

Antianaemic Potential of *Swertia chirata* on Phenylhydrazine Induced Reticulocytosis in Rats

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ABSTRACT

The antianemic potential of *Swertia chirata* extracts on phenylhydrazine induced anemia in rats was investigated. The ethanolic extract of *Swertia chirata* leaves is evaluated on anaemia model of rat induced by intraperitoneal injection of phenylhydrazine at 40 mg/kg for 2 days. Oral administration of this plant extracts at 200 mg/kg/day and 400 mg/kg/day, to the rats previously treated with phenylhydrazine, increased the concentration of haemoglobin, red blood cells number, haematocrit and reticulocytes rate.

Keywords: *Swertia chirata*, antianemic potential, Phenylhydrazine, Hemoglobin.

INTRODUCTION

Anaemia is a common blood disorder that affects people of all ages, although the people at greater risk are the elderly, young women of child-bearing age and the infants. This condition is not a disease but could develop as a result of various diseases. There are over 400 types of anaemia, many of which are rare but in all cases there is lower than normal number of circulating red blood cells¹. Anaemia is characterised by the decrease of the haemoglobin rate less than 13 g/dl in male or 12 g/dl in female². In the tropical area,

between 10 to 20% of the population presents less than 10 g/dl of haemoglobin.

Among the different species of *Swertia* reported in India, *Swertia chirata* is considered the most important for its medicinal properties. The plant extract have been reported to possess anti-inflammatory³, antipyretic⁴, anti-viral⁵ anthelmintic⁶, Anticarcinogenic⁷, hepatoprotective⁸ activities. Early studies documented the presence of flavonoids, xanthenes, terpenoids, iridoid and secoiridoid glycosides in the *Swertia chirata* plant⁹.

Traditionally the plant was in use as antipyretic medicines; therefore it developed interest for its evaluation for their not. antianemic property, whether it is having or not.

MATERIALS AND METHODS

Plant Collection

The aerial part of the plant *Swertia chirata* was collected locally, from the wild region Navegaon forest. The sample of plant was identified and authenticated by Dr. Jagannath Gadhpayle, Botanist, S. N. Mor College of Science, Tumsar, Bhandara [MS] India.

Extraction

Freshly collected aerial parts of the plant *Swertia chirata* were washed, shade dried under room temperature for a period of three weeks. The dried plant material was made to a coarse powder and weighed quantity of the powder (800 g) was subjected to hot percolation in a soxhelt apparatus using ethanol, at a temperature range of 40-80°C. The marc was completely dried and weighed. The extract (EESC) were concentrated to a dry mass by concentrating on water bath and keeping it in desiccators¹⁰.

Preliminary Phytochemical Test

The defatting is done by petroleum ether. Ethanolic extract obtained by the above methods from *Swertia chirata* (EESC) were subjected to qualitative test for the identification of various plant constituents by the standard procedures^{11,12}.

Experimental Animals

Swiss albino rats (150-200 g) were obtained from the animal house of Shree Farms, Nimgaon, Pahela, Bhandara, India [1231/b/08/CPCSEA] and used for present study. The animals were housed in groups of six per polypropylene cages and maintained at 24°C ± 1°C with the relative humidity of

45-55 % and 12:12 h dark light cycle. The experiments were carried out between 10:00 to 17:00 h. The animals had free access to food (standard chew pallets, Trimurti foods, Nagpur, India) and water ad libidum. The institutional animal ethics committee [928/ab/06/CPCSEA] of Manoharbai Patel Institute of Pharmacy, Kudwa, Gondia, (MS), India approved the Pharmacological and acute toxicity protocol.

Induction of Anaemia

Anaemia was induced in rats by daily oral administration of phenylhydrazine (PHZ) at 10 mg/kg for 8 days^{13,14}. Rats that developed anaemia with haemoglobin concentration lower than 13 g/dl were recruited for the study¹⁵.

Treatment of the Animals

The anaemic rats were randomly divided into 4 groups (6 rats per group) and treated daily for 4 weeks as follows¹⁶. The first group received Tween 20 (10 ml/kg) (negative control). The group 2 animals received Vit B12 syrup (Becosules Syrup, Pfizer Ltd., India, 50 ml) (1 ml/rat). Animals in groups 3 and 4 received the EESC at 200 and 400 mg/kg respectively. All administrations were by oral intubation.

Analysis of Haematological Parameters

Blood was collected by ocular puncture after overnight fast. The blood was collected before induction of anaemia, after induction of anaemia with PHZ and during 0, 1, 2, 3 and 4 weeks of treatments. The volume of blood collected (0.25 to 0.45 ml) did not affect blood parameters as earlier reported¹⁶. The red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (Hb) and haematocrit were determined at weeks 0, 1, 2, 3 and 4 by Semi Auto-Biochemistry Analyzer RX-50V.

Statistical Analysis

Experimental data were analyzed using one way analysis of variance (ANOVA) and LSD multiple range test to determine significant differences between means.

RESULTS

The phytochemical screening of EESC revealed abundance of resins, alkaloids, and glycosides, and trace amounts of saponins, terpenoids, and carbohydrate. The previous studies on acute toxicity testing of *Swertia Chirata* revealed no death up to doses of 5000 mg/kg¹⁵. In the control rats phenylhydrazine induced significant ($p < 0.5$) decrease in Hb concentration (42.12%), RBC (72.31%), WBC (54.76%) and haematocrit (50.7%), indicating anaemia^{16, 17}. The administration of the extract evoked a significant ($p < 0.5$) increase in the haematological parameters. The PHZ-induced anaemia was significantly ($p < 0.05$) reversed within 1 week of treatment with the extract, reaching maximum by the second week (Figures 1 - 2). In the control rats, the Hb for instance increased naturally and progressively from 6.89 ± 1.70 g/dl at day zero to 11.30 ± 1.01 g/dl at week 4. For 100 mg/kg extract-treated rats, the Hb increased from 6.53 ± 1.30 g/dl at day zero to 13.00 ± 1.23 g/dl (week 4). Similar positive and significant ($p < 0.05$) changes were recorded in the other haematological parameters and at the other doses of the extract (Figures 1 - 2). The effects of Vit. B12 syrup was comparable to those of the extract.

DISCUSSION

This study aimed to evaluate the effect of EESC on the haemolytic anaemia induced by phenylhydrazine in albino rats. It has been demonstrated previously that intraperitoneal administration of phenylhydrazine decreased haemoglobin concentration, red blood cells number and

haematocrit. Also Agbor and colleagues (2001) demonstrated that oral administration of 10 mg/kg phenylhydrazine for 8 days reduced haematological indices by 50%. In this study, PHZ altered the function of RBC by haemolysis characterized by 74.06% decrease in RBC, 48.17% decrease in Hb concentration, 55.24% decrease in WBC and 41.68% decrease in PCV. However, these parameters were restored to normal range after treatment with EESC suggesting that leaves of *Swertia chirata* have some haematinic effect. The results of this study indicated that the whole methanol extract of *Swertia chirata* increased significantly the concentration of haemoglobin, red blood cell count, white blood cell count and the packed cell volume mainly one week after of treatment. The increase in the blood indices was progressive giving the highest effect on the second week of treatment. Under normal condition, the body can generate new RBCs to replace the lost red cells; this will take much longer time as shown in this study. The recovery time of two weeks for untreated rats has earlier been reported when rats were bled 20% of their total blood volume to induce haemorrhagic anaemia¹⁸.

The increases in the haematological indices exhibited by *B Swertia chirata* extract might not be unconnected with the vitamin and mineral contents of the leaves of *Picrorhiza kurroa*. These constituents are well known haemopoietic factors that have direct influence on the production of blood in the bone marrow. Most importantly, the leaf extract appears safe for use since the LD50 of the ethanolic extract was greater than 5 g/kg.

CONCLUSION

The extracts of *Swertia chirata* leaves reversed anaemia induced by phenylhydrazine model of anaemia similar to those induced by parasite such as *Plasmodium falciparum*¹⁷. The vitamin and

mineral constituents of the leaf appear most likely as the active ingredients responsible for the haematinic effect of *Swertia chirata* leaves. This result supports at least partially the traditional use of *Swertia chirata* in the treatment of anaemia.

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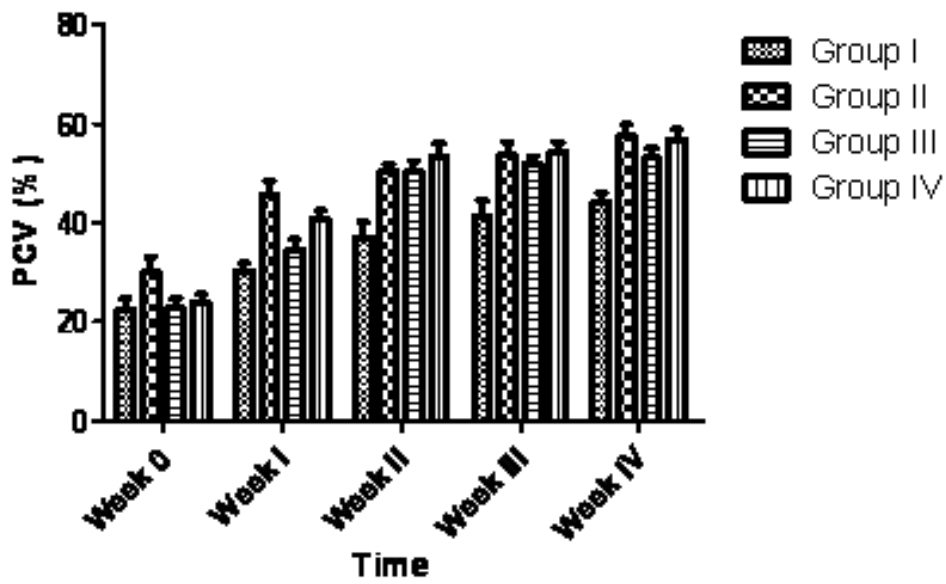


Figure.1. Effect of EESC on PCV after phenylhydrazine induced hemolysis in rats.

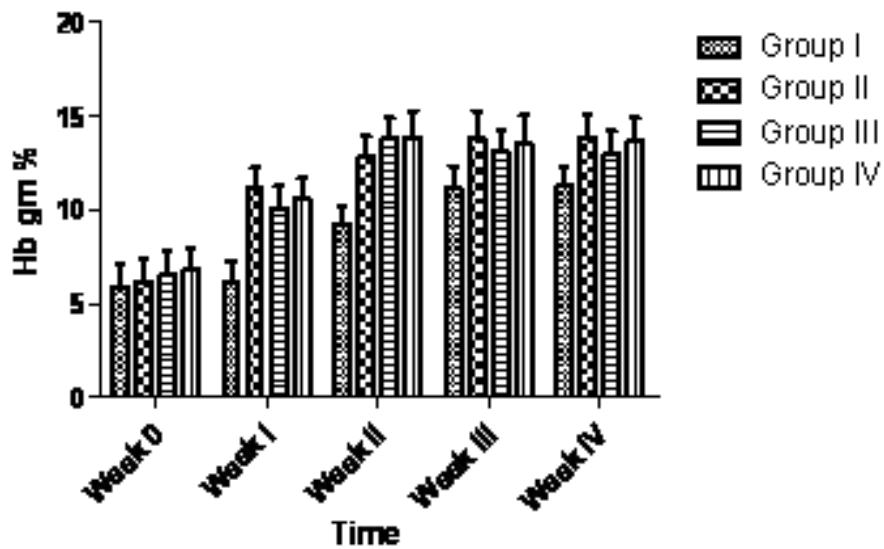


Figure.2. The Effect of EESC on Hb concentration after phenylhydrazine induced hemolysis in rats.