\text{L}$ ) in group C at day 7, then it increased at days 14 ( $10^9/\text{L}$ ) and 21 but remained low as compared to day 0 (Figure 1). However, in other groups which were simultaneously given papaya leaf juice in different doses the platelet count did not fall but was increased significantly between all reading times (t-test). Difference between all times was significant with p-value  $< 0.001$ .

Upon comparison it was noted that the difference among groups was significant at day 7, 14 and 21 with p-value  $< 0.001$ . Pair wise comparison among groups (post hoc Tukey's test) revealed that platelet count was significantly high in all treated groups as compared to group C at day 7, 14 and 21 with p-value  $< 0.001$ . It was also significantly high in M<sub>10</sub> group as compared to M<sub>5</sub> at day 7 and 14, while difference between F<sub>10</sub> and F<sub>5</sub> was significant at days 7 and 14 as well as 21. Difference between groups treated with male and female leaf juice was not significant (Table 1).

**DISCUSSION**

This study was designed to investigate the effect of papaya leaf juice on carboplatin induced thrombocytopenia in mice as animal model.

Carboplatin is a derivative of cisplatin. It is a second generation platinum drug that is clinically effective in malignant tumors. It was chosen because it causes less nephrotoxicity, neurotoxicity, ototoxicity, nausea and vomiting than cisplatin and is more myelotoxic.<sup>13</sup> Carboplatin acts by cross linking DNA, thus impairing cell replication. Effect on platelets is most marked, so carboplatin is used to induce thrombocytopenia in animal models.<sup>11</sup> Carboplatin causes a rapid decrease in circulating platelet number during day 5 to 8 followed by a sustained thrombocytopenia and then recovery starting between days 15 to 17<sup>11</sup> because it affects multilineage hemopoietic cell which are mature in nature and does not affect the stem cells.<sup>14</sup>

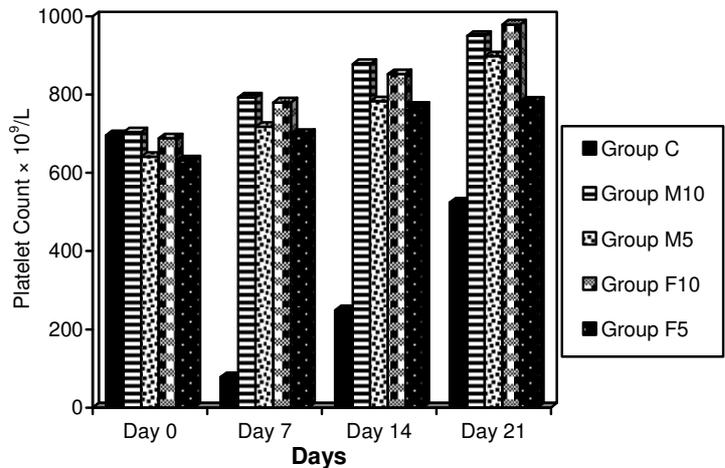
In the present study 55 adult healthy Swiss albino mice of either sex weighting 35 – 45 grams were divided into 5 groups (C, M<sub>10</sub>, M<sub>5</sub>, F<sub>10</sub>, F<sub>5</sub>). Carboplatin injection was given for induction of thrombocytopenia as a single intraperitoneal (IP) injection at a nonlethal dose (125 mg/kg) to all groups at the start of study. Papaya leaf juice was given for prevention of thrombocytopenia. In traditional medicine leaf of male papaya tree is used. To see any difference in effect of male and female tree leaf, juice of both types of leaves was used separately. Male papaya leaf juice was given to group M<sub>10</sub> (10 ml/kg) and M<sub>5</sub> (5 ml/kg) and female papaya leaf juice was given to F<sub>10</sub> (10 ml/kg) and F<sub>5</sub> (5 ml/g). To determine the platelet count blood samples were collected by cardiac puncture on days 0, 7, 14 and 21. Significance of difference was calculated.

After carboplatin injection platelet count decreased. Maximum effect was observed on day 7 and then spontaneous recovery started. Papaya leaf juice prevented fall in platelet count throughout study period. Platelet count was higher at all reading times in all experimental groups as compared to the same group at day 0. This shows that not only decrease in count was prevented but rather count was significantly increased above baseline. Multiple comparisons between groups showed that platelet count was significantly high in all experimental groups as compared to that of control at day 7, 14, and 21. Difference between male and female plant was not significant while higher dose (10 ml/kg) produced significantly high response as compared to low dose (5 ml/kg).

**Table 1:** Platelet count in carboplatin induced myelosuppressed mice treated by papaya leaf juice (n = 11).

Groups	Platelet Count × 10 <sup>9</sup> /L Mean ± SD			
	Day 0	Day 7	Day 14	Day 21
Group C	696 ± 53	78 ± 23	249 ± 81	524 ± 72
Group M <sub>10</sub>	704 ± 109	793 ± 104 <sup>ab</sup>	878 ± 78 <sup>ab</sup>	950 ± 109 <sup>a</sup>
Group M <sub>5</sub>	641 ± 50	718 ± 48 <sup>a</sup>	783 ± 83 <sup>a</sup>	898 ± 49 <sup>a</sup>
Group F <sub>10</sub>	688 ± 64	780 ± 67 <sup>ac</sup>	852 ± 86 <sup>ac</sup>	979 ± 73 <sup>ac</sup>
Group F <sub>5</sub>	632 ± 53	700 ± 38 <sup>a</sup>	769 ± 44 <sup>a</sup>	782 ± 59 <sup>a</sup>

Key: C = Positive control. M<sub>10</sub> = Male papaya leaf juice 10 ml/kg  
 M<sub>5</sub> = Male papaya leaf juice 5 ml/kg  
 F<sub>10</sub> = Female papaya leaf juice 10 ml/kg  
 F<sub>5</sub> = Female Papaya leaf juice 5 ml/kg  
<sup>a</sup> P-value ≤ 0.001 compared to control  
<sup>b</sup> P-value ≤ 0.005 compared to M<sub>5</sub>  
<sup>c</sup> P-value ≤ 0.005 compared to F<sub>5</sub>



**Figure 1:** Bar graph showing platelet count (mean ± SD) in carboplatin induced myelosuppressed mice treated by papaya leaf juice at 0, 7, and 21 days (n = 11).

**Key:** C = Positive control. M<sub>10</sub> = Male papaya leaf juice 10 ml/kg  
 M<sub>5</sub> = Male papaya leaf juice 5 ml/kg  
 F<sub>10</sub> = Female papaya leaf juice 10 ml/kg  
 F<sub>5</sub> = Female Papaya leaf juice 5 ml/kg

One animal study investigating effect of papaya leaf juice on platelet count on 5 healthy mice, showed increase in platelet count within hours, suggesting increase of platelets from bone marrow.<sup>15</sup> Gammulle et al.<sup>16</sup> studied effect of papaya leaf juice on hydroxyurea induced thrombocytopenia in rats. 7.2 ml/kg of

juice given for three consecutive days increased platelet count by 76.5% as compared with control. This is in concordance with our study.

Multiple mechanisms mediated by many active principles in papaya leaf juice may be responsible for increasing the blood cell counts. Papaya leaves contain active compounds papain, chymopapain, alkaloids, flavonoids, flavonoids, benzylglucosinolate and tannins. These compounds stimulate and / or improve the megakaryocytes to produce sufficient numbers of platelets to maintain a suitable platelet count in mammals, in particular during chemotherapy.<sup>17</sup> Biologically active compounds present in leaf are proteolytic enzymes, for example papain and chymopapain. As proplatelet formation is regulated by a caspase (protease) activation, protein digestion by these enzymes may increase platelet count.<sup>18</sup> The flavonols (such as kaempferol, quercetin, myricetin and fisetin), and flavonoids are used as ingredients in many pharmaceuticals composition. They are shown to have anabolic effect,<sup>19</sup> which may be responsible for stimulant effect on blood cell production. Tannins are large polyphenolic compounds containing sufficient hydroxyls and other groups (such as carboxyl) to form strong complexes with proteins and other macromolecules. As retraction events release individual pro-platelets,<sup>18</sup> the complex – forming properties of tannins may contribute to the positive effects on platelet count in blood.

One can **conclude** from the result of this study that papaya leaf juice prevents fall in platelet count induced by carboplatin in a dose dependent manner. There is no difference between male and female papaya leaf juice in this regard.

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#### REFERENCES

- Merriam – Webster Medical Dictionary [online] Available at <http://www.merriam-webster.com/medlineplus/Thrombocytopenia> [Accessed Dec 3, 2013].
- Murphy, M.P., Wainscoat, J. and Colvin, B.T. Hematological diseases. In: Kumar P, Clark M, eds. *Clinical Medicine: 6<sup>th</sup> Ed. An Imprint of Elsevier Saunders*; 2005; pp. 390-419.
- Craig, J.I.O., McClelland, B.D.L., and Ludlam, C.A. Blood disease. In: Boon NA, Colledge NR, Walkes BR, eds. *Davidson's principles and practice of medicine: 21<sup>st</sup> Ed.* Churchill Livingstone: Elsevier; 2010; pp. 985-1051.
- Fogarty PF, Minichiello T. Chapter 14. Disorders of haemostasis, thrombosis and antithrombotic therapy. In Papadakis MA, McPhee SJ, Rabow MW, Berger TG, eds. *CURRENT Medical Diagnosis and Treatment 2014*. New York: McGraw – Hill; 2013. <http://www.accessmedicine.com/content.aspx?aID=6176043> [Accessed Dec 1, 2013].
- Linkr CA, Damon LE, Damon LE, Andreadis C. Chapter 13. Blood Disorders in Papadakis MA, McPhee SJ, Rabow MW, Berger TG, eds. *CURRENT Medical Diagnosis and Treatment 2014*. New York: McGraw – Hill; 2013. <http://www.accessmedicine.com/content.aspx?aID=6386> [Accessed Dec. 1, 2013].
- Li, EQ., Zhao, A.B., Cao, K.I., Chen, X.Y., Dai, H.Y., and Wa, X.L. Effect of Liuwei Dihuang decoction, Buzhong Yiqi decoction and Compound Danshen decoction on the marrow suppressed mice. *China J Exp Tra Med Formulae*, 2010; 16: 153-57.
- Halim, S.Z., Abdulla, N.R., Afzan, A., Abdul Rashid, B.A., Jantan, I., and Ismail, Z. Acute toxicity Study of *Carica papaya* leaf extract in Sprague Dawley rats. *Journal of Medicinal Plants Research*, 2011; 5: pp. 1867-1872.
- Owoyele, B.V., Adebukola, O.M., Funmilayo, A.A and Soladoye, A.O. Anti-inflammatory activities of ethanolic extract of *Carica papaya* leaves. *Inflammo Pharmacol.*, 2008; 16: 168-73.
- Kumar, N., and Gupta, N. Papaya leaves for speedy rise of platelet count in Dengue, 2010. <http://dx.doi.org/10.1136/bmj.324.7353-1563>. [Accessed 15 Dec., 2013].
- Hettige, S. 2011. Papaya leaves for speedy rise of platelet count in Dengue. [Online]. Available at the College of General Practitioners of Sri Lanka on Web – (<http://www.cgpsl.org>) [Accessed 15 Dec., 2013].
- Ulich, T.R., del Castillo, J., Yin S., Swift, S., Padilla, D., Senaldi, G. Megakaryocyte growth and development factor ameliorates carboplatin – induced thrombocytopenia in mice. *Blood*, 1995; 86: pp. 971-76.
- Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother.*, 2010; 1 (2): 87-93.
- Rang HP, Dale MM, Ritter JM, Flower RJ. *Rang and Dale's Pharmacology*. 6<sup>th</sup> Edition. China: Elsevier; 2007.
- Chabner BA, Bertino J, Cleary J, Ortiz T, Lane A, Supko JG, Ryan D. *Chemotherapy of neoplastic diseases in: Brunton LL, Chabner BA, Knollmann BC, eds. Goodman and Gillman's. The pharmacological basis of therapeutics, 12<sup>th</sup> Edition. China. McGraw – Hill Companies; 2011: P. 1665-1770.*
- Salhasivam, K., Ramanathan, S., Mansor, S.M., Haris, M.H., Wernsdorfer, W.H. Thrombocyte counts in mice after the administration of papaya leaf suspension. *Wien Klin Wochenschr*, 2009; 121: 19-22.
- Gammulle A, Ratnasooriya WD, Jayakody JRAC, Fernando C, Kanatiwela C, Ugadama PV. Thrombocytosis and anti-inflammatory properties, and toxicological evaluation of *Carica papaya* mature leaf concentrate in a murine model. *Online International Journal of Medicinal Plant Research*, 2012; 1 (2): 21-30.
- Karim, A.B.M. Product and method for treating thrombocytopenia, 2009. [Online] Available at: <http://www.google.com/patents/WO2011028098A1> [Accessed on 15 Dec., 2013].
- Patel, S.R., Hartwig, J.H and Italiano, J.E. The biosynthesis of platelet from megakaryocyte proplatelet. *J Clin Invest.*, 2005; 115: 3348-54.
- Songlin, P., Xixin, Z.G., Xinluna, W., Pingchung, L., et al. Epimedium – derived flavonoids promote osteoblastogenesis and suppress adipogenesis in bone marrow stro-

mal cells while exerting an anabolic effect on osteoporotic

bone. *Bone*, 2009; 45: 535-44.