

Original Article

PREVENTION OF FALL IN WHITE BLOOD CELL COUNT BY CARICA PAPAYA LEAF JUICE IN CARBOPLATIN INDUCED LEUCOPENIA IN MICE

Nayyara Tahir and Rukhsana Kausar

Objective: To determine the effect of different doses of male and female papaya leaf juice on prevention of carboplatin induced leucopenia in mice.

Methods: A total of 55 Swiss albino mice were randomly divided into five groups (C, M10, M5, F10 and F5). Leucopenia was induced in all groups by a single intraperitoneal injection of carboplatin. Male papaya leaf juice was given to prevent leucopenia to groups M10 and M5 and female papaya leaf juice was given to F10 and F5. On days 0, 7, 14 and 21 blood samples were collected by cardiac puncture for white blood cell count. Significance of different was calculated by one way ANOVA.

Results: After carboplatin injection, white blood cell count decreased. Papaya leaf juice prevented fall in white blood cell count throughout the study period with p-value < 0.001. White blood cell counts were significantly high in all groups as compared to control on day 7 with no significant difference between gender and dose.

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

Keywords: leucopenia, carboplatin, carica papaya.

Introduction

A condition in which the numbers of leucocyte circulating in the blood is abnormally low¹ and which is most commonly due to a decreased production of new cells in conjunction with various infectious diseases, as a reaction to various drugs, or other chemical, or in response to irradiation.¹ It may be due to a reduction in all cell types of white cell (pancytopenia) or individual cell types usually neutrophils or lymphocyte. A reduction in neutrophil count usually less than $1.5 \times 10^9/L$ is called neutropenia.² The main causes are chemotherapy, infection e.g. viral, bacterial (salmonella), (protozoal (malaria), alcohol, BM infiltration as in leukemia, myelodysplasia, lymphoma, renal failure, sarcoidosis and drugs (cytotoxic)³ The lack of neutrophils allows the patient to become septicemic and shocked within hours.⁴ Lymphopenia ($<1 \times 10^9/L$) is caused by connective tissue diseases (inflammation), leukemia, lymphoma, renal failure, sarcoidosis, amyloidosis and drugs (corticosteroid, cytotoxic).² Treatment of leucopenia depends upon cause. WBC counts often improves when underlying cause is treated or causative medication stopped.³ Granulocyte concentrate transfusion are indicated in case of severe neutropenia ($<0.5 \times 10^9/L$) who are not responding to antibiotic therapy but it is not usually possible to give sufficient amounts. They

may transmit CMV infection and must be irradiated to eliminate the risk of causing GVHD⁵. The cytokines G-CSF, GM-CSF are involved in the production of granulocyte.

Thus the main stay of treatment is growth factors injection like filgrastim and sargramotism which can accelerate bone marrow.⁶ Bone marrow transplant is required when bone marrow is destroyed beyond self-repairing but it is difficult to find donors and also cost and toxicity limit its use.⁷

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 decoction (Yiqi, et al) on peripheral blood cells of cyclophosphamide treated mice were studied.⁸ This study established an increase in the cell count on the 14th and 28th day. Carica papaya belongs to the plant family Caricaceae. Papaya leaf juice is safe and has many pharmacological properties.⁹ Papaya leaf contains minerals, carbohydrates, dietary fiber, fat, protein, vitamins, and metals. Vitamins present in papaya leaf are thiamin (B₁), riboflavin (B₂), niacin (B₃), vitamin B₆, ascorbic acid (C) and especially B₁₇ (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,

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0.003 and 0.004 respectively. There was no significant difference between the four experimental groups.

Similarly the differences between all groups at day 14 and 21 were also insignificant(**Table.1**).

Table-1: White blood cells counts of the groups at various times presented by mean x 10⁹/L(n=11)

	Day 0		Day 7		Day 14		Day 21	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group C	8.8	2.3	3.9	2.1	7.1	2.6	8.4	2.1
Group M10	8.5	1.7	6.8	1.3	8.2	2.5	9.9	2.3
Group M5	8.1	2.1	7.1	2.3	8.2	2.0	9.3	1.5
Group F10	7.8	2.3	5.7	1.9	9.1	1.9	10.7	1.7
Group F5	7.8	2.1	1.0	2.2	8.1	2.0	8.9	1.6

Key: C= control, M10= Male papaya juice 10 ml/kg, M5=Male papaya juice 5 ml/kg F10=Female papaya juice 10 ml/kg, F5= Female papaya juice 5 ml/kg

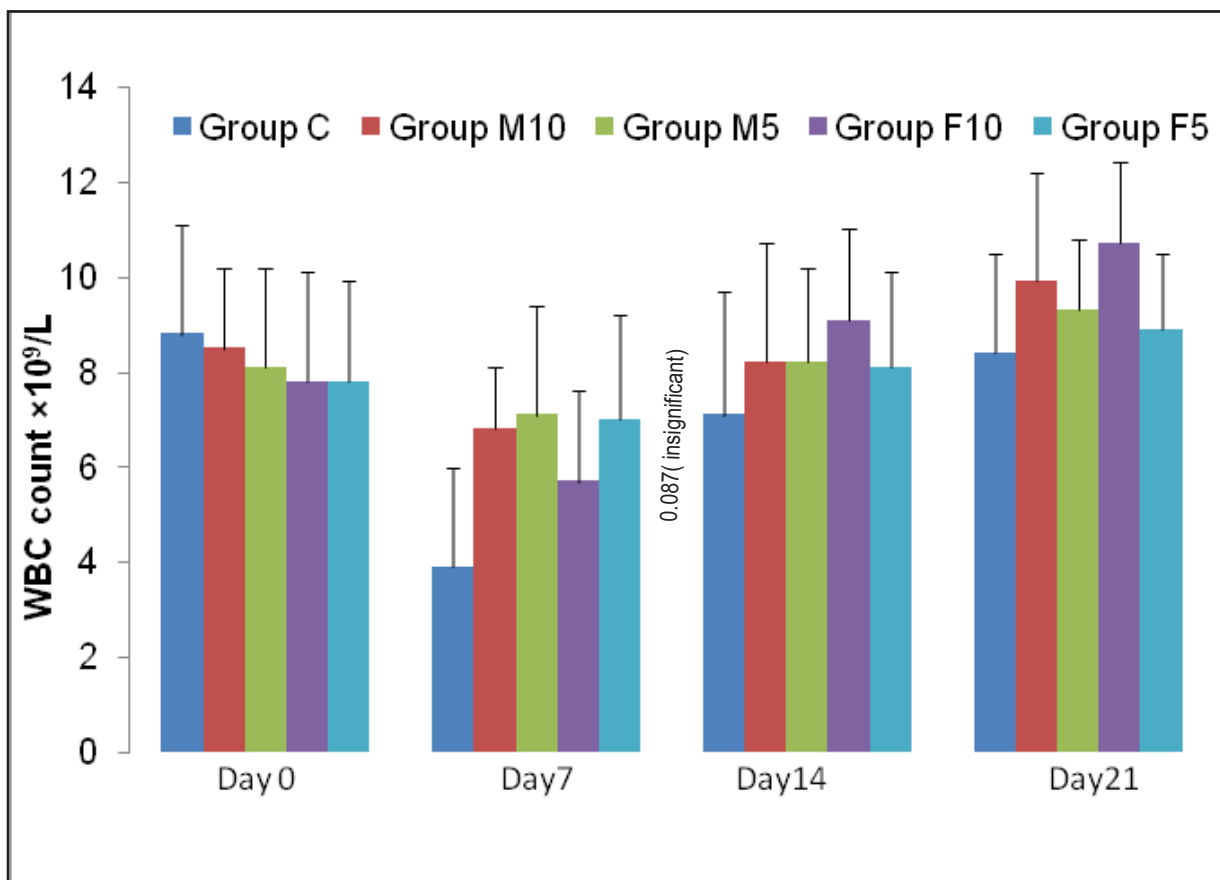


Figure-1: Bar graph showing WBC count mean±SD of five study group at 0,7,14 and 21 days (n=11)

Discussion

This study was designed to investigate the effect of papaya leaf juice on carboplatin induced leukopenia 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.¹⁵ carboplatin acts

by cross linking DNA, thus impairing cell replication. Carboplatin causes a rapid decrease in circulating white blood cell number.¹² because it affects multilineage hemopoietic cell which are mature in nature and does not affect the stem cells.¹⁶ In the present study 55 adult healthy Swiss albino mice of either sex weighting 35-45 grams were divided into 5 groups (C, M₁₀, M₅, F₁₀, F₅). Carboplatin injection was given for induction of leucopenia 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 leucopenia. 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 M10 (10ml/kg) and M5 (5ml/kg) and female papaya leaf juice was given to F10 (10ml/kg) and F5 (5ml/kg) to determine the WBC count blood samples were collected by cardiac puncture on days 0, 7, 14 and 21. Significance of difference was calculated.

After carboplatin injection WBC count decreased. Maximum effect was observed on day 7 and then spontaneous recovery started. Papaya leaf juice prevented fall in WBC count throughout study period. WBC count fell slightly at day 7, became equal at day 14 and slightly raised at day 21 as compared to day 0 in most of groups. This shows that not only decrease in count was prevented but rather count was increased above base line in all groups. Multiple comparisons between groups showed that WBC count was significantly high in all groups as compared to control on day 7 with no significant difference between gender and dose.

A study on a dengue patient revealed increased in WBC and platelet count following treatment with papaya leaf juice.¹⁷ Tham et al.¹⁸ studied effect of papaya leaf juice on lead acetate induced leucopenia in 48 rats in. The increase in granulocytes was more. 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, saponins, cardiac glycosides, steroids and tannins. These compounds stimulate and / or improve the immature cell to produce sufficient

numbers of mature cell to maintain a suitable cell count in mammals, in particular during chemotherapy. Biological active compound present in leaf are flavonols (such as kaempferol, quercetin, myricetin and fisetin), and flavonoids are used as ingredients in many pharmaceuticals composition. They are shown to have anabolic effect¹⁹ which may be responsible for stimulant effect on blood cell production. Many growth factors like IL-1, IL-3, IL-6, IL-11, Stem cell factors and megakaryocyte growth factor stimulate bone marrow. IL-1, IL-3 and IL-6 exert their primary effect in stem cell differentiation and are important for multiple blood lineage. IL-1 and IL-2 stimulate and regulate B lymphocyte and megakaryocyte colonies. Granulocyte colony stimulating factor (G-CSF), and granulocyte macrophage colony stimulating factor (GM-CSF) exert their effects in the differentiation cascade and their effects are lineage specific.²⁰ IL-6 also accelerate recovery from hematopoietic depression in mice by stimulating multilineage hematopoiesis.²¹ Thus active principles present in papaya leaf juice may stimulate G-CSF AND GM-CSF which is responsible for white blood cell production. However, this is a preliminary study and more work is needed to isolate and to identify the biologically active ingredients of papaya leaves that are responsible for these effects.

Conclusion

The result of this study that papaya leaf juice prevents fall in wbc count induced by carboplatin in a dose dependent manner. There is no difference between male and female papaya leaf juice in this regard. Thus papaya leaf juice may be used as a medicine to boost hematopoiesis when these have been suppressed by diseases.

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References

1. Merriam Webster Medical Dictionary [online] Available at <http://merriam-webster.com/medlineplus/leukopenia> [Accessed Dec 3, 2013].
2. 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: 21st Ed. Churchill Livingstone: Elsevier; 2010; pp.985-10513.
3. Murphy, M.P., Waincoat, J. and Colvin, B.T. Hematological diseases. In: Kumar P, Clark M, eds. Clinical medicine: 6th Ed. An Imprint of Elsevier Saunders; 2005; pp.390-419
4. Linker CA, Damon LE, Damon Le, Andreadis C. Chapter 13. Blood Disorders in Ppatakis MA, McPhee SJ, Rabow MW, Berger TG, eds. CURRENT Medical Diagnosis *D i a g n o s i s* <http://www.accessmedicine.com/content.aspx?aID=6386> [Accessed Dec.1, 2013].
5. Bain, B.G., Lewis, S.M. and Bates, I. Basic Hematological

- Technique, In: Dacie and Lewis Practical Hematology. 10 ed. Churchill Livingstone: An imprint of Elsevier, pp150-151.
- 6- Rang, H.P., Dale, M.M., Ritter, J.M and Flower R.J. Haemopoietic system. In Rang and Dale, s Pharmacology 6th ed. China: Elsevier; 2007.
- 7- Hope, S., and Rugo, MD. Cancer. In: Tierney, L.M., Mephee, S.J., Papadakis, M.A. Current medical diagnosis and treatment 45ed. McGraw Hill; Lange; 2006 pp.168
- 8- 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' 16:153-154
- 9- 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
- 10- 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
- 11- Deepak BSR, Girish KJ, Jadhav Lakshmi prasad L. Effect of papaya leaf juice on WBC and platelet count in dengue fever. A Case Report Journal of Ayurveda and Holistic Medicine. 2013; 1(3):44-47
- 12- 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].
- 13- Ulich, T.R., del Catillo, 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.
- 14- Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother, 2010' 1(2); 87-93
- 15- Rang, H.P., Dale, M.M., Ritter, J.M and Flower R.J., Flower R.J. Cancer chemotherapy. In: Rang and Dale, s Pharmacology 6th ed. Churchill Livingstone, 2006. pp.723.
- 16- Chabner BA, Bertino J, Cleary J, and Ortiz T et al. Chemotherapy of neoplastic diseases in: Brunton LL, Chabner BA, Knollmann BC, eds. Goodman and Gilman's. The pharmacological basis of therapeutics, 12th Edition. China. McGraw Hill Companies 2011. pp 1665-1770.
- 17- Ahmad, N., Fazal, H., Ajaz, M., Abbasi, B. H. Dengue treatment with Papaya leaf juice Asians Pac J Trop. Biomed. 2011: 330-333.
- 18- Tham, C.S., Chakravarthis, S., Haleagraharady of , N., Alwis, R.D. Morphological study of bone marrow to assess the effect of lead acetate on haemopoiesis and aplasia and the ameliorating role of papaya extract. Exp. The Med 2013; 5: 648-555
- 19- Songlin, P., Xixin, Z.G., Xinluna, W., Pingchung, L., et al. Epimedium derived flavonoids promote osteoblastogenesis and suppress adipogenesis in bone marrow stromal cells while exerting an anabolic effect on osteoporotic bone. Bone, 2009' 45: 535-44
- 20- Gordon, M. Stemcell and haematopoiesis, In: Hoffbrand VA, Catovsky D, Tuddenham DGE, eds. Postgraduate haematology 5th ed. Blackwell: 2005. pp.1-13.
- 21- Patchen, M.L., MacVittie, T.J. Williamarns, J.L., Schwartz, G.N., Souza, L.M. Administration of interleukin-6 stimulates multilineage hematopoiesis and accelerates recovery radiation-induced hematopoietic depression. Blood, 1991. 77: 472