



## RESEARCH ARTICLE

### ANTI-ANEMIC ACTIVITY OF HYDRO-ALCOHOLIC LEAF EXTRACT OF *AEGLE MARMELLOS* IN PHENYLHYDRAZINE INDUCED ANEMIC RATS

<sup>1,\*</sup>Joshi Ankur, <sup>2</sup>Soni Priyanka, <sup>1</sup>Vyas Narendra, <sup>1</sup>Khan Javed, <sup>1</sup>Malviya Sapna and <sup>1</sup>Kharia Anil

<sup>1</sup>Modern Institute of Pharmaceutical Sciences, Indore

<sup>2</sup>Acropolis Institute of Pharmaceutical Education and Research, Indore

(Shri Prabhat Chandra Kharia Research and Educational Society) Alwasa, Behind Rewti Range, Sanwer Road, Dist Indore (MP) Pin 453111

#### ARTICLE INFO

##### Article History:

Received 23<sup>rd</sup> January, 2017

Received in revised form

08<sup>th</sup> February, 2017

Accepted 24<sup>th</sup> March, 2017

Published online 20<sup>th</sup> April, 2017

##### Key words:

Anemia, Anti-anemic activity, *Aegle marmelos* and Vitamin B<sub>12</sub>.

#### ABSTRACT

The aim of the present study is to evaluate the anti-anemic activity of hydro-alcoholic leaf extract of *Aegle marmelos* against phenylhydrazine induced hemolytic anemia in rats. Phenylhydrazine (60mg/kg) was administered intraperitoneally for 2 days to induce anemia in rats. The animals were divided in to four groups of 6 animals each. Group I served as normal control, group II as anemic control, group III as reference control administered with Vitamin B<sub>12</sub> and group IV animals were treated with 200mg/kg, of hydro-alcoholic leaf extract of *Aegle marmelos*. All the test drugs were administered once daily for 28 days through oral route. On 29<sup>th</sup> day blood was withdrawn, through tail puncture under phenobarbitone anesthesia and subjected to the estimation of RBC, Hb and percentage Hematocrit. Both the hydro-alcoholic leaf extract of *Aegle marmelos* and Vitamin B<sub>12</sub> significantly increased the RBC, Hb and Hematocrit levels which conclude that, *Aegle marmelos* leaf extract exhibits anti-anemic activity.

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Citation: Joshi Ankur, Soni Priyanka, Vyas Narendra, Khan Javed, Malviya Sapna and Kharia Anil, 2017. "Anti-anemic activity of hydro-alcoholic leaf extract of *Aegle marmelos* in Phenylhydrazine induced anemic rats", *International Journal of Current Research*, 9, (04), 48928-48931.

#### INTRODUCTION

Anemia is a condition that develops when blood lacks enough healthy red blood cells or hemoglobin. Anemia affects the lives of more than 2 billion people globally, accounting for over 30% of the world's population which is the most common public health problem particularly in developing countries occurring at all stages of the life cycle (Ramesh and Lopamudra, 2010). Iron deficiency is the most common nutritional disorder in the world. Worldwide, Iron-deficiency anemia is a significant problem and especially in developing countries it is widespread yet the most neglected micronutrient deficiency disorder among children, adolescence girls, and pregnant women (WHO 2015). Iron deficiency is the most common nutritional disorder in become depleted and a restricted supply of iron to various tissues becomes apparent. This may result in depletion of Hemoglobin and iron-dependent intra-cellular enzymes participating in many metabolic pathways. Therefore, there is the need for proper management of micronutrient deficiencies most especially iron deficiency. Over the years, medicinal plants have been recognized to be of great importance to the health of individuals and communities. In many developing countries,

herbal medicines are assuming greater importance in primary health care (Eftekhari et al., 2003). *Aegle marmelos* is a slow-growing, medium sized tree, up to 12 to 15 m tall with short trunk, thick, soft, flaking bark, and spreading, sometimes spiny branches, the lower ones drooping (Dhankhar et al., 2011). Fruit, Leaf, Bark and Decoction of the bark have been used in traditional medicinal system for the treatment of various diseases (Figure 1).



Figure 1. *Aegle marmelos* plant

\*Corresponding author: Joshi Ankur,  
Modern institute of Pharmaceutical Sciences, Indore

## Fruit and Seeds

Fruits, yellowish green, with small dots on the outer surface, oblong to globose, 5.3 cm to 7.2 cm in diameter; weight, 77.2 g; volume, 73.7 ml; pulp, yellow and mucilaginous, the pulp of dried fruits retains its yellow, and also remains intact; rind woody, 4 to 5 mm thick (Hiremarh *et al.*, 1996). The Seeds are, numerous, embedded in the pulp, oblong, compressed, with white, cotton-like hairs on their outer surface. (Figure 2)



Figure 2. Fruit and Seed of *Aegle marmelos*

## Traditional Uses

Bael leaves are useful in jaundice and in the treatment of wounds. The extract of leaves is beneficial in the treatment of leucorrhoea, conjunctivitis and deafness. Fruits give feeling of freshness and energy. It is used as carminative and astringent. It finds good utility in thyroid related disorder. The other fine therapeutic uses reported in cardiac stimulant, swollen joints, pregnancy trouble, typhoid and coma. The dried powder of leaf is used in the treatment of irritable bowel syndrome (Sharma *et al.*, 2007).

## Chemical Constituent

Extensive investigations have been carried out on different parts of *Aegle marmelos* and as a consequence, varied classes of compound viz., alkaloids, coumarins, terpenoids, fatty acids and aminoacids have been isolated from its different parts (Yadav and Chanotia, 2009; Arul *et al.*, 2004).

## MATERIALS AND METHODS

### Plant material

The plant material is made up of the leaves of *Aegle marmelos*. The leaves was collected from medicinal garden of Modern institute of Pharmaceutical Sciences in August 2016. The sample of plant was identified and authenticated at Rajmata Vijayraje Scindia Krishi Vishwavidhalaya, College of Agriculture, Indore.

### Preparation of Extract

The collected leaves were, shade dried and then ground into coarse powder. The powder was then subjected to exhaustive extraction by a soxhlet extraction process using 70:30 ethanol and water as a solvent. The Hydro-alcoholic extract was concentrated by vacuum distillation to dry. The collected

extract was stored in desiccators and used for further pharmacological study.

## Animals

Healthy adult albino rats of Wistar strain of both sex, weighing about 150-200 g were obtained from the animal house of Modern Institute of Pharmaceutical Sciences, Indore. The rats of either sex were isolated and housed in separate cages during the course of experimental period and kept them at room temperature ( $24 \pm 2^\circ\text{C}$ ) with a 12 : 12 h light/dark cycle. The animals were fed with standard pellet diet and provided water *ad libitum*. All the procedures and protocols were reviewed and approved by the Institutional Animal Ethics Committee of MIPS, Indore.

## Anti-Anemic Activity

### Induction of Anemia

Anemia was induced in rats by intraperitoneal administration of phenylhydrazine (60mg/kg) daily for 2 days (Roque *et al.*, 2008). Rats that developed anemia with Hemoglobin concentration lower than 13 g/dl were recruited for the study.

### Treatment of the animals

The anemic rats were randomly divided into 4 groups six animals each. Group I was non anemic animals (normal control) received 1ml/kg of 0.1% Carboxy methyl cellulose solution. Group II was served as anemic control and group III served as reference control, received Vitamin B<sub>12</sub> syrup (1 ml/rat) and group IV animals received 200mg/kg of *Aegle marmelos* extract through oral administration, by suspending in CMC solution. All the test drug were administered orally, once daily for 28 days. On 29<sup>th</sup> day, blood was collected in EDTA coated tubes, by tail puncture under phenobarbitone (45mg/kg, ip) anaesthesia. The following parameters like, Red Blood Cell count (RBC), Hemoglobin (Hb) and Hematocrit percentage (HCT) were evaluated in blood (Ghai, 1995).

### Statistical Analysis

Data's were expressed as mean  $\pm$  SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's 't' test. P values < 0.05 were considered as significant.

## RESULTS

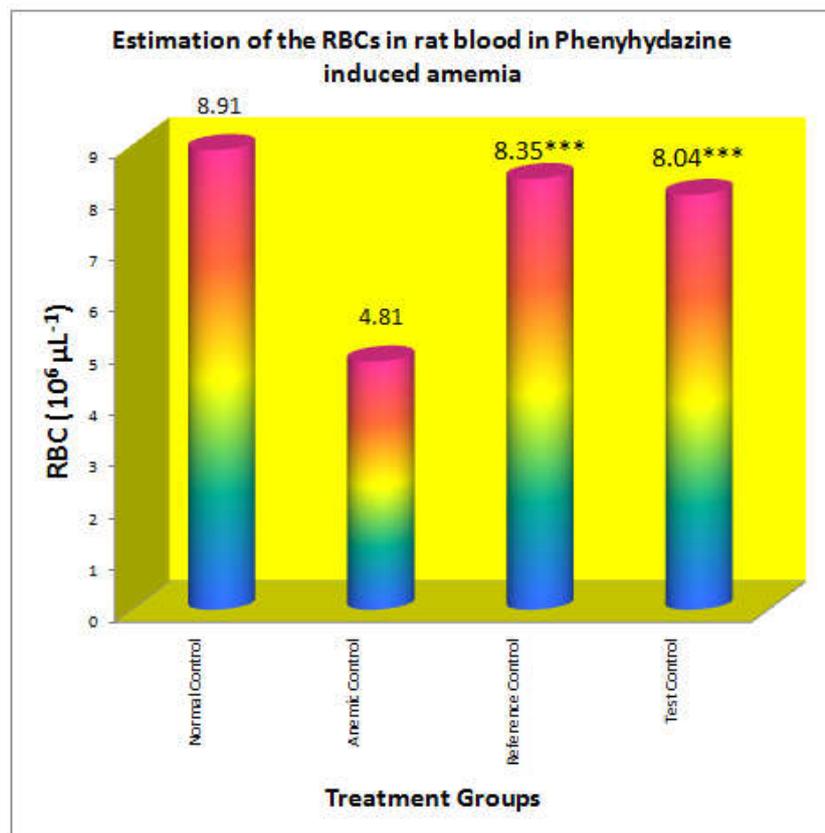
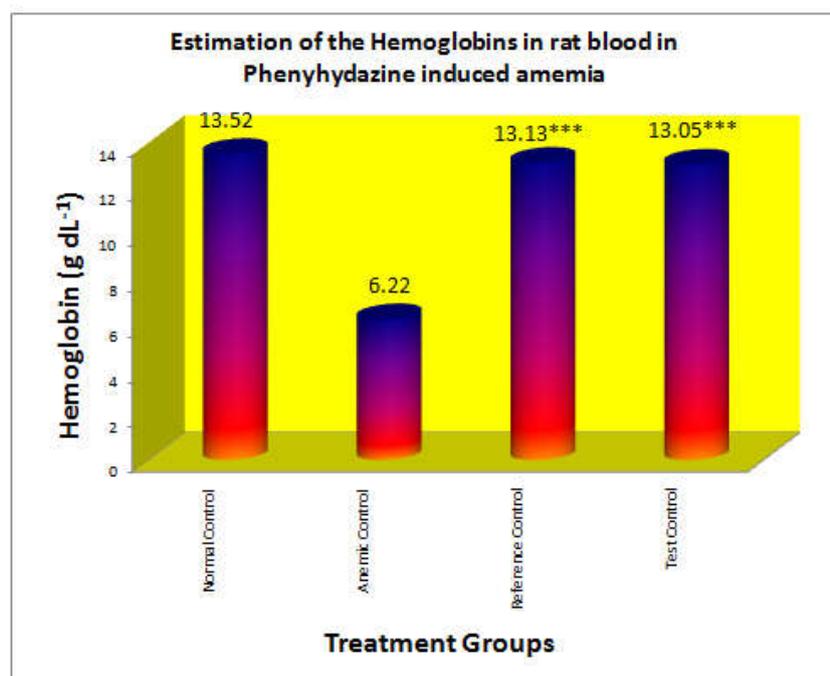
Anti-anemic activity of *Aegle marmelos* leaf extract on Phenylhydrazine induced hemolytic anemia in rats was studied and the results were shown on Table 1. The anti-anemic activity of *Aegle marmelos* leaf extract was assessed by determining the red blood cell count, hemoglobin and hematocrit percentage. Phenylhydrazine decreased the RBC, Hb and % HCT as compared normal control. There was significant (P<0.001) increase in RBC and Hb with both Vitamin B<sub>12</sub> and *Aegle marmelos* leaf extract against phenylhydrazine challenge. Also there was significant (P<0.01) increase in % HCT with both Vitamin B<sub>12</sub> and *Aegle marmelos* leaf extract. This shows that *Aegle marmelos* effective anti-anemic activity against phenylhydrazine induced hemolytic anemia in rats and it has comparable effect as that of the standard drug Vitamin B<sub>12</sub>.

**Table 1. Effect of *Aegle marmelos* leaf extract on Phenylhydrazine induced hemolytic anemia in rats**

S.No.	Drug treatment	RBC ( $10^6 \mu\text{L}^{-1}$ )	Hb (g $\text{dL}^{-1}$ )	HCT %
1	Normal Control (0.1% CMC)	8.91±0.61	13.52±0.55	47.88
2	Anemic Control Phenylhydrazine (60mg/kg)	4.81±0.14	6.22±0.23	28.42
3	Reference Control Vit B <sub>12</sub> (1ml/rat)	8.35±0.42***	13.13±0.73***	45.29**
4	Test Control <i>Aegle marmelos</i> (200mg/kg)	8.04±0.54***	13.05±0.74***	43.61**

Data were expressed as Mean ± SEM (n=6)

\*P<0.05, \*\* P<0.01 and \*\*\* P<0.001 Vs Anemic Control

**Figure 3. Estimation of the RBCs in rat blood in Phenylhydrazine induced anemia****Figure 4. Estimation of the Hemoglobins in rat blood in Phenylhydrazine induced anemia**

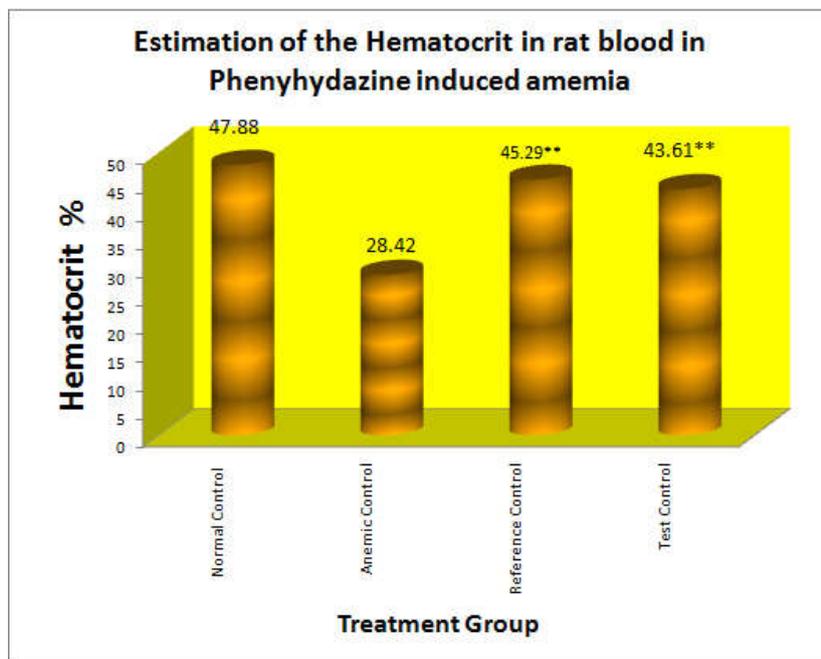


Figure 5. Estimation of the Hematocrit in rat blood in Phenyhydrazine induced anemia

## Conclusion

The Hydro-alcoholic leaf extract of *Aegle marmelos* exhibits anti-anemic activity against phenyhydrazine induced anemia in rats. The anti-anemic effect produced by the *Aegle marmelos* leaf may be due to its high content of iron which is present in the plant.

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