

**INHIBITORY POTENTIAL OF *PICRORHIZA KURROA* ROYLE EX. BENTH EXTRACTS ON PHENYLHYDRAZINE INDUCED RETICULOCYTOSIS IN RATS.**ASHISH TURASKAR\*<sup>1</sup>, SACHIN MORE<sup>1</sup>, RIZWAN SHEIKH<sup>1</sup>, J. GADHPAYLE<sup>2</sup>, DR. S. L. BHONGADE<sup>1</sup><sup>1</sup>Manoharbai Patel Institute of Pharmacy [B.Pharm.] Gondia [MS] India, <sup>2</sup>S. N. Mor College of Science, Tumsar, Bhandara [MS] India.  
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Received: 3 March 2013, Revised and Accepted: 22 March 2013

**ABSTRACT**

The antianemic potential of *Picrorhiza kurroa* extracts on phenylhydrazine induced anemia in rats was investigated. The ethanolic extract of *Picrorhiza kurroa* leaves is evaluated on anaemia model of rat induced by intraperitoneal injection of phenylhydrazine at 40 mg/kg for 2 days. Oral administration of these plant extracts at 100 mg/kg/day and 200 mg/kg/day, to the rats previously treated with phenylhydrazine, increased the concentration of haemoglobin, red blood cells number, haematocrit and reticulocytes rate.

**Keywords:** *Picrorhiza kurroa*, Reticulocytosis, Anemia, 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 cells [1]. Anaemia is characterised by the decrease of the haemoglobin rate less than 13 g/dl in male or 12 g/dl in female [2]. In the tropical area, between 10 to 20% of the population presents less than 10 g/dl of haemoglobin.

*Picrorhiza kurroa* (Scrophulariaceae) is an important herb in the traditional Ayurvedic system of medicine and has been used to treat liver and bronchial problems. Other traditional uses include treatment of dyspepsia (Similar to gentian in its bitter quality), bilious fever, chronic dysentery and scorpion sting. The most important active constituents of *Picrorhiza kurroa* are the cucurbitacin glycosides, apocynin, drosin, iridoid glycoside picrosides and kutkin [3,4]. *Picrorhiza kurroa* has hepatoprotective effect against Amanita poisoning [5], Carbon tetra chloride [6], and Aflatoxin B1 [7]. Bioactivity studies on *Picrorhiza kurroa* established its anti-inflammatory [8], immunomodulatory [9] and hydrocholeretic effects in rats and dogs [10] and antiviral activity on vaccinia virus [11].

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

**MATERIALS AND METHODS****Plant Collection and Extraction**

The dried powder of *Picrorhiza kurroa* was purchased from the local market parts and weighed quantity of the powder (800 g) was subjected to hot percolation in a Soxhlet apparatus using petroleum ether and ethanol, at a temperature range of 40-80°C. Before and after every extraction, the marc was completely dried and weighed. The extracts were concentrated to a dry mass by concentrating on water bath and keeping it in desiccators. [12]

**Preliminary Phytochemical Test**

The defatting is done by petroleum ether and ethanolic extract obtained by the above methods from *Picrorhiza kurroa* (EEPK) were subjected to qualitative test for the identification of various plant constituents by the standard procedures. [12, 13]

**Induction of anaemia**

Anaemia was induced in rats by daily oral administration of phenylhydrazine (PHZ) at 10 mg/kg for 8 days [14, 15]. Rats that developed anaemia with haemoglobin concentration lower than 13 g/dl were recruited for the study [16].

**Treatment of the animals**

The anaemic rats were randomly divided into 4 groups (6 rats per group) and treated daily for 4 weeks as follows [17]. The first group received Tween 20 (10 ml/kg) (negative control). The group 2 animals received Vit B12 syrup (Marketed Preparation) (1 ml/rat). Animals in groups 3 and 4 received the EEPK at 100 and 200 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 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 reported [18]. 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.

**Statistical analysis**

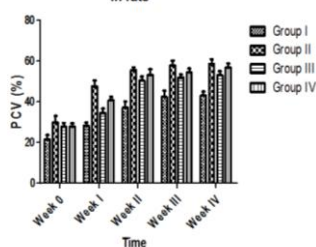
Experimental data were analyzed using one way analysis of variance (ANOVA) and LSD multiple range test to determine significant differences between means.

**RESULTS**

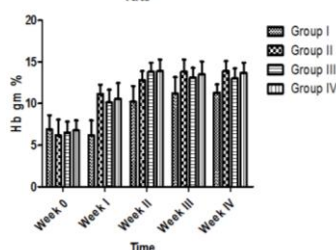
The phytochemical screening of EEPK revealed abundance of resins, alkaloids, and glycosides, and trace amounts of saponins, terpenoids, and carbohydrate. The acute toxicity testing revealed no death up to doses of 5000 mg/kg. In the control rats phenylhydrazine induced significant ( $p < 0.5$ ) decrease in Hb concentration (43.3%), RBC (71.1%), WBC (55.2%) and haematocrit (49.7%), indicating anaemia. 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 - 3). In the control rats, the Hb for instance increased naturally and progressively from  $6.89 \pm 1.70$  g/dl at day zero to  $11.30 \pm 1.01$  g/dl at week 4. For 100 mg/kg extract-treated rats, the Hb increased from  $6.53 \pm 1.30$  g/dl at day zero to  $13.00 \pm 1.23$  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 - 3). The effects of Vit. B12 syrup was comparable to those of the extract.

The effect of EEPK on Packed cell Volume after phenylhydrazine induced hemolysis in rats



The effect of EEPK on Hb concentration after phenylhydrazine induced hemolysis in rats



## DISCUSSION

This study aimed to evaluate the effect of EEPK 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 EEPK suggesting that leaves of *Picrorhiza kurroa* have some haematinic effect. The results of this study indicated that the whole methanol extract of *Picrorhiza kurroa* 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 anaemia [16].

The increases in the haematological indices exhibited by *B Picrorhiza kurroa* 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. In conclusion the extracts of *Picrorhiza kurroa* leaves reversed anaemia induced by phenylhydrazine model of anaemia similar to those induced by parasite such as *Plasmodium falciparum* [18]. The vitamin and mineral constituents of the leaf appear most likely as the active ingredients responsible for the haematinic effect of *Picrorhiza kurroa* leaves. This result supports at least partially the traditional use of *Picrorhiza kurroa* in the treatment of anaemia.

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