



## Comparative anti anemic activity of *Azadirachta indica* leaves and its combination with *Emblica officinalis* in phenyl hydrazine induced anemia using rats

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### ABSTRACT

*Azadirachta indica* (neem leaves) and its combination with *Emblica officinalis* (amla) were evaluated for their antianemic activity in phenyl hydrazine induced anaemic animals. Rats were divided into 7 groups of 6 each. Group 1 was given normal saline and served as control and all other groups were given 60 mg/kg b.w of phenyl hydrazine for 2 days to induce anemia. Group 2 served as positive control (only treated with phenyl hydrazine) and Group 3 was treated with standard ferrous sulfate containing drug Fefol<sup>®</sup> (0.012mg/kg.p.o.) and served as standard. Group 4 and 5 were treated with different doses (200 and 400 mg/kg b.w.) of aqueous extracts of *Azadirachta indica* leaves respectively. Whereas Group 6 and 7 were treated with combination of aqueous extract of *Azadirachta indica* leaves and *Emblica officinalis* fruit (200 and 400 mg/kg b.w.) respectively. All the treatments were given orally and continued up to 14 days. On 15<sup>th</sup> day blood samples were collected by retroorbital puncture and hematological parameters such as haemoglobin (Hb) concentration, RBC count and WBC count were estimated. Results showed that both *Azadirachta indica* leaves and its combination with *Emblica officinalis* fruit showed significant anti anemic activity but compared to *Azadirachta indica* leaf extract alone, its combination with amla showed better activity. This synergistic action of plants may be due to increase in absorption of iron from neem leaves due to vitamin C in amla fruit and this combination may be an alternative to synthetic iron therapy in anemia.

**Keywords:** Phenyl hydrazine, anaemia, *Azadirachta indica*, *Emblica officinalis*, haematological parameters.

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### INTRODUCTION

Anemia is a hematological condition characterized by the reduction in the concentration of hemoglobin accompanied by reduced number of circulating RBC. Main function of RBC is the transportation of oxygen into the tissues of body and in anemia there is decrease in oxygen carrying capacity which is detrimental to the body. Anemia is caused due to various factors such as inadequate absorption or intake of iron, reduced intake of vitamin B12 or folic acid, destruction of red bone marrow, hereditary conditions etc. Gradually it is recorded that large number of population is affected by anemia so it has become a prime importance to find out the remedy to treat it [1, 2].

Phenylhydrazine is a strong oxidant and its metabolites include reactive oxygen species, phenyldiazene, the phenylhydrazyl radical, and benzenediazonium ions through automatic oxidation, which damages RBCs by oxidation and causes severe hemolytic anemia by oxidating HbG [3].

*Azadirachta indica* is commonly known as neem belongs to family Meliaceae and all parts of the tree have medicinal properties known since ancient times and have been extensively used in ayurveda [4]. Many compounds such as limonoids, terpenoids, azadirone, azadirachtin, and flavonoids have been isolated from various parts of neem tree and have been evaluated for their pharmacological actions and plausible medicinal applications. Studies have shown that neem possesses anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, antiulcer, antifungal, antianemic, antibacterial, and antitumor activities [5–9].

*Emblica officinalis* is commonly called as amla and it belongs to Euphorbiaceae. Amla fruits are acrid, cooling, refrigerant, astringent, diuretic and laxative. The fermented liquor prepared from fruits is used in jaundice, dyspepsia and cough. *Emblica officinalis* fruits contain vitamin C, tannins, flavonoids, ellagic acid, gallic acid, quercetin and other constituents which are responsible for their therapeutic actions [10].

Leaves of *Azadirachta indica* were used to treat anemia traditionally and it is proved to normalize hematological properties in experimental rats [11]. Neem leaves were found to contain flavonoids, iron and other minerals which may be one of the factor contributing to its antianemic action [11, 12]. Amla fruits contain vitamin C and other constituents [10] which may increase the absorption of iron from neem leaves. So, in this context we want to find out whether amla fruit when given in combination enhances the antianemic property of neem leaves.

## EXPERIMENTAL SECTION

### Plant extracts

*Azadirachta indica* leaf aqueous extract and its combination with *Emblica officinalis* fruit aqueous extract were gift samples from Green Chem Herbal Extracts and Formulations, Bangalore.

### Animals

Sprague Dawley rats of either sex (200-250 gms) were obtained from the animal house of Krupanidhi Pharmacy college, Bangalore, India approved by CPCSEA (378/01/ab/CPCSEA). All the animals were maintained in a well ventilated room and given access to feed and water *ad libitum*.

### Experimental protocol

Animals were divided into 7 groups of six each. Group 1 served as control and received normal saline. Phenyl hydrazine 60 mg/kg, i.p for 2 days was given to induce anemia [13]. Group 2 received phenyl hydrazine alone whereas other groups received treatment after induction of anemia. Group 3 served as standard and received Fefol<sup>®</sup> at dose 0.012mg/kg, p.o [14]. Group 4 and 5 received aqueous extracts of neem leaves at doses of 200 and 400 mg/kg, p.o respectively [11]. Groups 6 and 7 received combination of aqueous extracts of neem leaves and amla fruit in 1:5 ratio at doses of 200 and 400 mg/kg, p.o respectively.

Treatments were continued for 14 days and on 15<sup>th</sup> day hematological parameters were measured.

### Biochemical estimations

On day fifteen blood samples were withdrawn by retro orbital route under mild ether anaesthesia. Fresh blood was immediately collected into heparinised tubes for measurement of routine hematological parameters. Haemoglobin estimation was done using Sahli's method [15]. Red blood cell count and White blood cell count were done using Neubauer chamber method.

Mean Cell Haemoglobin was calculated from following equation:

$$\text{MCH (pg)} = [\text{Hb (gm \%)} \times 10] / \text{RBC count (mil/mm}^3\text{)} .$$

**Statistical analysis**

The results of this study are expressed as mean  $\pm$  Std error of mean (Mean  $\pm$  SE). Statistical analysis was done by using One-way ANOVA followed by Dunnet multiple comparison test. All values of  $p < 0.05$  were considered statistically significant.

**RESULTS AND DISCUSSION****Effect on haematological parameters:**

Phenylhydrazine treatment significantly decreased haemoglobin and RBC ( $p < 0.001$ ). There is no significant change in MCH (Table 1).

All the test groups and standard has shown increased haemoglobin ( $p < 0.001$ ) when compared with phenylhydrazine treated group. Both the extract treated groups at different doses normalized RBC. Comparatively neem leaf extract when given in combination with amla fruit at high dose has shown better response similar to standard ( $p < 0.001$ ). There is no significant change in MCH in all groups (Table 1).

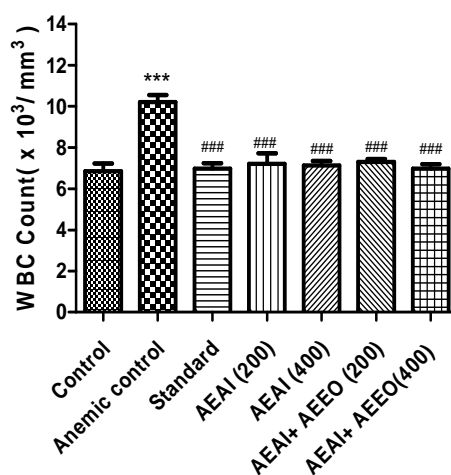
**Table 1: Effect of different groups on haematological parameters**

Groups/Parameters	Hb content (gm/dl)	RBC count ( $\times 10^6$ cells/ $\text{mm}^3$ )	MHC(pg)
Group I-Control	14.73 $\pm$ 0.23	6.515 $\pm$ 0.50	23.18 $\pm$ 1.47
Group II-Anemic control	9.73 $\pm$ 0.20 <sup>***</sup>	3.86 $\pm$ 0.09 <sup>***</sup>	25.24 $\pm$ 0.89
Group III-Standard	14.57 $\pm$ 0.25 <sup>###</sup>	6.49 $\pm$ 0.30 <sup>###</sup>	22.69 $\pm$ 1.24
Group IV-AEAI(200)	13.37 $\pm$ 0.28 <sup>###,*</sup>	5.77 $\pm$ 0.37 <sup>#</sup>	23.47 $\pm$ 1.59
Group V-AEAI(400)	13.65 $\pm$ 0.51 <sup>###</sup>	5.98 $\pm$ 0.44 <sup>#</sup>	23.73 $\pm$ 2.62
Group VI- AEAI+AEEO(200)	14.08 $\pm$ 0.20 <sup>###</sup>	5.92 $\pm$ 0.15 <sup>#</sup>	23.82 $\pm$ 0.69
Group VII -AEAI+AEEO(400)	14.67 $\pm$ 0.11 <sup>###</sup>	6.31 $\pm$ 0.30 <sup>###</sup>	24.03 $\pm$ 1.38

Values are given as mean  $\pm$  SEM, (n=6/group). <sup>\*</sup> $p < 0.05$ , <sup>\*\*\*</sup> $p < 0.001$  when compared with control. <sup>#</sup> $p < 0.01$ , <sup>###</sup> $p < 0.001$  When compared with Phenyl hydrazine treated group.

AEAI(200): Aqueous extract of neem-200 mg/Kg, AEAI(400):Aqueous extract of neem 400 mg/Kg AEAI+AEEO(200): Aqueous extract of neem with amla-200 mg/Kg, AEAI+AEEO(400):Aqueous extract of neem with amla-400 mg/Kg.

Phenyl hydrazine treatment elevated WBC count when compared with control. There is decrease of WBC count in standard and test groups ( $p < 0.001$ ) when compared with phenyl hydrazine treated group (Figure 1).

**Figure 1-Effect of different groups on WBC count**

Values are given as mean  $\pm$  SEM, (n=6/group). <sup>\*\*\*</sup> $p < 0.001$  when compared with control. <sup>###</sup> $p < 0.001$  When compared with Phenyl hydrazine treated group.

AEAI(200): Aqueous extract of neem-200 mg/Kg, AEAI(400): Aqueous extract of neem 400 mg/Kg AEAI+AEEO(200): Aqueous extract of neem with amla-200 mg/Kg, AEAI+AEEO(400): Aqueous extract of neem with amla-400 mg/Kg.

There are reports on the reduction in erythrocyte count, haemoglobin levels on administration of phenylhydrazine in rats [1,13]. The present work supports this statement as evidenced by results and treatment with neem leaves and its combination with amla reversed the above mentioned effects with phenyl hydrazine.

Phenyl hydrazine treatment causes haemolytic anemia by production of reactive oxygen species (ROS) which causes oxidative damage to RBC. Free radicals can also increase aging process of RBC cells. As a result there will be decrease in RBC and haemoglobin [16]. WBC cells are elevated as a result of body's defence mechanism to get rid of haemolytic products [1].

Neem has medicinal properties known since ancient times and have been extensively used in ayurveda. Neem leaves are rich source of minerals like iron, zinc and magnesium which may help in haemoglobin formation [17]. They also contain flavanoids, terpenoids and limonoids [4]. Flavanoids and limonoids are antioxidants which may prevent the damage caused by ROS formed in phenylhydrazine treatment.

Amla fruits contain vitamin C, tannins, flavonoids, ellagic acid, gallic acid, quercetin and other constituents [10]. Since neem leaves are a rich source of iron and amla contains ascorbic acid which facilitates iron absorption, the synergistic effect seen with the combination in this work is convincing.

Thus, the present work reveals the fact that neem leaves or its combination with amla has beneficial action on phenylhydrazine induced anemia in rats.

### CONCLUSION

The present work supports that phenyl hydrazine is capable of causing marked alteration in some haematological parameters inducing anemia. Neem leaves and its combination with amla has beneficiary role. Further, combination of both neem leaves and amla fruit proved to be a better alternative than neem leaves alone in preventing the symptoms of anemia produced by phenyl hydrazine. This might be due to synergistic action of drugs.

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