ORIGINAL ARTICLE

Effects of Papaya Seed on Quantitative Total Leukocyte & Differential Cell Counts in Rabbits

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ABSTRACT

Objective: To observe the effects of papaya seeds on quantitative total white blood cells and differential counts.

Methods: This experimental study was conducted at Pharmacology Department, Peoples University of Medical and Health Sciences foe women, Nawabshah from Oct to Dec 2017, thirty adult and healthy female rabbits were selected and divided equally into two groups, Group A (Control) given only fresh hay & water and Group B (treated) given papaya seeds powder as dose of 500mg once a day for 60 days along with fresh hay & v .ter provided ad libitum, then blood samples were sent to Diagnostic & Research Laboratory PUMHSW, for assessment of total leukocytes and differential cell counts.

Results: Total leukocyte count raised, highest (18.78%) increase seen on Day-30 in Group-B (treated), Lymphocytes increased significantly with ($p<0.05$) in comparison to the control group on day-3o&45. Neutrophil count remained in the normal range in Group-B, Eosinophils count decreased with ($p<0.05$) on Day-30. Whereas Monocytes count decreased from 3.1±0.36 to 1.8±0.17 in group-B.

Conclusion: An increment was seen in total leukocyte count due to increased lymphocyte count. Neutrophils remained within normal limits, whereas eosinophils & monocytes decreased.

Keywords: Papaya seeds, Total Leukocyte count, Differential Leukocyte count, Rabbits.

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INTRODUCTION:

Papaya is one of the most investigated plants nowadays; it is an evergreen blossoming plant having abundant properties of a natural remedy for treatment of various diseases[1].

All its parts like fruit, leaves, latex, peel, bark, and seeds are known to have active biochemicals which can be utilized in various hematological diseases and in other diseases[2].

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Papaya contains enzymes papain, lycopene, isothiocyanate, important vitamins (B complex and C), carbohydrates, flavonoids and cartenoides etc, so all these ingredients take part in various processes of immunity boosting, fighting against viral and bacterial diseases, and act as anti-oxidant, anti-cancer, anti-diabetic, anti-sickling, antifungal, anti-helminthic and anti-amoebic activity[3].

White blood cells are the cellular components of blood that lack hemoglobin but possess a nucleus. White blood cells are also motile, and their main function is to protect the body from infections and diseases[4]. WBCs are of different types most abundant are Neutrophils which fight infections by ingesting microbes like bacteria and fungi. Eosinophils fight large parasites like intestinal worms, these cells also secrete IgE antibodies to combat allergens. Basophils secrete histamine during allergic reactions. Lymphocytes consist of 3 cells B cells, T cells, and natural killer (NK) cells. B cells recognize viruses and release antibodies against them, while T cells and NK cells fight cells

infected by viruses and cancer. Monocytes these convert into macrophages and ingest cell debris[5].

White blood cells always remained a thought of physicians when they treat the patients having very low count in the disease of viral infections, Congenital disorders, Cancer, autoimmune disorders, malaria, Dengue fever, Poor nutrition, Alcohol abuse, cyclic neutropenia, drugs induced leucopenia (phenothiazines, phenylbutazone, allopurinol), antibiotics, anti-neoplastic and radiation therapy agents[6].

Although the low white blood cell count is treatable with pharamacological preparations like granulocyte colony stimulating factor 9 (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF) which stimulates the proliferation and differentiation by interacting with specific receptors found on myeloid progenitor cells in bone marrow but clinical trials have not shown improved survival in cancer patients treated with G-CSF. And these factors also cause febrile episodes[8].

Nevertheless the new drugs in these groups are drug of choice in postchemotherpy induced neutropenia and in acute myloid leukemia, aplastic anemia, myelodysplasia and also used in lymphoma, solid tumors and in patients who received allogenic bone marrow transplantation. These growth factors are not without the side effects including the fever, artharlgia, bone pain, mylagia, capillary leak syndrome, pleural effusion, allegic reaction and very serious but rare dose dependent splenic rupture can ocour[8].

Non pharmacological & natural remedies are very useful for treating the low white and differential cell count probably with much less / no side effects, these including the Lavender Essential Oil, Garlic, Spinach, Papaya Leaves and seeds, Vitamins, Yogurt, Omega-3 Fatty Acids, Zinc, Broccoli, Selenium, Kiwi, Citrus Fruits[9]. These natural fruits, vegetable and remedies contains bioactive chemicals, flavonoids, cartenoids and lot of other ingredients which take part in therapeutic activities[10].

METHODS

This experimental study was conducted at PUMHSW, Nawabshah from Oct to Dec 2017. Thirty adult healthy female rabbits were used at animal house of Pharmacology Department of PUMHSW, age of rabbits was between 16-2 months, weight was 1 kg to 3 kg, all animals belonged to domestic rabbit (Oryctolagus canaliculus), these two groups were kept in separate rabbit cages in well ventilated atmosphere, with maintained temperature of 27-30°C, 12 hour natural light and 12 hour darkness, with free access to tap water and fresh hay.

The animals were divided into two group A & B, each group consists of 15 animals. Group A served as (Control) and received the fresh water and hay. Group B served as (Treated) and received 500mg of papaya seed powder mixed with 5cc distilled water once daily for 60 days orally along with fresh hay and water.

Sample Administration & Schedule of Blood Collection:

The papayas were bought from the local market in Shaheed Benzir Abad Sindh, and they were cut in two pieces, was hed under running fresh water dried under room temperature. Seeds were ground into fine powder using a domestic dry grinder and packed in plastic envelopes as 500mg dose.

Blood Sample Analysis:

The blood samples of both groups were drawn on 0, 15th, 13th, 45th and 60th day of treatment. The blood was drawn from large, visible auricle veins by using the 1cc syringe, then blood was immediately transferred in EDTA tube, and sent to research laboratory at PUMHSW, for analysis of total leukocyte and differential cell count on (Nihon kohden Mek6420 k) autoanalyser Machine (Japan).

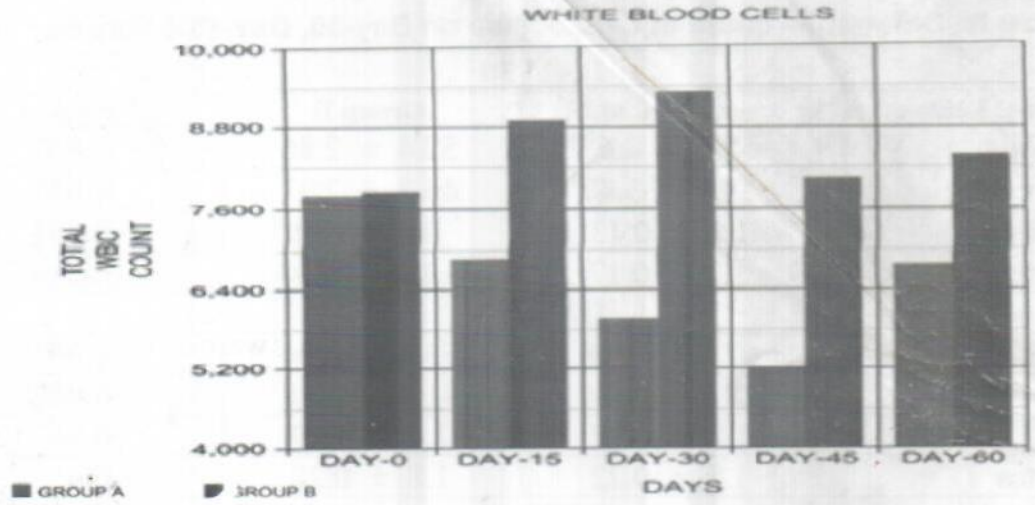
Data Analysis:

The Results of this study were analyzed by using the SPSS (version 19) and data presented as a standard error of mean (M± SEM), and groups were analyzed by applying the student t-test.

RESULTS

The present study was designed to observe the effects of papaya seeds on Leukocytes, the observation and results on blood tests compared with control group.

Papaya seeds raised the TLC (Total Leukocyte Count) ($p<0.05$) in Group-B (study), from Day-30 to Day-45 (18.78% increment), maximum increment observed on Day-30 in comparison of baseline Day-0 (Table I) and (Graph I).



Graph I. Total Leukocyte Count in Groups A and B.

Table I. Total Leukocyte Count of Female Rabbits

DAY	Hematological parameter	Group-A (Control)n= 15 (Mean ±SD)	Group-B (treated) n= 15 (Mean ±SD)	p-value
0	TLC	7800± 322.19	7840± 413.33	0.940
15	TLC	6860± 364.08	8893±1107.87	0.092
30	TLC	5960± 758.31	9313 ±1035.37	0.014
45	TLC	5220± 756.04	8046 ±939.04	0.028
60	TLC	6753± 317.41	8366 ±666.24	0.037

Table II. Differential leukocyte counts from Day-0 to Day 15.

DAY-0			
Differential Leukocytes	**Group-A**	**Group-B**	**P-value**
Neutrophils	44.7 ± 2.92	48.0 ± 2.59	0.401
Lymphocytes	49.8 ± 2.61	46.5 ± 2.61	0.375
Eosinophils	2.5 ± 0.27	2.4 ± 0.34	0.879
Monocytes	3.0 ± 0.29	3.1 ± 0.36	0.886
DAY-15			
Neutrophils	58.4 ± 5.28	56.6 ± 2.39	0.758
Lymphocytes	37.8 ± 5.08	39.4 ± 2.32	0.786
Eosinophils	2.1 ± 0.23	1.8 ± 0.24	0.430
Monocytes	1.7 ± 0.27	2.0 ± 0.26	0.167

Table III. Differential count of Leukocytes on Day-30, Day-45 & Day-60.

DAY-30			
Differential Leukocytes	**Group-A**	**Group-B**	**P-value**
Neutrophils	68.3 ± 3.63	52.4 ± 2.86	**0.002**
Lymphocytes	28.3 ± 3.47	45.2 ± 2.97	**0.001**
Eosinophils	1.6 ± 0.13	1.0 ± 0.21	**0.001**
Monocytes	1.2 ± 0.17	1.0 ± 0.14	0.434
DAY-45			
Neutrophils	67.3 ± 3.53	53.8 ± 3.04	**0.007**
Lymphocytes	28.8 ± 3.34	41.7 ± 2.88	**0.007**
Eosinophils	1.8 ± 0.17	2.0 ± 0.29	0.562
Monocytes	1.5 ± 0.22	1.8 ± 0.28	0.456
DAY-60			
Neutrophils	54.9 ± 2.23	48.5 ± 3.37	0.121
Lymphocytes	41.3 ± 2.02	47.9 ± 3.29	0.095
Eosinophils	2.0 ± 0.34	1.8 ± 0.17	0.603
Monocytes	1.6 ± 0.24	1.8 ± 0.17	0.500

Neutrophils remained in normal limits (34-70%) during the whole study period, but statistically significant difference seen with $p<0.05$ in both groups on Day-30 & Day-45 where higher values seen in Group-A (control).

No statistically significant change observed in counts of Lymphocytes, Eosinophils and Monocytes up to Day-15 as shown in table II and III, then lymphocytes count showed a statistically significant increment on Day-30 with p-value 0.001 & on Day-45 with p-value 0.007 in comparison of Group-A (control), Where values were 28±3.47 & 28.8±3.34 on Day-30 and Day- 45 in Group-A and in Group-B 45.2±2.97 & 41.7±2.88 on Day-30 and Day-45, so lymphocytes increased higher on Day-30 with p value 0.001. These findings show the immunity boosting ability of papaya seeds.

No statistically significant count difference occurred in Eosinophils and Monocytes count except in Eosinophils on Day-30 where higher values seen in Group-A in comparison of Group-B. No significant changes observed in Eosinophils and Monocytes counts (Table II & III) On Day-60. No statistically significant changes observed in Differential leukocyte counts of both groups (Table III).

Cumulative counts of Eosinophils and Monocytes in the study group decreased from day 15 to day 60, this decline is useful for prevention of the allergic and inflammatory conditions.

DISCUSSION:

The drugs derived from the plant kingdom are being increasingly used along with conventional medicine to improve the general health and well being for preventing and curing the disease conditions.[11] The aim of the science of nutrition is the determination of the amounts and kinds of eatable foods that promote the health, well-being, and disease-free states. Both the over nutrition and under nutrition conditions are problematic[12].

Although the exact mechanism for the effect of papaya seed powder cannot be explained by this study, but the results show that seeds have effects on white blood cells.

The rise of WBC count also seen in our study in female rabbits where the count was 7840±413.33/cmm on Day-0, 8893±1107.87/cm on Day-15 and 9313±1035.37/cmm on Day-30, so (18.78%) increment seen from baseline to Day-30 (Table I), It means papaya seed increased the cells of defense and may have the immunity boosting ability. Then gradual variation was seen by means of lower and higher counts up to the day 60. It indicates that papaya seeds consumption for 30 days in cases where white blood cells count increase is required can be taken in a safe dose of 500mg/day. Isaiah in his study on wistar rats by the using the papaya seeds and investigated the hematological parameters and anti spermatogenic effects, obtained the results that

that there was a slight stimulatory effects as the total white blood cell increased according to the varying doses of 100mg and 200mg respectively (6.56±0.38 and 6.96±0.10) with mean packed cell volume of 38.0±0.84 and 37.4±1.03 also noticeable is the slight rise in the Lymphocytes from the control group to the varying doses of exposure 70±2.06 and 68.6±1.12.() as our study also shows in Table-3 an increment in lymphocytes from 39.4±2.32 on day 15 to 45.2±2.97 on day 30 ($p<0.05$) in comparison to control group ,further statistically non-significant rise in lymphocytes seen in study group 47.9±3.29 (p- value 0.09) on day. Explains the immune s .mulatory properties of the papaya seeds[13].

In our study as shown in (Table 2,3) an increment in lymphocytes seen from 39.4±2.32% on Day-15 to 45.2±2.97% on Day-30 ($p<0.05$) in comparison to control group, further statistically non-significant rise in lymphocytes seen in Group-B 47.9±3.29% (p-value 0.09) on Day-60. These findings explain the immune stimulatory properties of the papaya seeds.

Nghonjuyia et al investigated the Papaya seeds in chicks for toxicological effects on the hematological and biochemical parameters. This study showed that, analyses did not show significant differences in any of the parameters examined in female or male groups, with the exception of a transient rise in white blood cell counts at high doses (640 mg/kg)[14].

Kipyegon AN et al studied the effect of oral administration of ripe Carica Papaya seed powder on testicular histology of Sus scrofa domestica boars was done, the hematological parameters were measured for 8 weeks, the highest wbc count was noticed at 4th week of study, values were 11.4 m/mm3 on day 0 to become 15.9 m/mm3 on day 30, it means 39.47% increment seen, without any obvious illness[15].

Nwangwa EK et al reported that, Carica papaya seed extract may have a stimulatory effect on haematopoeisis which may have resulted from the available bioactive and Vitamins components of its phytochemistry. They resulted that dose dependent statistically significant ($p<0.05$) increased in all haematological parameters (RBC, WBC, Platelets, PCV and lymphocyte count)[16].

Eosinophils play a significant role in the inflammation related to allergies, eczema, and asthma. Present study shows that Eosinophils count statistically decreased from 2.4±0.34 on day 0 to 1.0±0.21 ($p<0.001$) on day 30, this is also finding of study of Assadullah et al[3], there was also decrease of an eosinophil count from 2.0±0.00 on day 0 to 1.5±0.55 on day 45. As eosinophil take part in allergic conditions and their values increase in allergies, so decrease of this count shows that papaya seeds can be beneficial in allergic and inflammatory conditions.

This study was aimed at qualitative evaluation of the ethanol seed, leaf and pulp extracts of C. papaya for bioactive compounds and also to investigate their effect on the haematology in male albino rats. A 3 x 4 factorial experimental layout using randomized complete design was adopted. Results show that the phytochemicals found in seed, leaf and pulp were almost the same but however, in varying proportions. Present result also revealed that there were significant effects ($p < 0.05$) of the extracts on the heamatology of the treated rats, which was blamed on the varying and different variants ofbioactive compounds found in the extracts they were administered with. Suggestively, C. papaya extracts could be used to enhance the production of selected blood parameters, taking issue of dosage into consideration[4].

An Egyptian study was conducted on fishes (mature male tilapia). Fishes were stocked in the pond and normal feed and Papaya seeds ground powder was given for 4 weeks in fishes with higher dose resulted in progressive fall in RBCs, hemoglobin, and hematocrit levels, while higher levels were seen in white blood cells count[17], Same an increase of total leukocytes also seen in our study.

CONCLUSION:

The papaya seeds influence the white blood cells count in female rabbits, and increases the Lymphocyte count. Papaya seed consumption offers a cheap, natural, harmless, readily available mono-therapy and preventive strategy in low white blood counts. More sophisticated scientific studies are required for authentication of seeds for human use.

REFERENCES:

1. Milind P, Gurditta. Basketful benefits of Papaya. Int Res J Pharmacol. 2011;2(7):6-12.

2. Arvind G, Bhowmik D, Duraivel S, Harish G. Traditional, and medicinal uses of Carica papaya. J Medicinal Plants Studies. 2013; 1 (1):7-15.
3. Assadullah, Ahmed A, Maheshwari MP, Jahan F. Effects of Papaya seeds on qualitative Hemoglobin analysis in an animal model. Medical Channel. 2016; 22(1): 33-7.
4. Ikpeme EV, Ekaluo UB, Kooffreh ME, Udensi. Phytochemistry and hematological potential of ethanol seed leaf and pulp extracts of Carica papaya (Linn). Pak J Biol Sci. 2011;14 (6):408-11.
5. Kumar, Abbas, Aster. Robbins and Cortana Pathologic basis of disease. South Asia edition. Elsevier.2015;630-631,635-638.
6. Kasper DL, Fauci AS, Hauser SL, Longo DL, Jameson JL, Loscalzo J. Harrison's internal medicine.19th edition. McGraw Hill education. 2017;678-81.
7. Bertram. G Katzung. Basic and Clinical Pharmacology.14th edition. McGraw Hill education. 2018; 602-3.
8. McCullough J, Clay M, Herr G, Smith J, Stroncek D. Effects of granulocyte-colony-stimulating factor on potential normal granulocyte donors. Transfusion. 1999 Oct;39(10):1136-40.
9. Premkumar, Louis. Fascinating facts about phytonutrients in spices and healthy food. (2014). Available from; www.researchgate .net/publication/301675796_Fascinating_Facts_About_Phytonutrients_in_Spices_and_Healthy_Food.
10. Xu DP, Li Y, Meng X, Zhou T. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. Int J Mol Sci. 2017;18: 96.
11. Ahmed M, Jabeen F, Mahjabeen, Jehan N, Alam SM. Noval investigation on Thuja occidentalis extract on rabbit hematological and biochemical parameters. Inter Res J Pharmacy. 2013; 4(3):1
12. Parks N. What Is Over nutrition & under nutrition. Available from; https://www.livestrong.com/ article/518819-what-is-overnutrition-and-undernutrition/. Accessed on 12 October 2018.
13. Isaiah IN and Ibhoje UU. Haematologic Indices and Anti-Spermatogenic Effects of Dietary Supplemented Carica papaya seeds on Wister Rats. AJMI. 2016;1 (2):18-23.
14. Nghonjuyia NW, Tiambo CK, Taïwe GS, Toukala JP, Lisita F, Juliano RS, et al. Acute and sub-chronic toxicity studies of three plants used in Cameroonian ethnoveterinary medicine: Aloe vera (L.) Burm. f. (Xanthorrhoeaceae) leaves, Carica papaya L. (Caricaceae) seeds or leaves, and Mimosa pudica L.(Fabaceae) leaves in Kabir chicks. J Ethnopharmacol. 2016;178: 40-9.
15. Kipyegon AN, Mutembei HM, Tsuma VT, Oduma JA. Effects of Ripe Carica Papaya Seed Powder on Testicular Histology of Boars. Inter J Veter Sci. 2012;1(1):1-4.
16. Nwangwa EK., Hoye EI, Ugoji AE. A study of haemato stimulatory effects of a graded dose of aqueous Carica papaya seed extract in Wistar Rats. Res J Pharm Biol Chem Sci. 2013;4:129-36.
17. Abbas HH, Abbas WT. Assessment study on the use of pawpaw, Carica Papaya seeds to control Oreochromis niloticus breeding. Pak J Biol Sci. 2011;14 (24):1117-23.