

Phenylhydrazine Induced Haematoxicity and its Amelioration by *Dillenia indica* Extracts

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ABSTRACT: PHZ exposure has been shown to affect haematopoietic cells in adults. In developmental experimental animal studies, prenatal exposure of PHZ resulted in fatal effects like reduction in weight, skeletal malformation and increased incidence of the miscarriage and persistent decrease in the no. of blood forming cells. PHZ exposure is also associated with respiratory difficulties in children including bronchitis, asthma and wheezing. Acute exposure to PHZ can result in skin and eye irritation or burning, as well as dizziness, nausea, vomiting and suffocation. Here the PHZ was used to induce haemolytic anaemia into the rats. Anaemia is a very commonly known blood disorder. It affects the people of all ages and both the sexes and is posing the threat to the global healthcare and modern society. Hemolytic anaemia is the kind of anaemia which can be inherited or acquired type of anaemia resulting from the intravascular and extravascular blood cell destruction. Due to this PHZ exposure splenomegaly was clearly seen as there was difference of more than double in the size of the spleen if compared to the control sample. The administration of the *Dillenia indica* plant extract brought the Hgb and RBC count to normal but the WBC count were still on the higher side.

KEYWORDS: *Dillenia indica*, haemolytic anaemia, Phenylhydrazine, Haematoxicity.

I. INTRODUCTION

In India a large number of herbal extracts are used in folk medicine to treat various types of disorders. The discovery of new and novel pharmaceutical products from plants used in traditional system of medicine for the treatment of the incidence of anemia.

Dillenia indica is an evergreen large shrub or small to medium-sized, an important medicinal plant found in Assam North-east India. The plant has been blessed with numerous medicinal properties with edible constituents as well. The fruit is the main yield of the plant. The plant grows abundantly in Assam. But due to lack of knowledge and not knowing its medicinal values, most of the fruits of this plant and other parts are wasted. The leaf, bark and fruit of the plant are used in the indigenous system of medicine. It has many medicinal properties like relieving pain and regulating the body heat.¹ It is also used as cooling beverage in the treatment of fever.² It tones up the nervous system and removes fatigue. Traditionally they are used as laxative and carminative. It also helps in relieving fatulence.³ Fruit juice is also used as cardio tonic. Barks and leaves of the plant are used as laxative and astringent. Due to its various biological activities including anti-diabetic and anticancer properties the plant is gaining importance as a valuable medicinal plant.^{4,5} In this review emphasis is given on detailed exploration on the traditional claims, pharmacological activity and photochemical compounds of *Dillenia indica*.

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II. MATERIAL AND METHOD

Chemicals: Methanol and Phenylhydrazine were purchased from Sigma Aldrich (USA). All other chemicals used in the study were of Analytical R grade and available locally.

Plant materials:

The fresh plant sample of *Dillenia indica* whole plant was obtained from a local commercial source in Lucknow (India). The plant was identified at Division of Botany, CSIR-Central Drug Research Institute Lucknow.

Preparation of plant extracts:

The whole plant was dried at room temperature and ground into powder. Dry powder (2kg) was macerated in 70% Methanol and then kept 48 h at room temperature. The resulting extract was filtered. The filtrate was concentrated in an oven at a temperature of 40°C for 24 h to a constant. The extract was stored at -20 °C.

Hematoprotective activity:

Test animals:

Charles Foster rats of both sexes were obtained from National Laboratory Animal Center (NLAC), Central Drug Research Institute, Lucknow (India) and were allowed to acclimate to animal room conditions for 7 days prior to experimentation. They were kept in a controlled environment at 25±2°C and 30–60% relative humidity with a 12 h light and dark cycle. The animals were fed a standard rodent pellet diet and water ad libitum. Animal studies were conducted according to the regulations of the Institute Animal Ethics Committee.

Induction of Anemia:

Basal hematological profile determined for healthy Charles Foster rats using the fully automated MS9 hematology analyser. Phenylhydrazine (PHZ) induced anemia is a model for the study of hematinic effects.^{7,8} In present study Phenylhydrazine was used to induce anemia (10mg/kg B. Wt. for seven consecutive days). After 7days the hematological profile was determined again and the rats with a ≥30-40% reduction in red blood cells count and hemoglobin concentration were considered anemic and used for this study.

Experimental procedure:

The rats were divided into four groups of 10 Rats each group (5M+5F) and were subjected to the following chemical treatment.

Group I: Control; distilled water (7 days) (1% Gum Acacia)

Group II: 10 mg/Kg PHZ (7 Days) then 125 mg/Kg *Eclipta alba*

Group III: 10 mg/Kg PHZ (7 Days) then 250 mg/Kg *Eclipta alba*

Group IV: 10 mg/Kg PHZ (7 Days) then 500 mg/kg *Eclipta alba*

At the end of the experiment, animals were anesthetized with di-ethyl ether and sacrificed.

Body Weight

Body weight was recorded at day 0, 7 and at the time of termination of study.

Food and water consumption:

Monitoring 24-hour food and water consumption of the animals in a group was done at the beginning and end of the study by giving a measured quantity of water and pellet diet followed by estimation of the amounts remaining at the end of 24 hours. Average food and water consumption per animal was calculated for each group.

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Hematological analysis:

Blood samples were collected at 0, 7 and 14 days through tail vein in EDTA coated vials. Hematological parameters were analysed using MS-9 fully automated hematology analyzer. The following parameters were determined of blood samples. Erythrocyte (RBC), total and differential leukocyte(WBC), hematocrit(Hct), hemoglobin (Hb), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), platelet distribution width (PDW), and red distribution width (RDW). Absolute and relative organ weight: At the end of the study (14days), all organs were collected using standardized surgical procedures. The abdominal cavity of each animal was dissected and organs namely the heart, liver, lungs, spleen, kidney and brain were quickly removed, cleaned with normal saline, weighed and preserved in 10% formalin. Statistical analysis: All in vitro antioxidant assay were done in triplicate manner and results are expressed as mean \pm S.D. Data were analyzed with one-way ANOVA. Statistically significant effects were further analyzed. The statistical significance was determined at $p < 0.05$.

III. EXPERIMENTAL RESULTS

Induction of anemia:

Experimental animals were exposed with 10mg/kg body weight with Phenyl hydrazine by oral route. After the 7 day of Phenyl hydrazine administering a significant reduction ($p > 0.001$) in RBC (percentage reduction 52%), Hgb (Percentage reduction- 48%). There were no significant differences were observed in Hct, MCH, MCHC, RDW and Pct values between the normal and anemic animals(Fig. 1&2).

Body weight

The body weight was taken before the start of the experiment, and then after the doses of phenyl hydrazine and at last after the doses of the plant extract before the final hematology. There is no such significant difference in the body weight of the rats in all the groups in both the sexes.

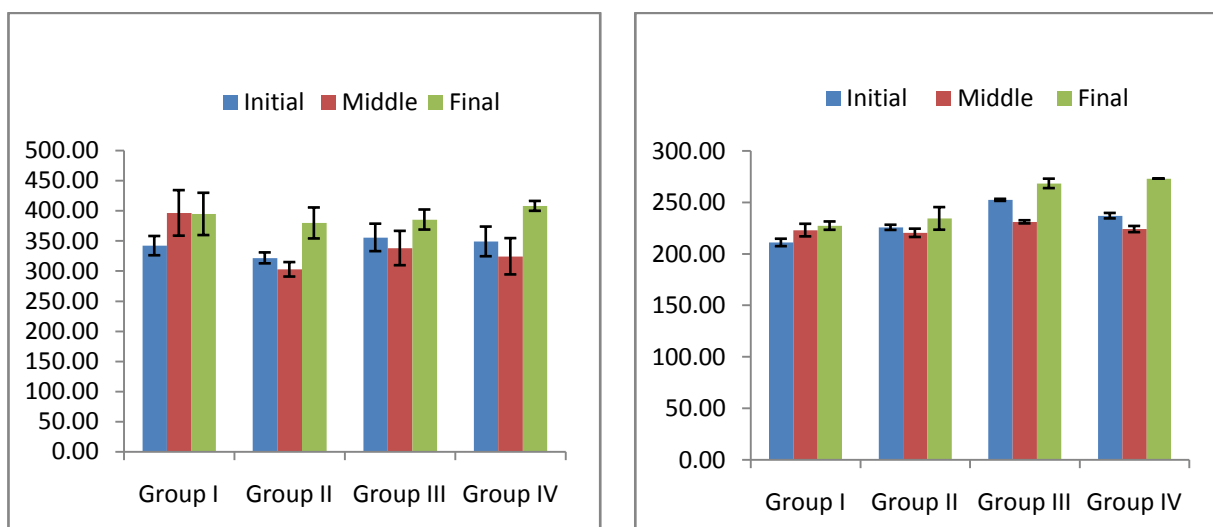


Fig. 1: Showing the Body Weight of Group 1-4 (Group I- Control, Group II- 10 mg /kg B.Wt. PHZ then 125mg/kg B. Wt. EA, Group III-10 mg/kg B. Wt. PHZ then 250mg/kg B. Wt. EA, Group IV-10 mg/kg B. Wt. PHZ then 500 mg/kg B.Wt. EA) both Male and Female SD Rats.

Food and Water Record

The food and water was taken for individual group before the start of the experiment, and then after the doses of phenyl hydrazine and at last after the doses of the plant extract before the final hematology. There is no such significant difference in the food and water of the rats in all the groups in both the sexes. 200gm was food was given each time and 250ml of water was given and after 24 hours the remaining pellets were weighed and water was also measured. This process was done each time while taking the food and water.

The rats treated with PHZ at dose of 10 mg/kg (7days) showing decrease in the consumption of food and water as compare to control group but not significant. There is no significant difference were seen in the food water consumption of animals of different treatment groups treated with *Dillenia indica* at dose 125, 250, 500 mg/kg body weight.

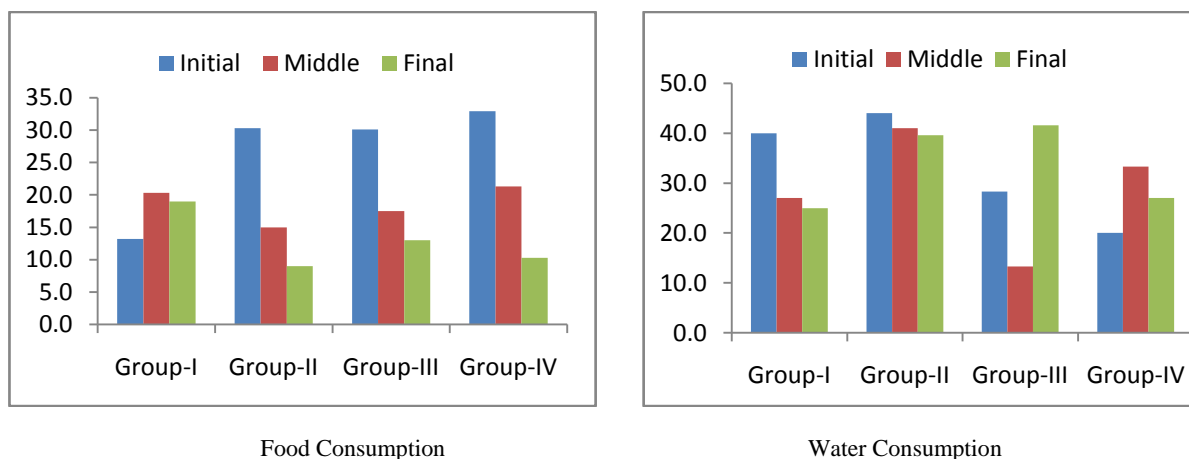


Fig. 2: Showing the Food and Water consumption of male SD rats of different group 1-4 (Group I- Control, Group II- 10 mg/kg B.wt. PHZ then 125mg/kg B. Wt. EA, Group III-10 mg/kg B. Wt. PHZ then 250mg/kg B. Wt. EA, Group IV-10 mg/kg B. Wt. PHZ then 500 mg/kg B.Wt. EA)

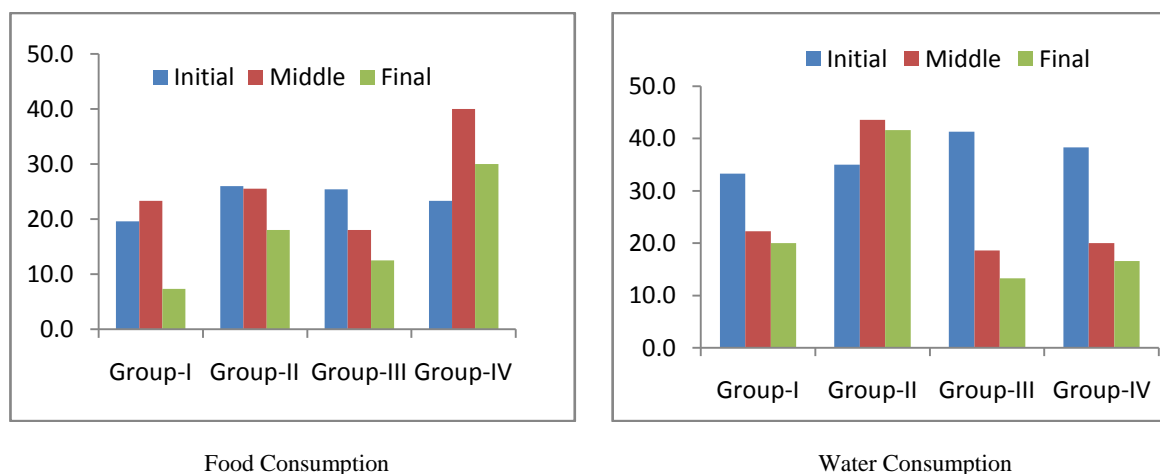


Fig. 2: Showing the Food and Water consumption of female SD rats of different group 1-4 (Group I- Control, Group II- 10 mg /kg B. Wt. PHZ then 125mg/kg B. Wt. EA, Group III-10 mg/kg B. Wt. PHZ then 250mg/kg B. Wt. EA, Group IV-10 mg/kg B. Wt. PHZ then 500 mg/kg B.Wt. EA)

In vivo Hematoprotective activity:

Compared to the respective control group the hematology parameters of different phenylhydrazine treated groups there were decrease in Hgb, T-RBC and increase in TLC mean value. After treatment with plant extract the hematology parameters (Hgb& T-RBC) are reverted back as compared to control group.

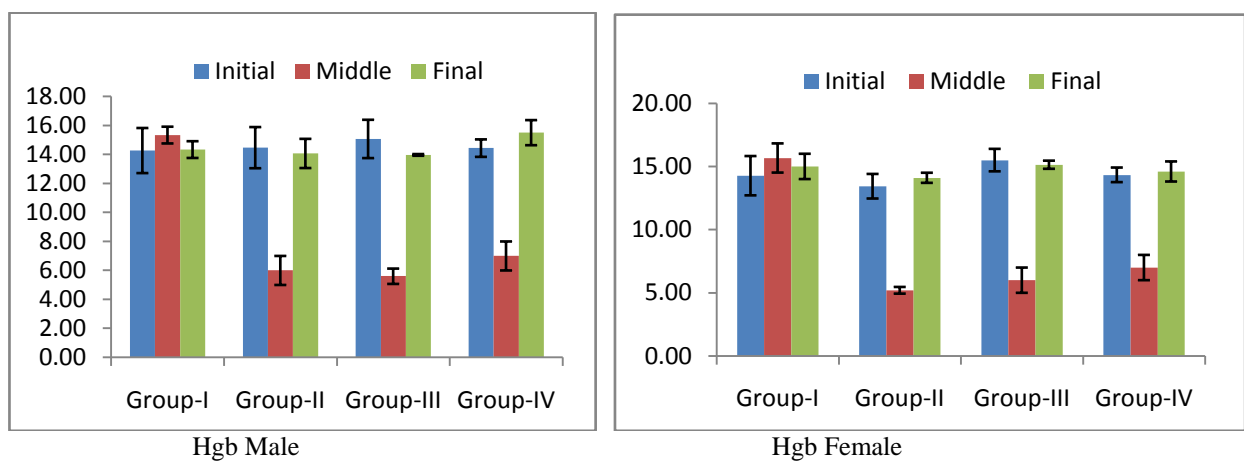


Fig. 3: Showing the number of Hgb (gms/dl) from Group 1-4 in male and female SD Rats

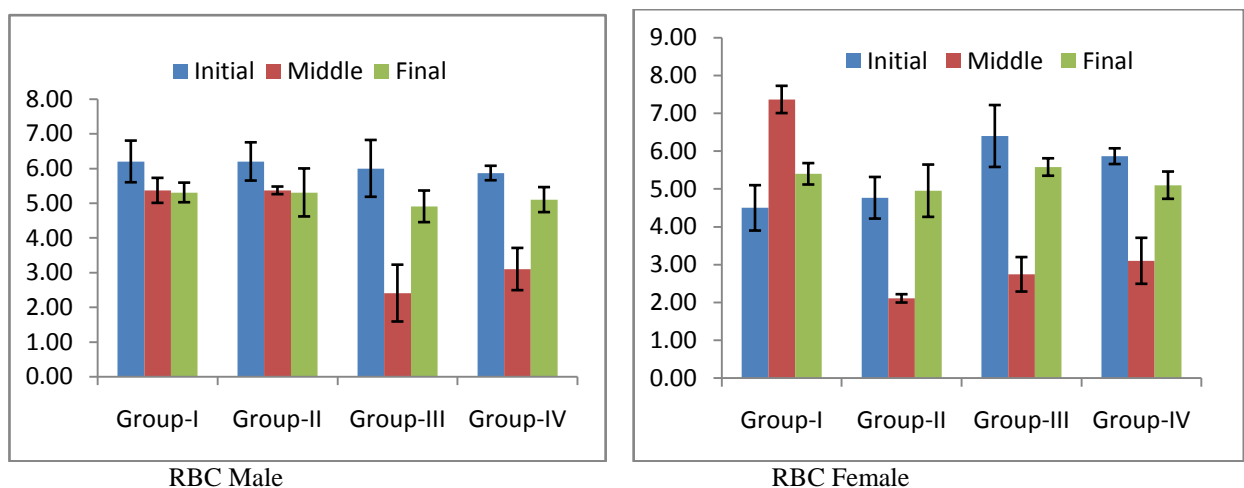


Fig. 4. Showing the number of total RBC (x10⁶/mm³) from Group 1-4 in male and female SD Rats

IV. DISCUSSION

In present study *Dillenia indica* plant extract administered at dose 125 mg/kg, 250mg/kg, 500mg/kg. *Eclipta alba* showing strong antioxidant activity. The radical scavenging activity of *Dillenia indica* seems to be correlated with its polyphenolic constituents though active components could play important roles in its antioxidative effect. Polyphenols act as reducing agents and antioxidants via several mechanisms including the scavenging of free radicals, chelation of

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transition metals, as well as the mediation and inhibition of enzymes⁹. Consequently, it is possible that the total phenolic constituents may contribute to antioxidant activity of *Dillenia indica*. Three different doses of *Dillenia indica* (150mg, 250mg and 500 mg) were evaluated in phenylhydrazine induced anemic rats. Phenylhydrazine is used for the induction of haemolytic anemia and the study of its mechanism in rats¹⁰. Phenylhydrazine generates ROS within both human and rat erythrocytes; ROS production was associated with extensive binding of oxidized and denatured hemoglobin to the membrane cytoskeleton. Thus, phenylhydrazine-induced haemolytic injury seems to be derived from oxidative alterations to red blood cell proteins rather than to membrane lipids¹¹. Phenylhydrazine-induced anemia is a model for the study of hematinic effects⁶. In this present study, the hematological parameters of Phenylhydrazine (10mg/kg) administration experimental rats are significantly increased or decreased as compare to control. Hemoglobin levels, RBC count were significantly reduced in all phenylhydrazine treated anemic rat groups (Group 2 to 4) as compared to the normal control group. MCV level was increased in all anemic rat groups, whereas no significant change in MCH, MCHC level. This result indicated the development of hemolytic anemia after administration of phenylhydrazine for seven days. The result of one week treatment of *Dillenia indica* at three different dose level (150mg, 250mg and 500 mg) were indicated that phenylhydrazine induced oxidative alterations were reversed by the administration of *Dillenia indica* for one week. Among the three different doses studied, The *Dillenia indica* possesses an excellent effect on haemopoietic system on phenylhydrazine induced anemic rats. During the treatment period, the *Dillenia indica* significantly increased (hematinic related disorders since they possess both antioxidant and Hematoprotective potentials.

V. CONCLUSION

Dillenia indica is a medicinal plant having good antioxidant activity due to the presence of secondary metabolites. Due to high antioxidant activity *Dillenia indica* decrease the risk of anemia by reducing the oxidative stress and it also increase the hemoglobin concentrate and RBC count, in this it can be predicted that *Dillenia indica* showing good anti-anemic activity, but further studies required to use it as a candidate drug.

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