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ORIGINAL ARTICLE

Effect of Carica papaya Leaf Juice on Bone Marrow Aspirate of Busulfan-induced Chronic Bone Marrow Aplasia in Mice

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ABSTRACT

Introduction: Aplastic anemia is a disorder characterized by peripheral pancytopenia and hypoplastic bone marrow. *Papaya*, botanical name *Carica Papaya*, is a tropical fruit with a wide range of benefits. In past couple of years, researches have been carried out on beneficial outcomes of *papaya* leaf juice on blood and bone marrow, particularly platelet count.

Aim: Taking into consideration the beneficial effects of *papaya* leaves on bone marrow, current research was done to probe the effects of *C.papaya* leaf juice on bone marrow differential cell count and cellularity in myelosuppressed mice where busulfan was used to induce myelosuppression.

Study Design: Experimental.

Place and Duration of Study: our study was performed in Post Graduate Medical Institute, Lahore in one year time.

Materials & Methods : It was an animal experimental study of 23 days in which 36 adult healthy swiss albino mice weighing 30-35 g of either sex were segregated into three groups with 12 mice in each group. Busulfan at a dose of 14mg/kg was given orally to the busulfan group to induce myelosuppression and to the *C.papaya* +busulfan group on 8 occassions (0, 3,8,11,15,18,21&23) for a duration of 23 days. To observe the myeloprotective effect of *Carica papaya* leaf juice 10ml/kg of juice was given to the *C.papaya* + busulfan group daily for 23 days. After the final dose of busulfan and *Carica papaya* leaf juice on day 23, the samples for bone marrow aspirate were collected on same day.

Results: The current study has revealed that *C.papaya* leaf juice not only prevented a decrease in all parameters of bone marrow aspirate but also maintained their normal values close to normal. The megakaryopoiesis was however found to be significantly higher than the normal. Keeping in view these beneficial effects *carica papaya* leaf juice can be used in chemotherapy induced thrombocytopenia and other related clinical conditions.

Conclusion: it can be concluded from this study that C.papaya can be used in all such conditions that lead to fall in chemotherapy induced thrombocytopenia because of its property to increase megakaryopoiesis.

Keywords: *C.papaya*, Busulfan, Megakaryopoiesis, Bone marrow failure.

INTRODUCTION

Bone marrow failure (BMF) disorders are part of a large group of hematological conditions defined by uni- or multi-lineage and/or peripheral blood cytopenia(s). They can be classified as acquired or inherited. A typical example of acquired bone marrow failure is aplastic anemia in which cytotoxic T-cell mediated autoimmune cells target hematopoietic stem and progenitor cells [1]. This leads to pancytopenia due to either reduced or absent hematopoietic precursor [2]. Among the most common treatment options are immunosuppressive therapy and bone marrow transplantation. However, new therapies are being explored and essentially changing the way Aplastic anemia is treated [3].

Carica papaya is a herbaceous, non-woody, tree like plant that belongs to the *caricaceae* family. It is world widely recognized for its curative and nutritious qualities [4]. With the progress of science and technology, traditional medicine is fascinating researchers, particularly those who are preferring alternative medicine, not only based on their traditional uses but also in advanced formal medications. Regarding the future prospects of papaya as a herbal medicinal product, the leaves are the most important talked about part of the plant in folk and pharmaceutical preparations [5].

The *Carica papaya* leaves are full of nutritional elements such as papain, tocopherol, ascorbic acid and glucosinolates [6]. Studies have also shown that leaves are rich in bioactive compounds like alkaloids, flavonoids, saponins, and tannins [7]. which are not only a source of enhancing antioxidant activity in the blood but also reduce lipid peroxidation levels [8].

The *Carica papaya* leaves are ubiquitously being used in treating different forms and stages of medical complexities [9] and are of particular importance in the treatment of dengue viral infection [10]. The alkaloid carpaine in *carica papaya* leaves has displayed anti-thrombocytopenic activity in many researches [11]. Carpaine and quercitin in *papaya* leaves promotes platelet production, inhibits platelet damage and maintains platelet membrane via gene expression activity and ceasing of viral proteases, respectively [12].The papain enzyme has also been reported to reverse immune mediated platelet destruction[13]. The current literature shows that *Carica papaya* leaf juice augments platelet activity.

MATERIAL AND METHODS

The study was carried out at Postgraduate Medical Institute (PGMI), Lahore, over a period of one year after the final approval of Institutional Review Board. The sample mice were chosen from healthy adult Swiss Albino mice of either sex weighing 30-35 grams. They were kept in hygienic conditions at the Animal House at PGMI. The room temperature was kept at 25 ± 2 °C.

Carica Papaya Leaf Juice preparation: *Carica Papaya* plant grown in the home garden was the source of leaves that were used in research. These leaves were washed and dried thoroughly. The veins and petioles were separated following which the leaf blades were pestled and squeezed using a muslin cloth. Approximately 10ml juice was procured from 25g of leaf blades. Fresh juice was made daily as needed [8].

Induction of Chronic Bone Marrow Aplasia: Busulfan (GlaxoSmithKline) was used for induction of bone marrow aplasia. 14 mg/kg was administered orally to each mouse on days 0, 3, 8, 11, 15, 18, 21 & 23 over a period of 23 groups. Group B (Busulfan) and Group C (Busulfan and *Carica papaya*) [9].

Experimental Design: Sample size of 12 per group was calculated using 90% power and 5% level of significance [8]. The mice were arbitrarily arranged into three groups, Group A: Normal, Group B: Busulfan, and Group C: Busulfan and *Carica papaya.* Group B and C were administered Busulfan as described above to induce bone marrow aplasia. Groups A and B were given distilled water of 10 ml/kg daily for 23 consecutive days while 10ml/kg of *Carica papaya* leaf juice was given to Group C daily for 23 days as a single morning dose.

The content from the right femur was aspirated, using 5 ml syringe, into 0.2 ml ice cold phosphate buffered solution (PBS) and a bone marrow smear was prepared. Femoral marrow smear was stained with Geimsa stain and analyzed for differential count for myelopoiesis, erythropoiesis, megakaryocytes, lymphocytes, and plasma cells. Entire length of the tissue was scanned and viewed at 100x. A 6-point scale was used to assess each parameter displaying 0= absent 1= very slight 2= slight 3= moderate 4= marked 5= very marked [10].

Statistical Analysis: SPSS 20 was used for statistical analysis. Gad Pad Prism was used for graphical representation. Comparison between groups was obtained by using one way ANOVA in addition to post hoc Tukey's test and a p value of < 0.05 was considered as convincing.

RESULTS

Granulopoiesis: The mean granulopoiesis count ± SD of all groups is given in table 1.a and figure1.

The comparison of means of all the groups by ANOVA revealed a significant difference between the group means with a p- value of < 0.001 (Table 1.a).

Table 1.a: Effect of *Carica papaya* leaf juice on (Mean +/- SD) of granulopoiesis in busulfan induced myelosuppressed mice (n=12)

Groups	Mean ± SD	ANOVA (p value)
Normal (A)	3.41 ± 0.51	***
Busulfan (B)	2.08 ± 0.51***	<0.001
Busulfan + *Carica papaya* (C)	3.00 ± 0.42≠≠≠	
***p-value ≤ 0.001 vs Normal group ≠≠≠ p-value ≤ 0.001 vs Busulfan group		

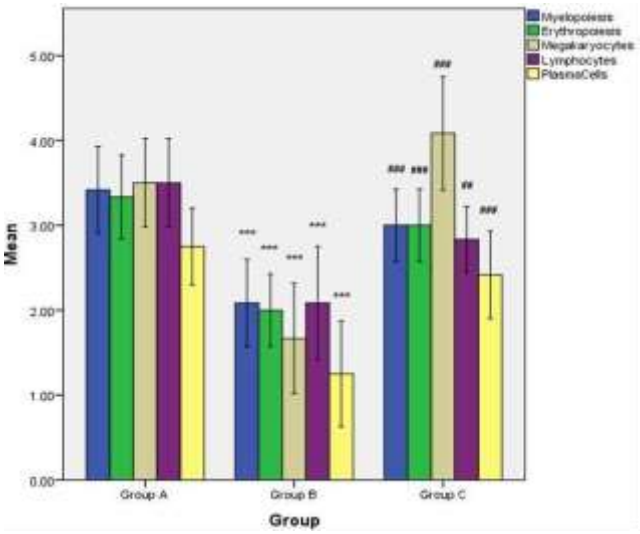

Figure 1: Effect of Carica Papaya leaf juice on myelopoiesis of busulfan induced myelosuppressed mice (n=12)

Multiple comparison by post hoc Tukey's test at day 23 revealed that granulopoiesis in Group B was significantly lower as compared to Group A and C whereas the difference between group A and C was statistically insignificant (Table 1.b).

Table 1.b: Multiple comparison of granulopoiesis by Tukey's test (n=12)

Groups (I)	Groups (J)	Mean (I-J)	Sig.
Normal (A)	Busulfan (B)	1.33	<0.001
	Busulfan + *Carica papaya* (C)	0.41	0.106
Busulfan (B)	Busulfan + *Carica papaya* (C)	-0.91	<0.001

1. Erythropoiesis: The mean erythropoiesis count ± SD of all groups is given in table 2.a and figure 1.

The comparison of means of all groups by ANOVA revealed significant difference between the group means with a p- value of < 0.001 (Table 2.a)

Table 2.a: Effect of *Carica papaya* leaf juice on (Mean ± SD) of erythropoiesis in busulfan induced myelosuppressed mice (n=12)

Groups	Mean ± SD	ANOVA (p value)
Normal (A)	3.41 ± 0.51	***
Busulfan (B)	2.00 ± 0.42***	<0.001
Busulfan + *Carica papaya* (C)	3.00 ± 0.42≠≠≠	
***p-value ≤ 0.001 vs Normal group ≠≠≠ p-value ≤ 0.001 vs Busulfan group		

Multiple comparison by post hoc Tukey's test at day 23 revealed that Erythropoiesis count of Group B was significantly lower as compared to Group A. Erythropoiesis count was significantly higher in Group C as compared to Group B (Table 2.b)

Table 2.b: Multiple comparison of erythropoiesis by Tukey's test (n=12)

Groups (I)	Groups (J)	Mean (I-J)	Sig.
Normal (A)	Busulfan (B)	1.41	<0.001
	Busulfan + *Carica papaya* (C)	0.41	0.081
Busulfan (B)	Busulfan + *Carica papaya* (C)	-1.00	<0.001

2. Megakaryocytes: The mean megakaryocyte count ± SD of all groups is given in table 3.a and figure1.

The comparison of means of all the groups by ANOVA revealed a significant difference among the group means with a p value of < 0.001 (Table 3.a).

Table 3.a: Effect of *Carica papaya* leaf juice on (Mean ± SD) of megakaryocytes in busulfan induced myelosuppressed mice (n=12)

Groups	Mean ± SD	ANOVA (p value)
Normal (A)	3.50 ± 0.52	***
Busulfan (B)	1.66 ± 0.65***	<0.001
Busulfan + *Carica papaya* (C)	4.08 ± 0.66≠≠≠	
***p-value ≤ 0.001 vs Normal group ≠≠≠ p-value ≤ 0.001 vs Busulfan group		

Multiple comparison by Post Hoc Tukey's test at day 23 revealed that megakaryocyte count of Group B is significantly lower than Group A. Megakaryocyte count was significantly higher in Group C as compared to Group B and the difference between group A and C was statistically insignificant (Table 3.b).

Table 3.b: Multiple comparison of megakaryocytes by Tukey's test (n=12)

Groups (I)	Groups (J)	Mean (I-J)	Sig.
Normal (A)	Busulfan (B)	1.83	<0.001
	Busulfan + *Carica papaya* (C)	-0.58	0.068
Busulfan (B)	Busulfan + *Carica papaya* (C)	-2.41	<0.001

3. Lymphocytes (Bone Marrow): The mean lymphocyte count ± SD of all groups is given in Table 4.a and Figure 1. The comparison of means of all the groups by ANOVA revealed a significant difference among the group means with a p value of < 0.001 (Table 4.a).

Table 4.a: Effect of *Carica papaya* leaf juice on (Mean ± SD) of lymphocytes in busulfan induced myelosuppressed mice (n=12)

Groups	Mean ± SD	ANOVA (p value)
Normal (A)	3.50 ± 0.52	***
Busulfan (B)	2.08 ± 0.66***	<0.001
Busulfan + *Carica papaya* (C)	2.83 ± 0.38≠≠≠	
***p-value ≤ 0.001 vs Normal group ≠≠≠ p-value ≤ 0.001 vs Busulfan group		

Multiple comparison by post hoc Tukey's test at day 23 revealed that lymphocyte count of Group B was significantly lower as compared to Group A. Lymphocyte count was significantly higher in Group C as compared to Group B (Table 4.b).

Table 4.b: Multiple comparison of megakaryocytes by Tukey's test (n=12)

Groups (I)	Groups (J)	Mean (I-J)	Sig.
Normal (A)	Busulfan (B)	1.41	<0.001
	Busulfan + *Carica papaya* (C)	0.66	0.05
Busulfan (B)	Busulfan + *Carica papaya* (C)	-0.75	0.005

4. Plasma cell: The mean plasma cell count ± SD. Of all groups are given in Table 5.a and Figure 1.

The comparison of means of all the groups by ANOVA revealed a significant difference among the group means with a p value of < 0.001 (Table 5.a)

Table 5.a: Effect of *Carica papaya* leaf juice on (Mean ± SD) of plasma cells in busulfan induced myelosuppressed mice (n=12)

Groups	Mean ± SD	ANOVA (p value)
Normal (A)	2.75 ± 0.45	***
Busulfan (B)	1.25 ± 0.62***	<0.001
Busulfan + *Carica papaya* (C)	2.41 ± 0.51###	
***p-value ≤ 0.001 vs Normal group ### p-value ≤ 0.001 vs Busulfan group		

Multiple comparison by Post Hoc Tukey's test at day 23 revealed that plasma cell count of Group B was significantly lower as compared to Group A. Plasma cell count was significantly higher in Group C as compared to Group B (Table 5.b).

Table 5.b: Multiple comparison of megakaryocytes by Tukey's test (n=12)

Groups (I)	Groups (J)	Mean (I-J)	Sig.
Normal (A)	Busulfan (B)	1.50	<0.001
	Busulfan + *Carica papaya* (C)	0.33	0.29
Busulfan (B)	Busulfan + *Carica papaya* (C)	-1.16	<0.001

DISCUSSION

An acquired or congenital disorder aplastic anemia causes bone marrow failure which ultimately leads to pancytopenia[11]. Even though medicine has come a long way in treatment options, Immunosuppressive therapy and stem cell transplant is the mainstay therapy for aplastic anemia[12]. All the same, acute stages of aplastic anemia are still challenging to treat and require more research[13].

This study was performed to observe *Carica papaya* leaf juice effect on prevention of busulfan induced bone marrow aplasia in mice. Previous literature shows a range of factors that have been used for induction of bone marrow aplasia, among which are: benzene & chloramphenicol[14], busulfan[10,15] and cyclophosphamide [16]. Busulfan was selected for this study as it causes irreversible damage to the bone marrow. Our study shows reduced levels of the estimated parameters in the blood, bone marrow aspirate and biopsy. This is believed to be followed by the method of administration of busulfan as described by Gibson et al[10].

Bone marrow smear results showed that there was a marked fall in myelopoiesis, erythropoiesis, megakacaryopoiesis, lymphocytes and plasma cells of the busulfan group in comparison to the normal group showings a p-value of < 0.001. Thams *et al*. showed that *Carica papaya* leaf extract on rats for 14 days induced protective effect as well as stimulated hematopoiesis, particularly the myeloblasts and megakaryocytes in lead acetate induced oxidative stress in bone marrow [17]. This is in accordance with the present study. The active ingredients of *Carica papaya* leaf extract are known to stimulate ALOX 12 and PTAFR genes which in turn lead to increased production of megakaryocytes and its to platelets and this can possibly explain the mechanism of improved hematopoiesis[18] by *Carica papaya* leaf.

CONCLUSION

From this study we can conclude that *Carica papaya* leaf juice can prevent thrombocytopenia, anemia and leukopenia induced by chemotherapy.

REFERENCES

1. Giudice, V. & Selleri, C. Aplastic anemia: Pathophysiology. Semin. Hematol. 59, 13–20 (2022).
2. Moore, C. A. & Krishnan, K. Aplastic Anemia. in Stat Pearls (StatPearls Publishing, 2022).
3. Peslak, S. A., Olson, T. & Babushok, D. V. Diagnosis and Treatment of Aplastic Anemia. Curr. Treat. Options Oncol. 18, 70 (2017).
4. Bere,A.W.,Mulati,O.,Kimotho,J.,Ong,A.F. Carica papaya leaf extract silver synthesized nanoparticles inhibit dengue type 2 viral replication in vitro. Pharmaceuticals.14,718(2021)
5. Hariono,M.,Julianus,J., Djunarko,I.,Hidayat, I.,Adelya,L., Indayani,F., Auw,Z., Namba,G., Hariyono,P. The future of carica papaya leaf extract as a herbal medicine product. Molecules.26,69229 (2021)
6. Singh, S. P. Therapeutic application of Carica papaya leaf extract in the management of human diseases. Daru 28, 735–744 (2020).
7. Nath,R., Dutta, M.Phytochemical and proximate analysis of papaya(Carica papaya) leaves. Sch.J.Agric.Vet.Sci. 3,85-87 (2016)
8. Abbasi, S. Y.,Kausar,R., Naz,H.,Chiragh,S. Effect of Carica papaya leaf juice on blood cell count of busulfan-induced chronic bone marrow aplasia in mice. Proc. AMIA Annu. Fall Symp. 35, 30–35 (2021).
9. Shubham, S., Mishra, R., Gautam, N., Nepal,M., Kashyap,N.,and Dutta, K. Phytochemical analysis of papaya leaf extract: Screening test. EC. Dent.Sci.3, 485-490, (2019).
10. Joy Ugo,N., Raymond Ade,A.,Tochi Joy,A., Nutrient components of carica papaya leaves extract. J.FoodSci.Nutr.Res. 2, 274-282, (2019)
11. Sarker,M.M.R.,Khan,F.Therapeutic potential of *Carica papaya* L. Leaves. Front.Pharmacol. 12, 610912(2021)
12. Munir, S., Liu,z., Tariq,T., kowalczewski, P., Lewandowiicz,J., Blecharczyk,A., Abid,M. Delving into the therapeutic potential of carica papaya leaf against thrombocytopenia. Molecules. 27(9) 2760, (2022).
13. Extraction & effects of papain obtained from leaves of *Carica Papaya* : A remedy to dengue fever. Extraction.3,44-46(2018).
14. Akhter, T., Khan, M. I. & Eva, E. O. Comparative evaluation of platelet augmentation activity of *Carica papaya* leaf juice and hydrocortisone in thrombocytopenic rats. Banglad. J. Physiol. Pharmacol. 30, 32–40 (2015).
15. Saraf, M. & Kavimandan, B. Animal trials of *Carica papaya* leaf extracts for increasing platelet count. Indian J. Public Health Res. Dev. 8, 782 (2017).
16. Tahir N, Zaheer Z, Kausar S, & Chiragh S. Prevention of fall in platelet count by carica papaya leaf juice in carboplatin induced thrombocytopenia in mice. Biomedica. 30(1) 21-24(2014)
17. Scheinberg P, Chen J. Aplastic anemia: what have we learned from animal models and from the clinic. SeminHematol. 50(2): 156-164(2015).
18. Gibson, F. M., Andrews, C. M., Diamanti, P., Rizzo, S., Machharia, G., Gordon, E. C., Williams, T., & Turton, J., 2003. A new model of busulphan-induced chronic bone marrow aplasia in the female BALB/c mouse. Int. J. Exp. P., 84 (1) : 31(2003).
19. Urbanowicz, I., Nahaczewska, W. & Celuch, B. Narrative review of aplastic anemia-the importance of supportive treatment. Ann. Palliat. Med. 10, 694–699 (2021).
20. Young, N. S., Bacigalupo, A. & Marsh, J. C. W. Aplastic anemia: pathophysiology and treatment. Biol. Blood Marrow Transplant. 16, S119–25 (2010).
21. Scheinberg, P., & Young, N. S. How I treat acquired aplastic anemia. Blood., 120 (6) : 1185-1196(2012).
22. Chen, J. 2005. Animal Models for Acquired Bone Marrow Failure Syndromes. C. M. R., 3 (2) : 102-108.
23. Turton, J. A., Sones, W. R., Andrews, C. M., Pilling, A. M., Williams, T. C., Molyneux, G., Rizzo, S., Gordon-Smith, E. C., & Gibson, F. M., 2006. Further development of a model of chronic bone marrow aplasia in the busulphan-treated mouse. Int. J. Exp.Pathol., 87 (1) : 49-63.
24. Feng, L., Huang, Q., Huang, Z., Li, H., Qi, X., Wang, Y., Lliu, Z., Liu, X., & Lu, L. Optimized Animal Model of Cyclophosphamide-induced Bone Marrow Suppression. B. C. P. T., 119 : 428-435(2016).
25. Tham, C. S., Chakravarthi, S., Haleagrahara, N., & Alwis, R. Morphological study of bone marrow to assess the effects of lead acetate on haemopoiesis and aplasia and the ameliorating role of Carica papaya extract. Exp. Ther. Med., 5 : 648-652(2013)
26. Subenthiran, S., Choon, T., Cheong, K., Thayan, R., Boon Teck, M., Muniandy, P., Afzan, A., Abdullah, N. & ismail, Z. Carica papaya leaves Xjuice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. C. A. M., ID 616737(2013).