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Anti myelosuppressant and hematopoietic activities of ethanolic fraction obtained from seeds of *Carica papaya* L.

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Abstract: Bone marrow suppression is one of the serious consequences of treatment with cytotoxic chemotherapeutic agents such as doxorubicin (DOX). It is very difficult to treat bone marrow suppression caused by anti-cancer drugs. This study was aimed to evaluate hematological effects particularly the antimyelosuppressant effects of ethanolic extract of papaya seeds at 200, 400 and 600 mg/kg daily dose for three weeks in doxorubicin induced hematopoietic suppression in rat model. Hematological parameters were assessed on weekly basis on days 0, 1, 7, 14 and 21. The alcoholic extract was found to cause remission of induced myelosuppression as indicated by a dose dependent increase in WBCs, neutrophils, lymphocytes, platelets, RBCs, Hb, hematocrit & mean corpuscular volume. However, the maximum dose (600mg/kg) of the extract showed maximum activity ($p<0.05$) in normalizing hematological parameters when compared with group B (induced group) and group A (controlled animals). These effects were compareable with those produced by Filgrastim 5µgm/kg used as standard or reference drug during these experiments. It is concluded from the results that papaya seeds possess myelostimulant activity and can be used to treat myelosuppression caused by chemotherapy. The drug can also be used for curing anemia, thrombocytopenia and immunological disorders characterized by myelosuppression.

Keywords: Myelosuppression, thrombocytopenia, immunological disorders, papaya, anemia.

INTRODUCTION

Cancerous cells do not respond to normal growth controls and divide unlimitedly at a rapid pace to form a tumor. (Anand *et al*., 2008). Anticancer drugs usually produce their effects by causing cell demise indescriminately (Cragg and Newman, 2005). These drugs can not distinguish between cancerous cells and those healthy cells that normally grow rapidly, as a consequence are equally toxic to both (Jackson and Bartek, 2009). As bone marrow cells divide rapidly, their growth is adversely affected by antineoplastic drugs like doxorubicin and others. This myelosuppression results in life threatening hematological toxicities like anemia, immunosuppression and frequent infections (Anand *et al*., 2008). This myelosuppression not only limits the use of cytostatic agents in cancer but also adversely affects prognosis (Nurgalieva *et al*., 2010).

Therefore, the development of clinically safe and useful myeloprotective drugs which could improve the quality of life by restoring normal physiological conditions is the need of the hour (Guzmán *et al*., 2005). Herbal therapy can be exploited as an alternative treatment to synthetic pharmaceutical products (Imaga *et al*., 2010). *Carica papaya* L. belongs to family Caricaceae. Great deal of research work has been reported on various parts of the plant including leaves, fruit, root and seeds (Sathasivam *et al*., 2009). Role of the leaves in the treatment of dengue fever is well known (Subenthiran *et al*., 2013). Keeping in view the traditional uses of the plant, we investigated the potential role of the seeds of papaya in reverting the myelosuppression caused by chemotherapeutic agents. During our experiments we studied the myeloprotective effects of the seed extract at different doses in rats by inducing myelosuppression with doxorubicin. Effects on different parameters like leukocyte count, differential leukocyte count platelet count, erythrocyte count, hemoglobin, hematocrit and mean corpuscular volume in controlled and drug induced myelosuppressed rats were studied.

MATERIALS AND METHODS

Sample Collection and Preparation of Plant Material

Ripped papaya fruits were purchased from a market in Lahore, Pakistan to obtain seeds. The plant material was identified by Dr. Zaheer-ul-Din, a taxonomist and in-charge at G.C. University, Lahore. The voucher specimen (GC.HERB.BOT.2961) was submitted in the herbarium for future reference. Collected seeds (600g) were dried in shade for 7 days prior to grinding and extraction.

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Preparation of Ethanolic Papaya Seed Extract by Maceration

Ethanolic extract was prepared by soaking 300g of powdered seeds in 1liter of 80% ethanol for three days. The extract was filtered via Watman No.1 filter paper. The marc was re-extracted with alcohol twice more to extract the maximum. After filtration, the alcoholic extract was dried by rotary evaporator under vacuum at 40°C to yield 31.8g of dried extract.

Qualitative Phytochemical Screening

Powdered plant material was screened to determine its chemical composition by standard chemical methods (Khandelwal, 2008, Harborne, 1998).

Animal Selection

White albino rats were purchased from the University of Veterinary and Animal Sciences, Lahore. Rats weighed between 150 and 200g. Animals were maintained at normal diet and water *ad libitum* for 14 days prior to experiments in order to get them acquainted with the environment (Kalabharathi *et al*., 2015). Animals were handled in accordance with the set of guidelines provided by the Ethical Research Committee (ERC), Institute of Pharmacy, Lahore College for Women University (LCWU), Lahore. The extract was diluted with normal saline to prepare concentrations of 200mg/kg, 400 mg/kg & 600 mg/kg for oral administration (Mannaa *et al*., 2014).

Experimental Model

Rats (N=45) were randomly divided into four groups, A, B, C, D. Each of groups A, B, and C consisted of five rats while groups D was subdivided into groups D1, D2 and D3 with 5 rats in each group. Prior to experiments, base line blood values were noted by with drawing 0.5ml of blood from periorbital venous plexus after anesthetizing rats with ketamine/xylazine (Parasuraman *et al*., 2015). Experiments were divided into phase1(induction phase) and phase 2 (treatment phase). During phase 1, Myelosuppression was induced in experimental groups (B, C, D1, D2, D3) by injecting 2mg/kg of DOX intraperitonially (IP), daily for 3 days according to the method described by Sheng *et al*. 2000. Group A (control group) received 1ml/kg of normal saline during the experiment, while group B received no treatment for induced myelosuppression. Immunoprotective reference drug, filgrastim (5ug/kg/day s.c.) was administered to the reference group (group C) for 10 days after induction phase (Sheng *et al*. 2000). 200, 400 and 600 mg/kg of extract was given to treated groups (D1, D2 and D3 respectively) for 3 weeks by orogastric cannula.

Hematological Analysis

0.5- 1ml of blood was collected as sample in EDTA tubes by bleeding periorbital venus plexus for baseline readings, to confirm myelosuppression on the 3rd day of phase1 and at the end of 1st, 2nd and 3rd week (Struck *et al*. 2011 and (TAHIR *et al*., 2014a). Sysmex hematology analyzer XP-300 was used to determine total WBCs, differential WBCs, platelets, RBCs, hemoglobin, hematocrit %, and mean corpuscular volume.

STATISTICAL ANALYSIS

Graph pad Prism version 5.00 for windows was used to analyze the data statistically. Results were shown as mean ± standard deviation and compared with ANOVA (one way analysis of variance) among groups. For post hoc analysis, Tukey's test was used. P value ≤ 0.05 was considered significant.

RESULTS

Phytochemical Screening

The extract was found to have alkaloids, tannins, glycosides, flavonoids, saponins and steroids.

Evaluation of Antimyelosuppressant Activity:

Anti myelosuppressant activity of the extract was determined in rats after inducing myelosuppression with i.p. doxorubicin 2mg/kg. Extract was used at three different concentrations (200, 400 and 600mg/kg). Antimyelosppressant activity of the extract was determined by observing changes in blood parameters like WBCs, differential WBC count, platelet count, RBCs, levels of hemoglobin, hematocrit and mean corpuscular volume. Filgrastim 5µg/kg was used as standard drug to compare the effecects of the extract. Base line readings for hematological parameters are shown in tables 1-7.

Intraperitoneal injection of DXR induced significant myelosuppression. Decreasesed peripheral blood counts as shown in tables 1-7.

Effects of the Extract on Total WBC and Differential WBC Counts in Myelosuppressed Rats.

The mean and standard deviation (SD) values of total WBCs, granulocytes (neutrophils) and agranulocytes (lymphocytes) of albino rats (N= 45) are summarized in tables 1-3.

Administration of graded doses (200, 400, 600 mg/kg) of ethanolic extract of papaya seeds caused significant dose dependent increase ($p<0.05$) in total and differential white blood cells counts across 7th, 14th and 21st day when compared with the Group B (induced group). Reference drug, Filgrastim in Group C also improved the counts white blood cells, neutrophils and lymphocytes. On the fourteenth day, ethanolic extract 600mg/kg increased the WBC, neutrophil and lymphocyte count to 8.82±0.34, 6.22±0.17 and 2.41±0.09 respectively in comparison to 5.1±0.26, 3±0.31 and 1.150±0.11 respectively (in Group B).

Table 1: Effect of the extract on mean WBC count ($\times 10^3$ / µL) ± SD

Days	Group A Normal (Normal saline)	Group B Induced (DXR 2mg/kg)	Group C Reference (Filgrastim 5µg/kg)	Ethanolic extract		
				Treated Group D1 (200mg/ Kg)	Treated Group D2 (400mg/ Kg)	Treated Group D3 (600mg/ Kg)
Pre-Administration	9.060±0.532	8.960±0.639	8.980±0.610	9.100±0.469	8.800±0.806	8.780±0.356
Induction Phase (day 3)	9.340±0.481	4.760±0.358[a]	4.800±0.448[a]	4.700±0.640[a]	4.640±0.462[a]	4.740±0.351[a]
Treatment Phase (day 7)	10.040±0.41	4.360±0.37[a]	7.460±0.53[ab]	6.340±0.658[ab]	6.720±0.47[ab]	7.420±0.37[ab]
Treatment Phase (day 14)	10.340±0.35	5.100±0.26[a]	9.040±0.33[ab]	7.480±0.807[abc]	8.100±0.47[abc]	8.820±0.34[ab]
Treatment Phase (day 21)	10.400±0.436	5.700±0.29[a]	9.840±0.546[b]	8.280±0.610[abc]	8.960±0.513[ab]	9.540±0.439[b]

WBC count (mean ± SD) evaluated in myelosuppressed rats at p value<0.05 for five five rats per group. Values followed by superscripts 'a', 'b', 'c' within rows indicates a = significant difference of Induced, Reference and Treated groups with Normal group b = significant difference of Reference and Treated groups with Induced group c = significant difference of Treated groups D1, D2, D3 with Reference group.

Table 2: Effect of the extract on mean neutrophils count ($\times 10^3$ / µL) ± SD

Days	Group A Normal (Normal saline)	Group B Induced (DXR 2mg/kg)	Group C Reference (Filgrastim 5µg/kg)	Ethanolic extract		
				Treated Group D1 (200mg/ Kg)	Treated Group D2 (400mg/ Kg)	Treated Group D3 (600mg/ Kg)
Pre-Administration	7.240±0.635	7.660±0.607	7.400±0.543	7.380±0.610	7.120±0.610	7.560±0.619
Induction Phase (day 3)	7.300±0.604	3.000±0.316[a]	3.300±0.424[a]	2.800±0.6205 [a]	2.740±0.623[a]	3.260±0.541[a]
Treatment Phase (day 7)	7.360±0.666	2.540±0.36[a]	5.320±0.64[ab]	3.760±0.503[abc]	4.540±0.41[ab]	5.100±0.52[ab]
Treatment Phase (day 14)	7.480±0.507	3.000±0.31[a]	6.660±0.607[b]	4.360±0.416[abc]	5.420±0.492[abc]	6.220±0.179[ab]
Treatment Phase (day 21)	7.620±0.507	3.320±0.34[a]	7.200±0.587[b]	4.940±0.462[abc]	5.940±0.305[abc]	6.840±0.351[b]

Neutrophils count (mean ± SD) evaluated in myelosuppressed rats at p value<0.05 for five experimental units per group. Values followed by superscripts 'a', 'b', 'c' within rows indicate a = significant difference of Induced, Reference and Treated groups with Normal group, b = significant difference of Reference and Treated groups with Induced group & c = significant difference of Treated groups D1, D2, D3 with Reference group.

Table 3: Effect of the extract on mean lymphocytes count ($\times 10^3$ / µL) ± SD

Days	Group A Normal (Normal saline)	Group B Induced (DXR 2mg/kg)	Group C Reference (Filgrastim 5µg/kg)	Ethanolic extract		
				Treated Group D1 (200mg/ Kg)	Treated Group D2 (400mg/ Kg)	Treated Group D3 (600mg/ Kg)
Pre-Administration	2.594±0.160	2.456±0.290	2.522±0.226	2.702±0.233	2.698±0.197	2.528±0.190
Induction Phase (day 3)	2.608±0.168	0.942±0.190[a]	0.888±0.152[a]	0.976±0.233[a]	0.886±0.188[a]	0.786±0.125[a]
Treatment Phase (day 7)	2.706±0.183	0.850±0.19[a]	2.236±0.18[ab]	1.432±0.164[abc]	1.808±0.09[abc]	1.998±0.17[ab]
Treatment Phase (day 14)	2.802±0.129	1.150±0.11[a]	2.524±0.15[ab]	1.904±0.145[abc]	2.102±0.187[abc]	2.412±0.09[ab]
Treatment Phase (day 21)	2.886±0.131	1.420±0.167[a]	2.818±0.146[ab]	2.214±0.126[abc]	2.434±0.125[abc]	2.700±0.121[b]

Lymphocytes count (mean ± SD) evaluated in myelosuppressed rats at p value<0.05 for five experimental units per group. Values followed by superscripts 'a', 'b', 'c' within rows indicates where a = significant difference of Induced, Reference and Treated groups with Normal group, b = significant difference of Reference and Treated groups with Induced group and c = significant difference of Treated groups D1, D2, D3 with Reference group.

Table 4: Effect of the extract on mean platelets count ($\times 10^3$ / µL) ± SD

Days	Group A Normal (Normal saline)	Group B Induced (DXR 2mg/kg)	Group C Reference (Filgrastim 5µg/kg)	Ethanolic extract		
				Treated Group D1 (200mg/ Kg)	Treated Group D2 (400mg/ Kg)	Treated Group D3 (600mg/ Kg)
Pre-Administration	900.80±38.388	862.60±50.905	897.00±40.509	874.40±38.805	896.80±29.945	873.20±51.075
Induction Phase (day 3)	913.00±38.981	365.80±62.38[a]	341.60±51.540[a]	334.00±49.558[a]	323.40±54.840[a]	376.40±32.631[a]
Treatment Phase (day 7)	920.00±34.511	280.20±66.76[a]	370.40±49.581[ab]	582.20±20.474[abc]	681.60±27.483[abc]	751.60±26.26[abc]
Treatment Phase (day 14)	924.00±37.014	370.80±47.24[a]	454.60±34.753[ab]	706.40±41.344[abc]	834.80±28.350[bc]	885.20±21.661[bc]
Treatment Phase (day 21)	941.600±37.287	461.80±53.42[a]	605.40±17.42[ab]	809.00±20.87[abc]	952.40±24.83[bc]	1005.60±56.12[bc]

Platelets count (mean ± SD) evaluated in myelosuppressed rats at p value<0.05 for five rats per group. Values followed by superscripts 'a', 'b', 'c' within rows indicates a = significant difference of Induced, Reference and Treated groups with Normal group, b = significant difference of Reference and Treated groups with Induced group & c = significant difference of Treated groups D1, D2, D3 with Reference group.

Table 5: Effect of extract on mean RBC count ($\times 10^6$ / µL) ± SD

Days	Group A Normal (Normal saline)	Group B Induced (DXR 2mg/kg)	Group C Reference (Filgrastim 5µg/kg)	Ethanolic extract		
				Treated Group D1 (200mg/ Kg)	Treated Group D2 (400mg/ Kg)	Treated Group D3 (600mg/ Kg)
Pre-Administration	7.282±0.456	7.018±0.341	7.106±0.186	7.204±0.471	7.602±0.468	7.438±0.504
Induction Phase (day 3)	7.320±0.462	3.800±0.288[a]	3.904±0.167[a]	3.978±0.194[a]	4.102±0.402[a]	3.608±0.405[a]
Treatment Phase (day 7)	7.386±0.452	3.562±0.34[a]	4.122±0.10[a]	5.400±0.149[abc]	5.836±0.41[abc]	6.094±0.15[abc]
Treatment Phase (day 14)	7.482±0.427	3.784±0.36[a]	4.690±0.12[ab]	5.546±0.621[abc]	6.296±0.237[abc]	6.644±0.28[abc]
Treatment Phase (day 21)	7.570±0.425	4.010±0.38[a]	5.256±0.195[ab]	6.392±0.280[abc]	6.698±0.280[abc]	7.410±0.267[bc]

RBCs count (mean ± SD) evaluated in myelosuppressed rats at p value<0.05 for five rats per group. Values followed by superscripts 'a', 'b', 'c' within rows indicate a = significant difference of Induced, Reference and Treated groups with Normal group, b = significant difference of Reference and Treated groups with Induced group & c = significant difference of Treated groups D1, D2, D3 with Reference group.

Table 6: Effect of extracts on mean hemoglobin concentration (g /dL) ± SD

Days	Group A Normal (Normal saline)	Group B Induced (DXR 2mg/ Kg)	Group C Reference (Filgrastim 5µg/ Kg)	Ethanolic extract		
				Treated Group D1 (200mg/ Kg)	Treated Group D2 (400mg/ Kg)	Treated Group D3 (600mg/ Kg)
Pre-Administration	13.622±1.845	13.742±0.816	14.172±0.538	14.056±0.773	14.358±0.679	13.752±0.904
Induction Phase (day 3)	13.494±1.607	7.410±0.845[a]	7.236±0.512[a]	7.498±0.587[a]	7.638±0.885[a]	7.622±0.945[a]
Treatment Phase (day 7)	13.710±1.64	7.728±0.58[a]	7.960±0.37[a]	9.940±0.503[abc]	10.760±0.619[abc]	11.156±0.500[abc]
Treatment Phase (day 14)	13.764±1.61	8.414±0.34[a]	9.040±0.30[a]	10.720±0.642[abc]	11.816±0.662[abc]	12.160±0.632[bc]
Treatment Phase (day 21)	13.976±1.47	8.738±0.32[a]	10.060±0.451[a]	11.672±0.771[abc]	12.460±0.723[bc]	13.020±0.438[bc]

Hb conc. (mean ± SD) evaluated in myelosuppressed rats at p value<0.05 for five rats per group. Values followed by superscripts 'a', 'b', 'c' within rows indicate a = significant difference of Induced, Reference and Treated groups with Normal group, b = significant difference of Reference and Treated groups with Induced group & c = significant difference of Treated groups D1, D2, D3 with Reference group.

Table 7: Effect of extracts on mean hematocrit (%) ± SD

Days	Group A Normal (Normal saline)	Group B Induced (DXR 2mg/ Kg)	Group C Reference (Filgrastim 5µg/ Kg)	Ethanolic extract		
				Treated Group D1 (200mg/ Kg)	Treated Group D2 (400mg/ Kg)	Treated Group D3 (600mg/ Kg)
Pre-Administration	43.840±2.492	44.700±1.637	43.580±2.376	44.120±2.292	45.240±3.063	42.620±2.105
Induction Phase (day 3)	44.080±2.486	28.160±1.877[a]	27.980±1.992[a]	27.840±2.305[a]	28.800±1.617[a]	28.420±2.410[a]
Treatment Phase (day 7)	44.462±2.73	25.400±1.669[a]	28.560±1.986[a]	34.240±1.316[abc]	36.220±0.870[abc]	39.320±1.480[abc]
Treatment Phase (day 14)	45.540±2.85	26.700±1.405[a]	30.560±2.107[ab]	38.080±1.446[abc]	40.380±0.769[abc]	42.400±1.832[bc]
Treatment Phase (day 21)	46.780±2.32	29.420±1.385[a]	32.220±2.022[a]	40.580±1.145[abc]	42.600±1.046[abc]	44.480±1.558[bc]

Hematocrit % (mean ± SD) evaluated in myelosuppressed rats at p value<0.05 for five experimental units per group. Values followed by superscripts 'a', 'b', 'c' within rows indicate a = significant difference of Induced, Reference and Treated groups with Normal group, b = significant difference of Reference and Treated groups with Induced group & c = significant difference of Treated groups D1, D2, D3 with Reference group.

Table 8: Effect of the Extract on Mean Corpuscular Volume (fL) ± SD

Days	Group A Normal (Normal saline)	Group B Induced (DXR 2mg/kg)	Group C Reference (Filgrastim 5µg/kg)	Ethanolic extracts		
				Treated Group D1 (200mg/ Kg)	Treated Group D2 (400mg/ Kg)	Treated Group D3 (600mg/ Kg)
Pre-Administration	56.200±3.514	56.660±1.906	57.120±1.258	58.700±2.649	58.720±2.736	58.140±4.261
Induction Phase (day 3)	56.260±3.228	53.660±2.506	54.820±1.277	55.420±2.380	55.900±3.295	54.760±3.580
Treatment Phase (day 7)	56.080±2.576	51.180±2.066[a]	56.280±1.827[b]	57.220±2.061[b]	58.080±2.980[b]	59.500±2.687[b]
Treatment Phase (day 14)	55.740±2.625	52.520±3.602	57.620±2.062[b]	59.600±2.689[b]	60.440±2.676[b]	63.340±2.485[abc]
Treatment Phase (day 21)	56.180±2.400	53.340±3.662	58.800±2.094[b]	62.080±1.831[ab]	63.260±2.536[ab]	65.720±1.881[abc]

Mean corpuscular volume (mean ± SD) evaluated in myelosuppressed rats at p value<0.05 for five experimental units per group. Values followed by superscripts 'a', 'b', 'c' within rows indicate a = significant difference of Induced, Reference and Treated groups with Normal group, b = significant difference of Reference and Treated groups with Induced group & c = significant difference of Treated groups D1, D2, D3 with Reference group.

Effect of the Extract on Platelets Count in Myelosuppressed Rats

All treated groups showed dose dependent significant increase ($p<0.05$) in platelets count on 7th, 14th and 21st day as compared to Group B (induced group) as shown in table 4. Extract showed greater improvement as compared to the reference drug filgrastim.

Effect of the Extract on Erythrocytic Parameter Profiles in Myelosuppressed Rats

Dose dependent significant increase ($p<0.05$) was observed in erythrocytes and related parameter profile when compared with the Group B (induced group). The mean and standard deviation (SD) values of RBCs, Hb, Hct and MCV are summarized in tables 5-8.

DISCUSSION

Uncontrolled cell growth results in cancer which affects surrounding healthy cells as well (Gonzales *et al*., 1991). Cytotoxic drugs destroy indiscriminately cancerous cells and fast dividing normal cells as in bone marrow, hair follicles, oral cavity, stomach and intestine (Kaelin Jr, 2005). Consequently, myelosuppresion is frequently caused by anti cancer drugs like doxorubicin. This myelosuppression results in immunodeficiency, anemia and thrombocytopenia which adversly affect the patient's health and often lead to cessation of therapy (Gonzalez-Casas *et al*., 2009).

Plant drugs have long been used to treat various ailments without causing much side effects. Natural compounds present in herbal drugs, work synergistically in treatment of different diseases. These phytochemicals can be beneficial in maintaining normal physiology in cancer patients. Papaya plant is a well-known medicinal plant in Asia however, not much research work has been reported on the seeds and antimyelosupressant effects of the plant. In the present study, the antimyrelosuppressant activity of

the ethanolic extract of papaya seeds was investigated in myelosuppressed rats.

Preliminary phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, glycosides, saponins and steroids in *Carica papaya* seed the ethanolic extract. The immuno stimulant components that are usually reported to be present in the plant extracts are flavonoids, alkaloids, phenolic compounds and tannins (Lakshmi *et al*., 2003, Dashputre and Naikwade, 2010). Flavonoids and phytosterols present is the plant are thought to play an important role in leukopoiesis and erythropoiesis (Gabriel *et al*., 2015).

Myelosuppression was induced by Doxorubicin 2mg/kg i.p. for 3 days at 24 hour intervals (Sheng *et al*., 2000). Filgrastim (5µg/kg/day s.c. for 10 consecutive days) was used as standard to evaluate the effects of alcoholic extract of papaya seeds in three doses 200, 400 and 600mg/kg using albino rat model (Sheng *et al.,* 2000). Results showed that the extract possessed powerful hematopoietic effects as it rapidly improved the numbers of WBC's, RBC's and platelets in myelosuppressed rats. It restored the total WBC count to normal on the 14^{th} day. This activity was comparable to that of standard, Filgrastim. Lymphocyte count was also increased to normal within two weeks of treatment. Platelet counts were significantly improved in treated groups as compared to reference group (treated by standard, Filgrastim) which shows the extract improved all haematopoietic parameters where as Filgrastim only improved total WBC count not having significant effects on other haematopoietic parameters. The significant increase in the counts of WBC's, differential leukocytes, thrombocytes and erythrocytes shows that papaya seeds can help recovery in cases of leukopenia, neutropenia, lymphocytopenia, thrombocytopenia and anemia commonly associated with chemotherapy for cancer.

Body's capability to fight against infections depends on the differential white blood cell count in the body. Neutrophils protect against blood pathogens (Ekanem *et al*., 2008). Elevation of lymphocyte count caused by the extract shows strengthening of immune system. Rise in red blood cells and related parameters indicate that papaya seeds may be of benefit in recovering from anemia associated with chemotherapy. These findings agree with the previously reported findings that administration of papaya seeds to normal rats via oral route increased blood profile (Naik and Indira). Rise in the levels of erythrocytes, hemoglobin and Hct by the extract may be attributed to the stimulation of erythropoietin in the kidneys (Khoshvaghti *et al*., 2014). High levels of MCV were probable due to increase in number of RBCs, caused by the extract of papaya seeds. Tannins and flavonoids (antioxidants) present in the extract can also activates hematopoiesis (Aher and Ohlsson, 2006). Flavonoids have been implicated in erythropoiesis and are know to possess anabolic activity owing to which they are frequently incorporated into many pharmaceutical products (Songlin *et al*., 2009).

The significant increase in platelets count indicates that papaya seeds have the potential to be used as therapeutic agent to treat thrombocytopenia caused by cytotoxic drugs like Doxorubicin. Thrombolcytopenia caused by carboplatin can be reversed by papaya seed and leaf juice (Tahir *et al*., 2014b). The substantial rise in the level of thrombocytes by the extract, can be due to phytoconstituents present in the extract e.g. tannins. Polyphenolic compounds like tannins make complex with proteins which increase thrombocyte count (Patel *et al*., 2005).

CONCLUSION

It can be concluded from the present study that ethanolic extract of papaya seeds have anti myelosupprerssant activity and can be used as powerful hematopoietic agent in reverting bone marrow suppression caused by chemotherapeutic agents like doxorubicin. The drug can also be used when platelet level is alarmingly low as in case of dengue fever.

REFERENCES

Aher SM and Ohlsson A (2006). Late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. *Cochrane Database Syst. Rev. 2 (2020).*

Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B and Aggarwal BB (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res.,* 25(9): 2097-2116.

Cragg GM and Newman DJ (2005). Plants as a source of anti-cancer agents. *J. Ethnopharmacol.,* **100**(1-2): 72-79.

Dashputre N and Naikwade N (2010). Immunomodulatory activity of Abutilon indicum linn on albino mice. *Int. J. Pharm. Sci. Res.,* **1**(3): 178-184.

Ekanem J, Kolawole O and Abbah O (2008). Trypanocidal potential of methanolic extract of *Bridelia ferruginea* benth bark in *Rattus novergicus. Afr. J. Biomed. Res.,* **2**(2): 045-050.

Gabriel NN, Qiang J, He J, Ma XY, Kpundeh MD and Xu P (2015). Dietary Aloe vera supplementation on growth performance, some haemato-biochemical parameters and disease resistance against Streptococcus iniae in tilapia (GIFT). *Fish Shellfish Immun,* **44**(2): 504-514.

Gonzales C, Kaufman D, Tobin A and Chesselet MF (1991). Didstribution of glutamic acid decarboxylase (M r 67 000) in the basal ganglia of the rat: An immunohistochemical study with a selective cDNA-

generated polyclonal antibody. *J Neurocytol,* **20**(12): 953-961.

Guzmán DC, Mejía GB, Vázquez IE, García EH, Del Angel DS and Olguín HJ (2005). Effect of testosterone and steroids homologues on indolamines and lipid peroxidation in rat brain. *J. Steroid Biochem. Mol. Biol.,* **94**(4): 369-373.

Harborne A (1998). Phytochemical methods a guide to modern techniques of plant analysis, Springer Science & Business Media, pp.1-22.

Imaga NA, Gbenle GO, Okochi VI, Adenekan S, Duro-Emmanuel T, Oyeniyi B, Dokai PN, Oyenuga M, Otumara A and Ekeh FC (2010). Phytochemical and antioxidant nutrient constituents of Carica papaya and Parquetina nigrescens extracts. *Sci. Res. Essays.,* **5**(16): 2201-2205.

Jackson SP and Bartek J (2009). The DNA-damage response in human biology and disease. *Nature,* **461**(7267): 1071-1078..

Kaelin Jr WG (2005). The concept of synthetic lethality in the context of anticancer therapy. *Nat. Rev. Cancer,* **5**(9): 689-698.

Kalabharathi H, Shruthi S, Vaibhavi P, Pushpa V and Satish A (2015). Diuretic activity of ethanolic root extract of *Mimosa pudica* in albino rats. *JCDR,* **9**(12): FF05.

Khandelwal KR (2008). Practical Pharmacognosy, Pragati Books Pvt. Ltd., 1-129

Khoshvaghti A, Derakhshanian S, Zamanzadeh A and Sarvestani AA (2014). The effects of *Fumaria officinalis* hydroalcoholic extracts on haematological profile of New Zealand rabbits. *Comp. Clin. Path.,* **23**(4): 869-873.

Lakshmi V, Pandey K, Puri A, Saxena R and Saxena K (2003). Immunostimulant principles from *Curculigo orchioides. J. Ethnopharmacol.,* **89**: 181-184.

Mannaa FA, Abdel-Wahhab KG and Abdel-Wahhab MA (2014). Prevention of cardiotoxicity of aflatoxin B 1 via dietary supplementation of papaya fruit extracts in rats. *Cytotechnology,* **66**: 327-334.

Naik NR and Indira P (2015). Effect of *Mesua ferrea* flower and *Carica papaya* seed extract on haematological studies in female albino rats. *Biolife,* **3**(3): 730-734.

Nurgalieva Z, Xia R, Liu CC, Burau K, Hardy D and Du XL (2010). Risk of chemotherapy-induced peripheral neuropathy in large population-based cohorts of elderly patients with breast, ovarian, and lung cancer. *Am. J. Ther.,* **17**: 148-158.

Parasuraman S, Zhen KM and Raveendran R (2015). Retro-orbital sample collection in rats: A video article. *PTB Reports,* 1.

Patel SR, Hartwig JH and Italiano JE (2005). The biogenesis of platelets from megakaryocyte proplatelets. . *Clin. Investig.* **115**(12): 3348-3354.

Sathasivam K, Ramanathan S, Mansor SM, Haris MRM and Wernsdorfer WH (2009). Thrombocyte counts in mice after the administration of papaya leaf suspension. *Wien Klin Wochenschr* **121**(3): 19.

Sheng Y, Pero R and Wagner H (2000). Treatment of chemotherapy-induced leukopenia in a rat model with aqueous extract from *Uncaria tomentosa. Phytomedicine,* **7**(2): 137-143.

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.

Subenthiran S, Choon TC, Cheong KC, Thayan R, Teck MB, Muniandy PK, Afzan A, Abdullah NR and Ismail Z (2013). Carica papaya leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. *Evid. Based Complementary Altern. Med.,* 1-7.

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

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