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Research Article/Özgün Araştırma

In vivo antianemic study of two species of Murva on Phenylhydrazine induced anaemia in rats

Sıçanlarda fenilhidrazin ile indüklenmiş anemi üzerine iki Murva türünün in vivo antianemik etkileri

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Abstract

Aim: The present study was carried out to evaluate the antianemic effects of methanol extracts of *Marsdenia tenacissima* and *Sansevieria roxburghiana* on Phenylhydrazine (PHZ) induced anaemia in rats.

Materials and Methods: PHZ (40mg/kg, i.p) injection was administered to all rats on Day 0 and Day 1 day except the group I. Group II was disease control, group III to IV treated with 100, 200 mg/Kg of methanol extract of *M. tenacissima* whereas group V & VI received 50, 100 mg/kg of methanol extract of *S. roxburghiana*. Group VII received the higher doses of both extracts continuously from the day 3 to 28. RBC, Haematocrit and Haemoglobin were analysed on day 0,2,7,14,21 and 28.

Results: Continuous 28 days oral administration extracts raised the RBC, haemoglobin, and Haematocrit periodically in rats which were treated with PHZ.

Conclusion: These plants possess antianemic activity and can be used for rapid recovery and management of anaemia.

Keywords: Murva; *Marsdenia tenacissima; Sansevieria roxburghiana;* Phenylhydrazine, Antianemic activity.

Öz

Amaç: Bu çalışma, farelerde *Marsdenia tenacissima* ve *Sansevieria roxburghiana*'nın metanol ekstraktlarının fenilhidrazin (PHZ) ile indüklenen anemi üzerindeki antianemik etkilerini değerlendirmek için yapıldı.

Gereç ve Yöntem: Grup I hariç 0. ve 1. gün tüm sıçanlara PHZ (40 mg/kg, i.p) enjeksiyonu uygulandı. Grup II hastalık kontrolüdür, grup III ila IV, *M. tenacissima*'nın 100, 200 mg/kg metanol ekstraktı ile tedavi edilirken, grup V & VI 50, 100 mg/kg *S. roxburghiana* metanol ekstraktı almıştır. Grup VII, her iki ekstrakttan en yüksek dozları 3. ila 28. günler boyunca sürekli olarak aldı. Eritrosit, hematokrit ve hemoglobin 0, 2, 7, 14, 21 ve 28. günlerde analiz edildi.

Bulgular: PHZ ile tedavi edilen sıçanlarda periyodik olarak 28 günlük oral metanol ekstraktlarının alınması eritrosit, hematokrit ve hemoglobini yükseltti.

Sonuç: Bu bitkiler antianemik aktiviteye sahiptir ve aneminin hızlı iyileşmesi ve tedavisi için kullanılabilirler.

Anahtar Kelimeler: Murva; *Marsdenia tenacissima; Sansevieria roxburghian;* Fenilhidrazin; Antianemik aktivite.

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Introduction

According to World Health Organization (WHO), anaemia is a condition where haemoglobin concentrations lower than 12g/dL in women and 13 g/dL in men.[1] Anaemia was caused by various nutritional and infectious factors. It is also associated with the cancer[2] and heart failure[3] in which, 40% morbidity is due to anaemia. Cancer associated anaemia is due to chemotherapy which causes myelosuppresion or production of cytokines[2] or poor nutritional status.[4] In advanced stage of cancer, there is an imbalance of iron status, redox status, erythropoietin production and energy metabolism. These factors contribute existence of anaemia condition in cancer patients.[4] Anaemia, which occurs in the heart failure is multifactorial where it causes cardiac myocyte dysfunction due to inadequate oxygen delivery to the tissue. It is reversible with iron dextran infusion but, in the case of Doxorubicin induced anaemia iron supplement worsen the cardiotoxicity.[5,6] However, a single drug which compromises the above all incident is quite difficult. Hence a new strategy was developed in pharmaceutical industries to establish a formulation with extended action of cardioprotection from DOX, anticancer activity with free from myelosuppresion and antianemic effect. Under this comprehensive concept, recently, medicinal herbs and their formulations received greater attention on the treatments of various lives threatening disease because of their efficacy and rapid curative properties. Among the herbal preparation, Ayurvedic formulations have been placed at the first position for more than thousands of years due to their less toxicity and wide acceptability.[7] One of such Ayurvedic plant is Murva. It is a controversial drug, combination of 11 medicinal plants roots found in different parts of India. *Marsdenia tenacissima* is an acceptable source of Murva whereas *Sansevieria roxburghiana* Schult and Schult.f. (S. zeylanica Roxb.) was consider as Murva in west Bengal.[8] Traditionally Murva is used for treatment of anaemia, diabetes, stomach disorder, typhoid, cough, fever and urinary tract infection.[9] According to the traditional use, Murva is mainly used to treat pandu (anaemia)[10,11] but, none of them experimentally proven its antianemic effect. Therefore the present study was aimed to evaluate the antianemic effect of methanol extract of *M. tenacissima* and *S.roxburghiana* on phenylhydrazine (PHZ) induced anaemia in rats.

Materials and Methods

Experimental animals

Wistar albino rats (200g-250g) were taken from the animal house of St. Joseph's College of Pharmacy, Cherthala, Kerala, India, then they were acclimatised for a week under standard controlled condition (12 h light/12 h darkness, at 25^{o}C). The study protocol (**SJCP/IAEC/2018-4/35**) was approved by Institutional Animal Ethics Committee (IAEC), St. Joseph's College of Pharmacy Cherthala, Kerala, India.

Extraction

The Roots of *Marsdenia tenacissima* (MT) Rhizome & roots of *Sansevieria Roxburghiana* (SR) were cleaned and dried at room temperature (shade dry). About 300g of defatted coarse powered drug was successively extracted in a Soxhlet apparatus with methanol (70-80^{o}C for 48 hours). Methanol extract of MT (MEMT), SR (MESR) and aqueous extract of MT (AEMT), SR (AESR) were collected by rotary evaporator followed by dried and stored in a well tight container for experimental purposes.

Antianemic study design

Male and female Wistar rats (180-220 g) were selected for this study. Total 42 animals were divided in to seven groups containing 6 animals each. Anaemia was induced by intraperitoneal injection of PHZ (40 mg/kg/body weight) on Day 0 and Day 1.[12,13] The treatment schedule as follows;

Group I served as normal control received NaCl (0.9%) on D0 and D1followed by distilled water daily during 28 days.

Group II anaemic control received PHZ (40 mg/kg,i.p) at days 0 (D0) and 1 (D1) then distilled water daily during 28 days.

Group III and IV received PHZ (40 mg/kg) i.p on day 0 (D0) and day 1 (D1) and followed by 100mg/kg and 200 mg/kg of MEMT, orally, once in a day from D3 to D28.

Group V and VI received PHZ (40 mg/kg) i.p on day 0 (D0) and day 1 (D1) and followed by 50 mg/kg and 100 mg/kg of MESR, orally, once in a day from D3 to D28.

Group VIII received PHZ (40 mg/kg) i.p on day 0 (D0) and day 1 (D1) and followed by 100mg/kg of MESR and 200mg/kg of MEMT, orally, once in a day from D3 to D28. The blood samples were collected in the rats by tail vein under anaesthesia at day 0 (D0), after induction of anaemia with PHZ and on day 2 (D2), followed by first, second, third and fourth weeks of treatment. The following parameters were analysed from the blood RBC, Haematocrit and Haemoglobin (Hb).

Statistical evaluation

In vivo data were expressed as the Mean±SEM of six values. The difference between experimental groups was compared to disease control and normal control by oneway analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test where, $p<0.05$ implied significance.

Results

The data were expressed as mean±S.E.M, n=6. The data were analysed by ANOVA followed by Newman-Keul's multiple comparison test. Where, a: $p<0.001$, b-: $p<0.01$, c: $p<0.05$ and d: $p>0.05$.

The data were expressed as mean±S.E.M, n=6. The data were analysed by One-Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test. Where, a: $p<0.001$, b: $p<0.01$, c: $p<0.05$ and d: $p>0.05$.

Table 1 &2 showed the effect of MEMT, MESR and combination of extracts on various haematological parameters such as RBC count, haemoglobin and haematocrit on Day 0, 2,7,14,21 and 28. Only PHZ treated rats showed the reduction of the above haematological parameters. After the induction of anaemia by 40 mg/kg of PHZ (i.p, two consecutive days), the intermittent observation (up to 28 days) showed there was a change in hematological parameters. On 29th day, after continuous oral single dose administration of extracts significantly ($p<0.001$) raised the level of Hb, RBC and haematocrit percentage in PHZ administered rats. There was a progressive improvement on the restoration of haematological parameters found with the intermittent observation report.

Discussion

Most of the Ayurvedic medicines are available in poly herbal formulation where they used more than one plant drugs because when combining the several medicinal herbs to achieve extra therapeutic effectiveness. Various study revealed that on combination, the different herbs acts on the different receptors at the same time it produces more therapeutic efficacy than the individual plants. Therefore, a poly herbal preparation is preferred to achieve better therapeutic effects which also reduce the harmful effect.[14]

In Ayurveda, Murva is a category of plants used for the treatment of various diseases. It is controversial drugs with 11 sources are reported and are available in different location in India.[8] Various phytochemical constituents were isolated and characterised from these plants. Different pharmacological activities were individually evaluated from these different sources of Murva. Treatment of anaemia is one of the important traditional uses of Murva.[10] Anaemia is characterised by the reduction in level of haemoglobin and RBC results in decrease in oxygen carrying capacity.[12]

PHZ is a well-known and effective haemolytic agent which induces chemical changes in the red cell membrane and causes oxidative denaturation of haemoglobin, which results in formation of altered haemoglobin known as Heinz bodies that shorten erythrocyte life span and the consequence is anaemia. Therefore PHZ is used as anaemia inducing agent in laboratory animals.[13] It was previously reported that intraperitoneal administration of PHZ reduces the haematological parameters such as haemoglobin, RBC count and

haematocrit.[13,15] The similar results were found in the present study which was corrected daily administration of MEMT, MESR and the combination of MEMT&MESR for 28 days. Generally, the study results suggested that it could be a effective medicine for the treatment of anaemia.

Table 1. Effect of MEMT, MESR and combination of MEMT&MESR on haemoglobin, RBC count and haematocrit on Day 0, 2 and 7

Treatment	Parameters	Day 0	Day 2	Day 7
Normal control	Hb(g/dl)	13.67±0.15	13.27±0.23	13.23±0.24
	RBC (Cells/ml x$10^{9)}$	8.38 ±0.28	8.39±0.28	8.46±0.31
	Haematocrit (%)	50.29±0.1.69	50.36±0.1.69	50.77±0.1.81
Disease control	HB(g/dl)	14.03±0.61	5.10±0.26	5.00±0.25
	RBC (Cells/ml x$10^{9)}$	8.66±0.24	3.67±0.24	3.60±0.0.21
	Haematocrit (%)	51.9±0.1.44	22.02±1.43	21.61±0.1.27
MEMT(100mg/kg)	Hb(g/dl)	12.90±0.16	5.37±0.24	5.63±0.17^{c}
	RBC (Cells/ml x$10^{9)}$	8.69±0.17	3.34±0.07	3.59±0.0.17^{d}
	Haematocrit (%)	52.13±1.03	20.03±0.42	21.53±0.1.00^{d}
MEMT(200mg/kg)	Hb(g/dl)	13.13±0.25	5.23±0.22	6.80±0.17^{a}
	RBC (Cells/ml x$10^{9)}$	8.33±0.0.41	3.33±0.7	4.23±0.09^{d}
	Haematocrit (%)	49.98±2.49	20.2±0.43	25.37±0.0.53^{d}
MESR(50mg/kg)	Hb(g/dl)	12.97±0.26	5.53±0.18	5.43±0.11^{d}
	RBC (Cells/ml x$10^{9)}$	8.71±0.21	3.71±0.0.09	3.84±0.08^{d}
	Haematocrit (%)	52.26±0.1.26	22.24±0.57	23.05±0.0.51^{d}
MESR (100mg/kg)	Hb(g/dl)	13.23±0.27	5.03±0.18	6.37±0.06^{a}
	RBC (Cells/ml x$10^{9)}$	8.43±0.21	3.71±0.0.17	4.21±0.03^{d}
	Haematocrit (%)	50.59±1.30	22.17±1.07	25.25±0.19^{d}
MESR (100mg/kg) + MEMT(200 mg/kg)	Hb(g/dl)	13.60±0.25	5.20±0.19	8.47 ±0.10^{a}
	RBC (Cells/ml x$10^{9)}$	8.66±0.47	3.85±0.10	4.997±0.0.19^{a}
	Haematocrit (%)	51.93±1.14	23.08±0.61	28.22±0.1.17^{a}

Table 2. Effect of MEMT, MESR and combination of MEMT&MESR on haemoglobin, RBC count and haematocrit on Day 14, 21 and 28

Treatment	Parameters	Day 14	Day 21	Day 28
Normal control	Hb(g/dl)	13.23±0.19	13.43±0.08	13.43±0.08
	RBC (Cells/ml x$10^{9)}$	8.46±0.31	8.40±0.0.27	8.31±0.27
	Haematocrit (%)	50.77±1.81	50.40±1.63	49.91±0.1.60
Disease control	HB(g/dl)	4.73±0.31	5.07±0.20	4.90±0.23
	RBC (Cells/ml x$10^{9)}$	3.64±0.21	3.72±0.0.24	4.08±0.0.26
	Haematocrit (%)	21.81±0.0.25	22.31±1.44	24.47±0.1.58
MEMT (100mg/kg)	Hb(g/dl)	5.67±0.27^{b}	7.00±0.18^{a}	7.80±0.21^{a}
	RBC (Cells/ml x$10^{9)}$	4.28±0.0.04^{b}	5.02±0.0.13^{a}	5.58±0.0.13^{a}
	Haematocrit (%)	25.66±0.25^{b}	30.13±0.77^{a}	33.48±0.0.79^{a}
MEMT (200mg/kg)	Hb(g/dl)	8.13±0.18^{a}	10.03±0.12^{a}	11.57±0.22^{a}
	RBC (Cells/ml x$10^{9)}$	4.895±0.04^{a}	6.01±0.12^{a}	6.81±0.25^{a}
	Haematocrit (%)	29.37±0.23^{a}	36.07±0.73^{a}	40.78±0.1.51^{a}
MESR (50mg/kg)	Hb(g/dl)	5.87±0.12^{b}	6.68±0.17^{a}	7.63±0.25^{a}
	RBC (Cells/ml x$10^{9)}$	4.31±0.0.07^{c}	5.14±0.0.09^{a}	5.69±0.17^{a}
	Haematocrit (%)	25.86±0.0.42^{c}	30.83±0.52^{a}	34.14±1.04^{a}
MESR (100mg/kg)	Hb(g/dl)	7.73±0.15^{a}	8.96±0.22^{a}	9.93±0.36^{a}
	RBC (Cells/ml x$10^{9)}$	5.51±0.11^{a}	5.85±0.08^{a}	6.32±0.16^{a}
	Haematocrit (%)	33.06±0.0.68^{a}	35.10±0.49^{a}	37.94±0.97^{a}
MESR (100mg/kg) + MEMT (200 mg/kg)	Hb(g/dl)	9.77±0.19^{a}	11.67±0.37^{a}	13.23±0.21^{a}
	RBC (Cells/ml x$10^{9)}$	6.10±0.13^{a}	6.76±0.0.17^{a}	8.28±0.28^{a}
	Haematocrit (%)	36.63±0.76^{a}	40.58±1.01^{a}	49.70±1.67^{a}

Limitation of the study

Only healthy rats with 180 to 220g were selected for this study.

Conclusion

The present study confirmed the traditional use of Murva for the treatment of "pandu"-anaemia by *in vivo* antianemic activity of methanol extract of *M. tenacissima* and *S.roxburghiana* on phenylhydrazine (PHZ) induced anaemia in rats. It can be used as an adjuvant medicine in cancer chemotheraphy where it protects the hematopoietic system and also synergistically act as an anticancer drug.

Ethics Committee Approval

The study protocol (**SJCP/IAEC/2018-4/35**) was approved by Institutional Animal Ethics Committee (IAEC), St. Joseph's College of Pharmacy Cherthala, Kerala, India.

Author Contributions

R.A.-Designed the research protocol and preparation of manuscript. A.A.M-Conducted animal study and parameter evaluation. D.V.T.-Statistical analysis and report writing.

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Conflict of Interest

There is no conflict of interest in this study.

Financial Disclosure

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