

***PUNICA GRANTUM L AND ZIZIPHUAS MAURITIANA* MITIGATE ANEMIA IN CANCER**

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ABSTRACT

Back ground: Anaemia is interpreted as the decrease in the number of the red blood cells in blood or decline in the quantity of haemoglobin. Chemotherapy can engender various side effects such as bone marrow depression with low count of erythrocyte and haemoglobin which lead to anaemia. With conventional medical remedy, it isn't always feasible to intercept and treat chemo- and radiotherapy side effects sufficiently. Hence the intent of this research was to perceive evidence if ayurvedic therapies can be a suitable and safe supportive to conventional drugs. **Aim:** To palliate the anaemia with *Punica grantum L* and *Ziziphus mauritiana* in cancer. **Materials and method:** Animals were divided into 12 groups of 12 mice. Group 1, 2, 3 mice served as normal, ehrlich ascites carcinoma control and standard (5- Flourouracil) respectively. Group 4, 5, 6, 7, 8, 9, 10, 11, 12 mice were treated orally with aqueous,

ethanol, chloroform extract of *Punica grantum L*, *Ziziphus mauritiana* and combination of both plants 200 mg/kg b.w, respectively, for nine days. After treatment, blood samples were collected and RBC, Hb content was reckoned and remaining mice were used to assess increase in life span. **Results:** Ethanol extract of combination of *Ziziphus mauritiana* and *Punica grantum* manifested remarkable percentage increase in life span and notably restored RBC and Hb content towards normal. **Conclusion:** Ethanol extract of combination of *Ziziphus mauritiana* and *Punica grantum* exhibited significant mitigation of anaemia during cancer.

KEYWORDS: *Punica grantum L*, *Ziziphus mauritiana*, anaemia.

INTRODUCTION

Anemia is a ubiquitous clinical concern among cancer patients, arising by virtue of malnutrition, chronic illness or as an adverse effect of cancer therapy.^[1] A coherent review of literature from 1966 to 2003 discerned the existence of anemia in 30% to 90% of patients with cancer.^[2] Anemia has been pinpointed in 55% to 100% of patients undergoing chemotherapy^[3] and in 48% to 57% of patients enduring radiotherapy.^[4] Anemia declines duration of survival in cancer patients, furthermore, their comprehensible quality of life.^[5,6] Ayurveda is an primitive Indian system of remedies that makes usage of herbs. Herbal medicines aid to refine immunity and conquer anaemia by up surging blood haemoglobin count and RBC production.^[7] The plant *Punica grantum* L (*Punicaceae*) is commonly known as “Anar” and “Dalima” in Unani and Ayurvedic system respectively. The plant *Ziziphus mauritiana* (*Rhamnaceae*) is also called as “Ber” and “Kola” in Unani and Ayurvedic system respectively. These plants have been traditionally used as a major component of many herbals formulations for healing of diabetics, atherosclerotic, diarrhea, ulcer and tumor. The current investigation was carried out to assess the mitigation of anaemia in tumour bearing mice by extracts of *Punica grantum* L and *Ziziphus mauritiana*.

MATERIALS AND METHOD

Plant material and Preparation of extracts

The seeds of *Punica grantum* L (*Punicaceae*) and *Ziziphus mauritiana* (*Rhamnaceae*) were assembled from Udipi District, Bangalore, Karnataka State, India and authenticated by Green Chem of India, Bangalore, Karnataka, India. The seeds were rinsed with water for the discharge of adhering material and sun dried. Seeds were powdered with a mechanical grinder, passing through sieve # 40 and stored in airtight container. The seed powder (1kg) was extracted in a soxhlet with hexane (4000ml) for 6h for the removal of fatty matters. The hexane extract was discarded and residues were successively extracted with distilled water, ethanol and chloroform (3200ml each) for 8h each. The extracts were filtered and concentrated under vaccum (Buchi, Switzerland) to get concentrated extracts (60g), which was dried in vaccum oven and stored in a desiccator.

Tumour cell line

EAC cells were supplied by Amala Cancer Research Center, Thrissur, Kerala, India and were maintained by weekly intraperitoneal (i.p) inoculation of 10^6 cells/mouse in the laboratory. Ehrlich Ascites Carcinoma (EAC) cells maintained in the peritoneal cavity of *Swiss albino*

mice were collected from an animal having 7 days old ascitic tumor by aspirating the ascitic fluid in sterile isotonic saline. The viable EAC cells were counted under microscope. A number of viable cells 10^6 cells were inoculated into the peritoneal cavity of each mouse.

Animals

Healthy adult *Swiss albino* mice weighing 25 ± 5 g was supplied from the Drug Control Laboratory (DCL), Bangalore, were housed in ventilated cage and animals had natural day and night cycle with temperature $25 \pm 3^\circ\text{C}$. The animals were housed in large spacious hygienic cages during the course of the experimental period. The animals were permitted free access to standard laboratory cube pellets and drinking water *ad libitum*. The study protocol was mandated by Institutional Animal Ethics Committee (IAEC), Visveswarapura Institute of Pharmaceutical Sciences, Bangalore. (Registration No: 152/1999, renewed in 2012).

Treatment schedule

A number of 1×10^6 Ehrlich ascites carcinoma (EAC) cells from the tumor bearing mice aseptically were inoculated. Group 1 mice (n=12) served as normal control, group 2 mice (n=12) were EAC control. Group 3 mice (n=12) received standard drug 5- fluorouracil 20 mg/kg b.w, i.p., group 4, 5 and 6 (n=12) mice were administered, orally with aqueous, ethanol, chloroform extract of *Punica grantum* L of 200 mg/kg b.w, respectively, for nine days, group 7, 8 and 9 (n=12) mice were administered, orally, aqueous, ethanol, chloroform extract of *Ziziphus mauritiana* of 200 mg/kg b.w, respectively, for nine days, whereas group 10, 11 and 12 (n=12) mice were administered, orally, aqueous, ethanol, chloroform extract of combination of both plants (ZP) of 200 mg/kg b.w, respectively, for nine days.

Tumor growth response

Percentage increase in life span (%ILS) was evaluated by using following formula.

$$\text{MST} = \frac{\text{day of first death} + \text{day of last death}}{2}$$

$$\% \text{ LS} = \frac{(\text{MST of treated group} - \text{MST of control group})}{\text{MTS of the control group}} \times 100$$

Hematological parameters

At the end of investigation, on day 10, after an overnight fasting, blood was collected by retro-orbital puncture and used for the assessing of the hemoglobin (Hb) content, red blood cells (RBC) count by standard procedures.

RESULTS AND DISCUSSION

Table shows the effect of different extracts of *Punica grantum* L and *Ziziphus mauritiana* on haematological parameters and % ILS against EAC. RBC count and Hb content in the EAC groups were significantly diminished as compared to normal group. Treatment with ethanol extract of combination of *Ziziphus mauritiana* and *Punica grantum* L, ZP(E) 200 mg/kg revealed significant ($r < 0.001$) rise in the RBC count and Hb content when compared to the EAC control group, and rehabilitated these values towards normal. The maximum %ILS was found to be 63.38 %, 84.51% in tumor bearing mice treated with ZP (E) and 5-Flurouracil respectively when compared with other treated animals.

Effect of different extracts of *Punica grantum* L and *Ziziphus mauritiana* on hematological parameters and percentage increase in life span in EAC tumor bearing mice.

TREATMEN T GROUP	RBC COUNT (x 10 ⁶ /mL)	Hb (g%)	ILS%
NORMAL	5.68 ± 0.11	15.99 ± 0.39	-----
EAC	2.17 ± 0.1 ^c	7.07 ± 0.42 ^c	-----
5-FU	4.65 ± 0.25 ^r	12.29 ± 0.56 ^{br}	84.51
P-E	3.95 ± 0.34 ^{bq}	9.74 ± 0.76 ^{cp}	52.11
P-Aq	3.61 ± 0.32 ^{cp}	8.66 ± 0.57 ^{cy}	39.44
P-Ch	3.07 ± 0.33 ^{cy}	8.26 ± 0.43 ^{cy}	33.80
Z-E	3.29 ± 0.25 ^{cx}	9.42 ± 1.00 ^{cx}	42.25
Z-Aq	3.07 ± 0.30 ^{cy}	8.93 ± 0.30 ^{cy}	30.99
Z-Ch	2.79 ± 0.37 ^{cy}	8.09 ± 0.68 ^{cz}	23.94
ZP-E	4.26 ± 0.31 ^{ar}	10.89 ± 1.00 ^{cq}	63.38
ZP-Aq	3.89 ± 0.32 ^{cq}	9.70 ± 0.81 ^c	42.25
ZP-Ch	3.66 ± 0.40 ^{cq}	9.27 ± 0.28 ^{cx}	36.62

$n = 6$, Values are mean ± S.E.M, one way ANOVA followed by Dunnet's multiple comparison test. p values: $a < 0.05$, $b < 0.01$, $c < 0.001$, compared to the normal group; $p < 0.05$, $q < 0.01$, $r < 0.001$, as compared with EAC control; $x < 0.05$, $y < 0.01$, $z < 0.001$, as compared with 5-Fluorouracil treated group.

Crucial rational of anemia in cancer patients is myelosuppressive throughout chemotherapy. Emendation of anemia can be accomplished by either treating the underlying etiology or issuing supportive care by transfusion with packed red blood cells (PRBC) or allocation of erythropoiesis stimulating agents (ESAs), with or without iron supplementation but there are risks of PRBC transfusion which include transfusion-related reactions, congestive heart failure, bacterial contamination and viral infections iron overload and ESAs transfusion also can cause to ascended mortality and tumor development so certain supportive and alternative medicines have intermittently been demanded to have anticancer potential with no or negligible side effects which can mitigate anemia in cancer patients.⁸ Data attained from results exhibited ethanol extract of combination of both plants ZP(E) at 200 mg/kg dose raised in life span and the rehabilitation of haematological parameter therefore it can palliate anaemia in cancer.

CONCLUSION

The current research manifest that ethanol extracts of combination of *Punica grantum* L and *Ziziphus mauritiana* can alleviate anemia in animals suffering from cancer and increased survival time of them so it can be appraised worthwhile for supportive therapy in anemia fostered during chemotherapy.

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