



EFFECT OF *CARICA PAPAYA* LEAF EXTRACT ON HYDROXYUREA INDUCED HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN WISTAR ALBINO RATS

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ABSTRACT

Background: Thrombocytopenia or anemia are common in patients who are exposed to chemotherapy and irradiation. The aim of the study was to observe the effect of *Carica papaya* leaf extract on hydroxyurea induced hematological and biochemical parameters in a rat model.

Materials and Methods: Thirty rats were divided into 5 groups (n = 6). *Carica papaya* leaf extract (7.2 ml/kg) was given orally to normal as well as to rats administered with 1.6 mg/kg of hydroxyurea for a period of five days. Blood was withdrawn from retro-orbital plexus at various time intervals by inducing light anesthesia with ketamine (1ml/kg i.p) to determine the blood cell count and liver enzymes. Data were analyzed using SPSS version 17.0.

Results: Platelet and RBC counts were significantly (p<0.05) increased in the group of rats administered with *Carica papaya* leaf extract (7.2 ml/kg p.o) alone for a period of five days, compared to that of normal controls. But there was only a compensatory rise in platelet and RBC counts in rats administered with *Carica papaya* leaf extract (7.2 ml/kg p.o) plus hydroxyurea (1.6 mg/kg p.o) for a period of five days. This could be due to the bone marrow suppression effect of hydroxyurea. However, WBC count, hematocrit (%), and SGPT values were not changed significantly (P ≥ 0.05) in any of the test groups, except in rats exposed to daily dose of hydroxyurea, where the changes were significant (p<0.05) on day 5.

Conclusion: Fresh *Carica papaya* leaf extract at a dose of 7.2 ml/kg restored normal blood cell counts in rats co-administered with hydroxyurea.

Keywords: *Carica papaya* L., blood cell count, hydroxyurea, bone marrow suppression

INTRODUCTION

During steady-state hematopoiesis in humans, new erythrocytes, leukocytes, and platelets are produced each day to replace those lost through natural aging processes¹. In response to hematological stress (e.g., hypoxia, infection), the numbers of a particular type of blood cell required to meet physiological demands can expand rapidly by >tenfold².

Many chronic systemic diseases such as inflammatory bowel disease, rheumatoid arthritis, and chronic infections (hepatitis C, dengue fever) are associated with anemia, neutropenia, and thrombocytopenia. Drugs that have also been implicated in the causation of these hematological variations include sulfonamides, chlorpromazine, procainamide, penicillin, cephalosporins, cimetidine, methimazole, phenytoin, chlorpropamide, and antiretroviral medications. Myelosuppressive drugs (especially hydroxyurea, interferon alfa-2b, anti-neoplastic drugs) cause diminished or absent megakaryocytes in bone marrow.

Papaya, Carica papaya (C. papaya) L., is one of

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the major fruit crops cultivated in tropical and sub-tropical zones. The different parts of papaya such as fruit, fruit juice, seed, root, leaves, bark, latex contain various chemical constituents that has been proved for various medicinal activities like antioxidant, antihypertensive, wound healing, hepatoprotective, anti-inflammatory, antimicrobial, antifungal, anti-fertility, histaminergic, diuretic, anti-amoebic, anticancer, anthelmintic, effect on smooth muscles (abortifacient), antimalarial, hypoglycemic activity, immunomodulatory activity, anti-ulcer activity, anti-sickling activity³. The focus of this study is on its leaves, which was shown to contain papain, chymopapain, cystatin, flavonoids, cyanogenic glucosides and glucosinolates, vitamin C & E, and minerals such as Zn, Mn, Fe⁴. These are antioxidants that reduce lipid peroxidation in a cell⁵.

To examine the potential role of *Carica papaya* on hematological and biochemical parameters, we analyzed in this study the effect of the fresh *Carica papaya* leaf extract on bone marrow in a rat model.

MATERIALS AND METHODS

Drugs

Drug used in this study is Hydroxyurea manufactured by Ambalal Sarabhai Enterprises Ltd, India.

Plant material and preparation of leaf extract

Carica papaya leaves were collected at the residence of Dwaraka Mayee, Kuthar padav, Munnur post, Mangalore, Karnataka. The leaves were washed with water to remove dirt and were cut into small pieces by using a scissor. The leaf material was ground into a fine paste using an electric grinder. Extract was prepared by squeezing and filtering the obtained fine paste. The suspension was kept in the refrigerator at a temperature of 4°C till use.

Animals

30 Albino rats of Wistar strain weighing 290±10g were obtained from the Animal House of the Department of Pharmacology, Nitte University, Mangalore, Karnataka. They were housed under standard conditions of temperature and light and fed with standard diet and water *ad libitum*. This study was approved by the Institutional Animal Ethics Committee (KSHEMA/IAEC/02/2014).

Dose selection

7.2 ml/kg of *Carica papaya* leaf extract and 1.6

mg/kg of Hydroxyurea were used in this study⁶.

Experimental set up

The rats were divided into five groups of six animals each:

- Group I served as normal control;
- Group II was treated with a single dose of hydroxyurea (1.6 mg/kg p.o);
- Group III was treated with hydroxyurea (1.6 mg/kg p.o) for a period of five days.
- Group IV was treated with *Carica papaya* leaf extract (7.2 ml/kg p.o) for a period of five days;
- Group V was treated with *Carica papaya* leaf extract (7.2 ml/kg p.o) along with hydroxyurea (1.6 mg/kg p.o) for a period of five days;

Collection and Evaluation of blood samples

Blood was withdrawn from retro-orbital plexus from all the groups after subjecting the animals to light anesthesia using ketamine (1ml/kg i.p). Hematology parameters were determined by using HemaTrue Hematology Analyzer. Biochemical parameters were measured with a semi automatic analyzer (Star 21) using experimental kits (AGAPPE) following the methods described by the manufacturers.

Statistical analysis

The data were presented in terms of mean and SD, analyzed by descriptive and analytical (Unpaired *t* test) statistics, and all analysis was made using the 17.0 (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.) and *P* value < 0.05 was considered, statistically significant.

RESULTS

A. Effect of hydroxyurea on hematological parameters (Table 1)

Administration of a single dose of hydroxyurea produced significant decrease in platelet count ($p < 0.05$) on day 1 compared to normal control. Animals exposed to single dose of hydroxyurea did not produce any significant ($p > 0.05$) decrease in platelet count on day 3 and day 5 compared to normal control. Furthermore time dependent decrease in platelet count was continued to be significant in animals exposed to daily dose of hydroxyurea on day 3 and day 5 compared to normal control. Red blood cell count and hematocrit were significantly decreased ($p < 0.05$) after administering

daily dose of hydroxyurea on day 3 and day 5 compared to normal controls. However significant reduction in white blood cell count ($p < 0.05$) was observed on day 5 in rats exposed to daily dose of hydroxyurea, compared to the baseline.

B. Effect of *Carica papaya* leaf extract on hematological parameters (Table 1)

Treatment with *Carica papaya* leaf extract produced a time-dependent increase in platelet count, red blood cell count and hematocrit which is significant ($p < 0.05$) on day 5 with respect to normal controls. No significant change in white blood cell count was observed in rats receiving *Carica papaya* leaf extract alone during the entire course of the study.

C. Effect of *Carica papaya* leaf extract along with hydroxyurea on hematological parameters (Table 1)

When *Carica papaya* leaf extract was given along with hydroxyurea, time dependent increase in blood cell count was observed from day 1 with restoration to pre-treatment value on day 5. This result shows that treatment with *Carica papaya* leaf extract and hydroxyurea for 5 days did not produce any significant ($p > 0.05$) decrease in blood cell count with respect to the normal control.

D. Effect of *Carica papaya* leaf extract and hydroxyurea on biochemical parameters (Table 2)

Treatments with *Carica papaya* leaf extract and combined doses of *Carica papaya* leaf extract and hydroxyurea produced no significant ($P \geq 0.05$) increase in liver enzymes (SGOT and SGPT) except on day 5 in rats administered with daily dose of hydroxyurea where SGPT levels were increased (from 64.30 ± 1.05 to 76.04 ± 12.20) which is statistically significant ($p < 0.05$).

Table 1: Effect of *Carica papaya* leaf extract and hydroxyurea and their combination on hematological parameters in rats.

GROUPS	DAY 0	DAY 1	DAY 3	DAY 5
Platelet count ($\times 10^3/\text{ml}$)				
I	817.66 \pm 45.96	801 \pm 152.42	812 \pm 74.81	806.66 \pm 131.85
II	724.66 \pm 97.17	466.33 \pm 40*	667 \pm 66.64	744 \pm 75.17
III	743 \pm 109.08	453.33 \pm 30.74*	361.33 \pm 36.82*	318.66 \pm 25.57*
IV	699.33 \pm 82.94	732.33 \pm 77.78	807.33 \pm 97.79	905 \pm 64.02*
V	757.66 \pm 95.37	582.66 \pm 83.72	648.33 \pm 183.91	697.66 \pm 212.36
Red blood cell count ($\times 10^6/\text{ml}$)				
I	9.5 \pm 0.97	9.32 \pm 0.7	9.23 \pm 1.2	8.89 \pm 1.46
II	8.70 \pm 0.44	7.92 \pm 0.76	8.49 \pm 0.59	8.52 \pm 0.44
III	8.56 \pm 0.43	8.18 \pm 0.45	7.76 \pm 0.22*	7.40 \pm 0.07*
IV	8.01 \pm 0.97	8.58 \pm 0.48	8.76 \pm 0.62	9.37 \pm 0.78*
V	8.37 \pm 0.47	8.23 \pm 1.16	9.01 \pm 1	9.03 \pm 1.08
White blood cell count ($\times 10^3/\text{ml}$)				
I	10.46 \pm 3.32	11.16 \pm 4	10.89 \pm 2.3	11.66 \pm 0.64
II	9.66 \pm 1.65	8.36 \pm 1.32	8.74 \pm 1.42	10.06 \pm 2.05
III	10.1 \pm 1.44	9.23 \pm 1.25	8.73 \pm 1.02	7.79 \pm 0.73*
IV	9.2 \pm 0.98	9.7 \pm 3.37	10.73 \pm 3.72	10.93 \pm 3.18
V	12.23 \pm 2.10	11.53 \pm 1.70	13.06 \pm 3.20	13.5 \pm 3.81
Hematocrit (%)				
I	56.06 \pm 3.21	54.26 \pm 2.85	55 \pm 2.04	54 \pm 8.92
II	50.46 \pm 3.95	48.56 \pm 2.74	48.7 \pm 3.46	49.26 \pm 1.77
III	55.9 \pm 2.19	51.96 \pm 6.41	49.2 \pm 1.75*	47.06 \pm 2.40*
IV	48.83 \pm 4.31	50.96 \pm 4.54	53.06 \pm 1.77	56.46 \pm 1.70*
V	54.43 \pm 8.14	51.3 \pm 6.95	54.8 \pm 4.9	54.83 \pm 7.30

All values are expressed as mean \pm SD, n=6, * means $p < 0.05$ in comparison to control;

Table 2: Effect of Carica papaya leaf extract and hydroxyurea and their combination on biochemical parameters in rats.

GROUPS	DAY 0	DAY 1	DAY 3	DAY 5
SGOT / ALT (IU/ml)				
I	26.1±2.94	27.99±4.39	24.52±3.89	21.74±4.73
II	24.17±1.49	29.34±1.10	27.13±2.81	27.86±1.16
III	23.99±7.90	26.45±3.20	25.42±3.56	24.05±3.55
IV	27.9±8.23	22.28±3.20	27.32±8.32	26.64±6.28
V	24.58±2.45	27.73±2.90	25.64±9.30	21.95±2.93
SGPT / AST (IU/ml)				
I	63.71±14.61	57.96±16.67	60.02±11.42	67.37±9.71
II	45.73±2.89	49.92±2.13	47.77±1.86	47.32±2.62
III	64.30±1.05	64.5±4.32	71.36±9.31	76.04±12.20*
IV	58.74±20.40	56.70±13.01	64.92±12.04	75.32±14.84
V	59.95±14.02	68.09±14.93	64.27±9.98	63.33±12.93

All values are expressed as mean±SD, n=6, * means p≤0.05 in comparison to control;

DISCUSSION

Many of the chemotherapy drugs temporarily stop cells from dividing, especially the cells that divide rapidly. Red blood cells, white blood cells and platelets are rapidly dividing cells and are made by the bone marrow. In most cases, no treatment is necessary and the primary management is to stop the culprit drug⁷.

When the anemia is severe or is adversely affecting the quality of life, then treatment involves either red blood cell transfusions or, more commonly, the administration of parenteral recombinant erythropoietin (epoetin alfa or darbepoetin). The use of recombinant erythropoietin is associated with an increased risk of venothromboembolism and arterial thrombotic episodes, and there is controversy about whether it is associated with reduced survival in patients with malignancy. The neutropenia following myelosuppressive chemotherapy is predictable and is largely ameliorated by the use of myeloid growth factors (filgrastim or sargramostim)⁸.

The severity and duration of chemotherapy-induced depressions in the platelet count are determined by the specific regimen used, although the platelet count typically resolves more slowly following a chemotherapeutic insult than does neutropenia or anemia, especially if multiple cycles of treatment have been given. Due to the associated risk of life-threatening hemorrhage, severe

thrombocytopenia associated with immune platelet destruction, bone marrow failure, or exposure to myelosuppressive agents has been treated urgently with platelet transfusions⁹. Recombinant IL-11 was the first cytokine approved for the prevention of chemotherapy-induced thrombocytopenia based on clinical trials demonstrating a reduction in this risk¹⁰. Studies evaluating the efficacy of recently approved platelet growth factors eltrombopag and romiplostim in the treatment of chemotherapy-induced thrombocytopenia are ongoing¹¹. In spite of their proven efficacy in some of the clinical trials the major limiting factor of all the recombinant myeloid growth factors is their cost.

The need of the hour is to find a natural compound which is easily available, less expensive, and having direct bone marrow stimulating effect. In this regard two studies have been conducted so far using cytotoxic drugs to induce thrombocytopenia. A 15 day study conducted with *C.papaya* leaf aqueous extract at concentrations of 400 mg/kg and 800 mg/kg in cyclophosphamide-induced thrombocytopenic rat model significantly increased the platelet counts¹². Another study using high dose (0.72ml/100g) of mature leaf concentrate of *C.papaya* also showed the same effect in hydroxyurea induced thrombocytopenic rat mode⁶. But the drawback in these studies is the duration of cytotoxic drug used. In the first study, cyclophosphamide was used only for first three days where as only one dose of hydroxyurea was used in the second study. The fact is that the cytotoxic drug

induced thrombocytopenia is transient with reversal in platelet count upon drug withdrawal. Considering that hydroxyurea is given daily in some of the cancer patients, this study was done to assess the hematological and biochemical changes brought about by the drug when given daily and to test the effect of *C.papaya* leaf extract on these changes.

Animal studies suggest that papaya leaf extracts have potential therapeutic effect in dengue fever by inhibiting hemolysis and causing increased blood cell counts¹³. But the fact remains that dengue is mostly a self-limiting disease with spontaneous increase in platelets during recovery.

Our study shows a compensatory rise in platelet and RBC count when *C.papaya* leaf extract is administered along with a drug having bone marrow suppression effect. This finding must be explored in patients on palliative cytotoxic chemotherapy. Interestingly, the dose of ketamine used to induce anesthesia is varied among different groups of rats. In rats administered with *C.papaya* leaf extract the dose of ketamine used was much higher than the dose used in normal control rats (3ml/kg vs 1ml/kg). The reason and significance of this is unknown.

CONCLUSION

C.papaya leaf extract demonstrated a significant increase in platelet and RBC count in normal as well as rats administered with hydroxyurea (1.6 mg/kg p.o) for a period of five days. This experimental evidence indicates that *C. papaya* leaf extract could have a potential therapeutic efficacy in patients on palliative cytotoxic cancer chemotherapy causing alteration in normal blood cell counts.

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