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Original article

Hematinic Effect of Hydro-Alcoholic Extract of *Persea americana* Miller Fruits in Phenylhydrazine Induced Anemic Rats

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ABSTRACT:
Objective: The aim of the present study is to evaluate the Hematinic Effect of hydro-alcoholic extract of *Persea american* Miller fruits against the Phenylhydrazine induced haemolyticanemia in rats. Methods: 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 B12 and group IV animals were treated with 300mg/kg, of hydro-alcoholic fruit extract of *Persea americana* Miller. All the test drugs were administered once daily for 28 days through oral route. On completion of the experimental period, the blood was collected with EDTA as an anticoagulant. Plasma was separated by centrifugation. Then Plasma was used for the estimation of various biochemical parameters like Haemoglobin, RBC and percentage Haematocrit. Results: Intervention with Hydro alcoholic extract of *Persea americana* significantly protected against the deteriorating effects of phenylhydrazine on the levels of RBCs, HGB, and HCT at each time-point of analysis, especially on day 7, the most critical time-point. There was significant ($P<0.001$) increase in RBC and Hb with both VitaminB12 and *Persea americana* fruit extract against phenylhyrazine challange. Also there was significant ($P<0.01$) increase in % HCT with both Vitamin B12 and *Persea americana* Miller fruit extract
Conclusion: The Hydro-alcholicextract of *Perseaamericana* Miller fruit exhibits Hematinic Effect against phenyhydrazine induced anemia in rats. The anti-anemic effect produced by the *Persea americana* Miller may be due to its high content of iron which is present in the plant.
Keywords: Anemia, Hematinic Effect, *Persea americana*, Haemoglobin, Hematocrit.

1. INTRODUCTION

Anaemia is one of the most common blood disorders afflicting people all over the world. Anemia is described as a loss in the blood's oxygen carrying capacity due to a drop in erythrocyte mass or haemoglobin concentration [1]. More than two billion people on the earth suffer from anemia [2]. It can appear at any stage of life, but it is more common in pregnant women and young children [3]. Due to the high incidence of malaria and other parasite illnesses in the tropics, anaemia is more common, which can result in a drop in circulating red blood cells or haemoglobin levels [4].The World Health Organization (WHO) classifies anaemia as a serious public health problem (prevalence > 40%) in 69 nations for children under the age of five and in 68 countries for pregnant women. According to the National Family Health Survey (NFHS3), anaemia affects 71 percent of people in the industrialised world, 84 percent of people in the developing world, and 79 percent of people worldwide [5]. Low iron levels are the leading cause of anaemia. Anemia, on the other hand, can be caused by a variety of factors, including malaria, parasite infection, dietary inadequacies, drug toxicity, and a genetic or acquired defect [6].For the treatment of anaemia, dietary modifications and iron supplements are often used. Oral iron therapy has a number of drawbacks, including inadequate absorption and poor compliance [7]. Furthermore, using large amounts of these iron supplements might result in major health problems, such as neurogenic illnesses or cancer [8]. All of these factors highlight the importance of having a safe and effective anaemia treatment option.

Medicinal plants have long been used to treat a variety of ailments, including anaemia. Many herbs are believed to be

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beneficial for anaemia in ancient medical systems such as Ayurveda [9]. Previous studies reported antianemic potentials of several Indian medicinal plants [10].

1.1. Objective

The fruits of *Persea americana*, was selected for the study of hematinic Effect as there are traditional claims to treat anaemia. Through the literature review it was found that the fruit of Persia Americana contains various phytoconstituents and minerals including Iron. Iron is required for the formation of Haemoglobin. The literature review shows that various works on the fruit.

1.2. Plant Profile

In many regions of the world, the fruit of *Persea americana* Miller of the Lauraceae family is consumed. Various components of the plants have been the focus of investigation in recent years. It has been proven that the fruit, in particular, has therapeutic benefits. The edible fruit pulp contains up to 33% oil rich in monounsaturated fatty acids [11], which are thought to alter fatty acid content in cardiac and renal membranes and improve absorption of /carotene and lutein [12]. A lower risk of cancer has been linked to the amount of carotenoids available in foods [13]. Wound healing [14] and hepatoprotection [15] are two more benefits of the oil. The seeds were subjected to a proximate analysis. Medicinal qualities have been reported in other portions of the plant. In animal studies, the aqueous leaf extract, has analgesic and anti-inflammatory [16], anticonvulsant [17], hypoglycaemic and hypocholesterolaemic [18], vasorelaxant and blood pressure lowering [19] activities.

2. MATERIALS AND METHODS

2.1. Plant material

The fruits of *Persea Americana* Miller were collected from the market in Indore in the month of February. Plant was identified by the Dr. S.N. Dwivedi, Prof. and Head, Department of Botany, Janta PG College, APS University, Rewa, M.P. and herbarium specimen was submitted in Department of Botany for future references. Plant authentication no. is JC/Bot./2020/PAF-073

2.2. Preparation of plant extract

The fruits of *Persea americana* Miller were cleaned, dried in the shade at room temperature, and then ground into a coarse powder. The powdered fruit material was placed in a sealed container with 70:30 ethanol and distilled water at room temperature for at least 7 days, with frequent agitation, until the soluble stuff had dissolved. After standing, the mixture is strained, the marc is crushed, and the combined liquids are clarified using filtration or decantation. In a rotary evaporator, the mixed extract was concentrated. For the in vivo experiments, the concentrated extract was used [20].

2.3. Animals

Animals Wistar strain male albino rats, weighing 100–150 g were selected for the study. The animals were taken from the animal house of Oriental College of Pharmacy and Research, Indore. The animals were housed individually in polypropylene cages under hygienic and standard environmental conditions (22 ± 3°C, humidity 30–70%, 12 h light/dark cycle). The animals were allowed to have standard feed and water ad libtum. They were acclimated to the environment for one week prior to experimental use [21]. All the animal testing was done under the approval of Institutional Animal Ethical Committee (IAEC) of Oriental College of Pharmacy and Research, Indore. The CPCSEA registration number is 1509/PO/RE/S/11/CPCSEA

2.4. Anti-anemic activity

Anemia was induced by intra peritoneal injection of phenyl hydrazine at 40 mg/kg for 2 days, following the injections; rats were divided into five groups of six rats each.

Group I-Control rats received 0.1% Carboxy methyl cellulose.

Group II-Phenyl hydrazine treated rats (40 mg/kg per day for 2 days).

Group III-Phenyl hydrazine treated rats with Vitamin B12 per day for 28 days.

Group IV-Phenyl hydrazine treated rats with a single dose of Hydroalcoholic extract of *Persea americana* Miller fruits (300 mg/kg) per day for 28 days.

On completion of the experimental period, the blood was collected with EDTA as an anticoagulant. Plasma was separated by centrifugation. Then Plasma was used for the estimation of various biochemical parameters like Haemoglobin, RBC and percentage Haematocrit [22, 23].

The dose of extract was selected on the basis of, the OECD guideline 420 (up & down) procedure was followed for the acute toxicity study [24].

3. STATISTICAL ANALYSIS

The results of the haematological estimations were presented as mean ± SD of six animals in each group. Total variations, present in a set of data were estimated by One Way Analysis Of Variance (ANOVA). P value of <0.05 was considered statistically significant.

4. RESULTS

Anti-anemic activity of *Persea americana* Miller extract on Phenylhydrazine induced hemolytic anemia in rats was studied and the results were shown on Table 1. The anti-anemic activity of *Persea americana* Miller 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 as shown in Table 1 and Fig 2, 3 and 4.

Table 1: Haematological parameters after the treatment with Hydro alcoholic extract of *Persea americana*, at the end of 28 days

S. No	Parameters	GROUP I	GROUP II	GROUP III	GROUP IV
1	RBC Count (10^6uL^{-1})	8.91±0.625	6.32±0.846	8.89±0.395***	7.68±0.639***

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2	Hemoglobin (gDL^{-1})	13.13 ± 0.31	9.23 ± 0.11	13.18±0.862***	12.46±0.248**
3	Hematocrit (%)	47.58±1.313	30.14±1.37	42.87±0.962**	40.62±0.527**

Data were expressed as Mean ± SEM (n=6) *P<0.05, ** P<0.01 and *** P<0.001 Vs Anemic Control

4.1. RBC Count

There is a significant decrease in RBC Count of wistar albino rats after the induction of Phenyl hydrazine on Third day in each anemic induced Group (II,III,IV)because of the destruction of RBC. The destruction of red blood cells is maybe due to the presence of oxidative stress .Group III receiving standard Drug Vit B 12 shows significant increase in RBC Count (8.89±0.395) as compared to disease control group (6.32±0.846) at the end of 28th day. Group IV receiving 300mg/Kg Hydroalcoholic extract of *Persea americana* Miller Fruits also shows the significant increase in RBC count (7.68±0.639) as compared to as compared to disease control group (6.32±0.846).

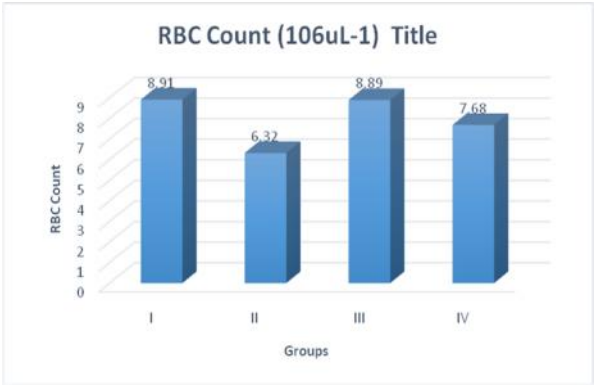

Fig 1: Estimation of the RBCs in rat blood in Phenyhydazine induced amemia

4.2. Hemoglobin (gDL^{-1})

There is a significant decrease in Hemoglobin (gDL^{-1}) of wistar albino rats after the induction of Phenyl hydrazine on Third day in each anemic induced Group (II, III, IV).Group III and IV receiving standard Drug Vit B 12 and Hydroalcoholic extract of *Persea americana* Miller Fruits shows significant increase in Hemoglobin (gDL^{-1}) (13.18±0.86) and (12.46±0.248) respectively as compared to disease control group (9.23 ± 0.11) at the end of 28th day.

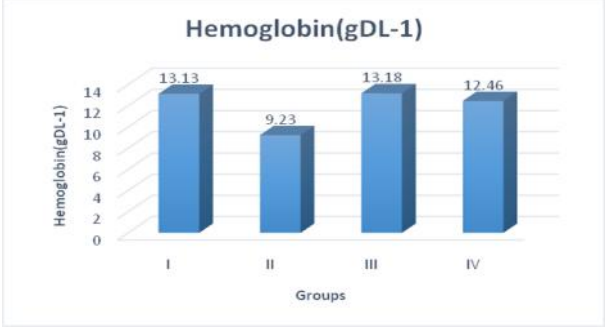

Fig 2: Estimation of the Hemoglobins in rat blood n Phenyhydazine induced anemia

4.3. Hematocrit (%)

It is also called as packed cell volume. It is the volume of packed red cells obtained after centrifugation of sample of anti-coagulated venous or capillary blood .There is a significant decrease in Hematocrit (%)of wistar albino rats after the induction of Phenyl hydrazine on Third day in each anemic induced Group (II,III,IV).Group III and IV receiving standard Drug Vit B 12 and Hydroalcoholic extract of *Persea americana* Miller Fruits shows significant increase in Hemoglobin (gDL^{-1}) (42.87±0.962) and (40.62±0.527) respectively as compared to disease control group (30.14±1.37) at the end of 28th day.

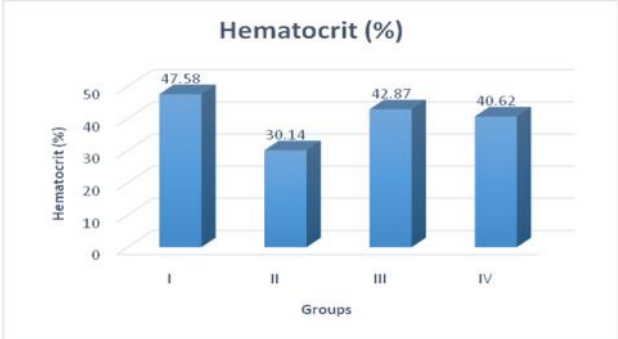

Fig 3: Estimation of the Hematocritin rat blood in Phenyhydazine induced anemia

5. DISCUSSION

Persea americana is traditionally used in ayurveda to treat fever, inflammation peptic ulcer. Analgesic, digestive, hair tonic diuretic, anti tussive, cardio protective, chemo preventive, free radical scavenging activity, antimutagenic, cancer, antidiabetic. Various parts of this plant have various applications in ethnomedicine, ranging from treatment of hypertension, diarrhea, dysentery, toothache, anemia, intestinal parasites to the area of skin treatment and beautification. In the present study, phenyl hydrazine intoxicated rats decrease hemoglobin levels, RBC, Haemocrit. The hemogram of the present study showed a significant reduction in RBC count, HGB concentration, and HCT values in rats on day's third post-injection of phenylhydrazine. Intervention with Hydro alcoholic extract of *Persea americana* significantly protected against the deteriorating effects of phenylhydrazine on the levels of RBCs, HGB, and HCT at each time-point of analysis, especially on day 7, the most critical time-point. The avocado fruit contains a lot of nutrients, and this includes its high content of essential minerals and vitamins. Several studies reported that *P. americana* is also a rich source of vitamins such as A, C, E, K, B1, B2, B6, B9 and minerals such as phosphorus, sodium, magnesium, potassium, iron and zinc, coenzyme Q10, beta-carotene, folic acid, lutein and zeaxanthin [25]. The anti-anemic effect produced by the *Persea Americana* Miller may be due to its high content of iron which is present in the plant.

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6. CONCLUSION

The Hydro-alcholic Fruit extract of *Persea americana* Miller exhibits anti-anemic activity against phenyhydrazine induced anemia in rats. The anti-anemic effect produced by the *Persea americana* Miller may be due to its high content of iron which is present in the plant. Further isolation and purification of bioactive compounds from *Persea americana* fruits may indicate the presence of a potent new anti-anaemic drug as well as the molecular mechanism behind its therapeutic effect.

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