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Research Article

EVALUATION OF HEMATINIC ACTIVITY OF NAGA BHASMA IN PHENYLHYDRAZINE-INDUCED ANEMIA IN EXPERIMENTAL RATS

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ABSTRACT

Objective: *Bhasmas* are biologically produced nanoparticles prescribed with several other medicines of Ayurveda. Metal-based drugs are prepared by transmutation of base metals into noble ones along with the use of plant extracts meant to eradicate the toxic effects of metal. Naga bhasma (NB) is one of such metallic preparation used in various diseases such as diarrhea, spleen enlargement, and diabetes. The present study aims to test the hematinic activity of NB against phenylhydrazine (PHZ)-induced anemia in rats.

Methods: The experiment was carried on Wistar rats of either sex (150–200 g). Anemia was induced by an oral administration of PHZ 40 mg/kg for a period of 2 days. Hemoglobin (Hb) was measured on the 3rd day. Rats with Hb <11 g/dl were selected for the study. The animals were divided into six groups containing six animals each. Group I served as normal control, Group II as anemic control, and Group III was considered as standard that received ferrous sulfate (100 mg/kg), while Groups IV, V, and VI were treated with test NB 100, 200, and 400 mg/kg, respectively, for 15 days. Hb was checked on the 0, 2nd, 7th, and 15th days.

Results: Oral administration of PHZ decreased Hb from 14 g/dl at day 0 to 11 g/dl at day 2. NB induced a significant increase in Hb concentration to 13 at the dose of 100 mg/kg p.o. after 48 h.

Conclusion: PHZ decreased Hb rate inducing anemia. NB caused an increase in the Hb values in anemic rats. Thus, it can be concluded that NB exhibits significant hematinic activity in rats.

Keywords: Naga bhasma, Anemia, Phenylhydrazine.

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INTRODUCTION

According to the World Health Organization (WHO), anemia is defined as hemoglobin (Hb) levels <12.0 g/dL in women and <13.0 g/dL in men. However, normal Hb distribution varies not only with sex but also with ethnicity and physiological status [1]. The prevalence of anemia has been attributed to various aggravating factors such as poor nutrition, high prevalence of blood parasites, prolonged use of nonsteroidal anti-inflammatory drugs, and exposure to toxic chemicals. Due to the high prevalence and the possibility of even further increase, there is the need to prevent it or seek for more cost-effective and better treatment strategies [2]. The consequences of anemia included general body weakness, frequent tiredness, and lowered resistance to disease. Anemic condition, if not treated, may lead to serious problems in pregnant women such as premature delivery and low birth weight. It may also hamper the physical and mental development of children. There are several types of anemia, and all are characterized by a reduction in a number of circulating red blood cells (RBCs) and Hb [3]. Iron deficiency is the most common nutritional disorder in the world. It is a significant problem, especially in developing countries. It is widespread yet the most neglected micronutrient deficiency disorder among children, adolescence girls, and pregnant women (WHO, 2015). Iron deficiency may result in depletion of Hb and iron-dependent intracellular enzymes participating in many metabolic pathways [4]. Different alternative systems of medicines, including Ayurveda, make use of herbal preparations for their curative effects. *Bhasmikarana* is a process in which metallic herbal preparations called *Bhasma* is prepared. *Bhasmikarana* process converts the metal into its especially desired chemical compound that eliminates the toxicity of the metal and has the necessary medicinal benefits [5]. In ancient times, lead was used as pill and as well as liquid jade. When lead is subjected to a programmed heat treatment with herbal ingredients in a controlled atmosphere, the reaction between the herbal and lead leads to the formation of a herbometallic preparation, called Naga bhasma (NB). This herbometallic preparation has been used in treating diabetes, diarrhea, spleen, and skin disorders. NB has shown testis regenerative potential on partially degenerated testes. Clinical studies have proven the antidiabetic activity of NB [6,7]. The empirical use of incinerated preparations in the treatment of anemia dates from ancient times. These incinerated preparations are Lauha Bhasma, NB, and Abhrak Bhasma [8,9]. Thus, the present study was undertaken to assess the hematinic activity of NB.

METHODS

Animals

Wistar rats of either sex weighing 150–250 g were used for the study. The animals were procured from the animal house of Seth Govind Raghunath Sable College of Pharmacy, Saswad, Maharashtra, India. Protocol of the experiments and animal usage were discussed in the institutional ethical committee meeting and permission was obtained to carry out the parameters selected for the study. Animals were housed individually in polypropylene cages, maintained under standard conditions (12-h light and 12-h dark cycle; 25±5°C; 35–60% humidity), the animals were fed with standard pellet diet and provided water *ad libitum*.

Acute toxicity study

Acute oral toxicity was carried out according to OECD guidelines 425. Albino mice (female) weighing between 25 and 40 g were used. Above study was done at dose of 2000 mg/kg and 5000 mg/kg body weight.

Induction of anemia

Anemia was induced in rats by intraperitoneal administration of phenylhydrazine (PHZ) (60 mg/kg) daily for 2 days [9,10]. Rats that developed anemia with Hb concentration lower than 13 g/dl were recruited for the study.

The anemic rats were randomly divided into six groups with six animals each.

- Group 1: Received distilled water daily (normal control)
- Group 2: Received 1% sodium CMC daily (anemic control)
- Group 3: Received ferrous sulfate (standard 100 mg/kg)
- Group 4: Received an oral single dose of NB (100 mg/kg)
- Group 5: Received an oral single dose of NB (200 mg/kg)
- Group 6: Received an oral single dose of NB (400 mg/kg).

Analysis of hematological parameters

Blood was collected by retro-orbital puncture of experimental animals after an overnight fast. The blood was collected before induction of anemia and after induction of anemia with PHZ and after 1 and 2 weeks of treatment with NB. The RBC count, white blood cell (WBC) count, and Hb concentration were determined at weeks 1 and 2. The mean cell volume (MCV), mean cell hemoglobin (MCH), and the mean cell hemoglobin concentration Mean corpuscular hemoglobin concentration (MCHC) were determined.

Mean corpuscular hemoglobin (MCH)

This indicates the weight of Hb in a single RBC and is expressed in picograms (pg) (1 pg=10 g)

MCH=Hb (g/dL)/RBC count million/cumm

Mean corpuscular hemoglobin concentration (MCHC)

This denotes the Hb concentration per 100 ml of packed RBC and is related to the color of the red cells. This is expressed as the percentage of packed cells.

MCHC=Hb (g/dL)/PCV%×100.

This is expressed as the volume in cubic microns or femtoliters of an average RBC.

Mean corpuscular volume

MCV=PCV%/RBC count million per/cumm×10.

Statistical analysis

Experimental data were analyzed using analysis of variance and Dunnett's test to determine significant differences between means.

RESULTS

The present study was carried out to assess the antianemic activity of NB. The changes in the hematological parameters of the rats during the study are presented in Tables 1-3. Table 1 represents the effect of PHZ on the hematological parameters. The RBC and Hb of rats administered with PHZ decreased significantly ($p<0.05$) while the MCV and MCH were increased compared to that of the control group (Table 1). Table 2 represents the changes in hematological parameters after the treatment with NB. Group II PHZ intoxicated rats showed a significant (**$p<0.01$) decrease in the content of Hb and RBC when compared to a normal control Group I. In Groups 4, 5, and 6, a significant increase (**$p<0.01$) in RBC and Hb was seen compared to the anemic control (Table 2). Significant increase (**$p<0.01$) in MCV and MCH was observed in the anemic control compared to normal control. It was further attenuated in the NB (100, 200, and 400 mg/kg) treated group. The Hb and RBC of Groups 4, 5, and 6 reached normal values after 1 week of treatment (Table 2) with maximum level of increase in the 2nd week (Table 3). The Hb and RBC of Group 3 were comparable to that of Group 4 (Table 3).

Table 1: Effect of PHZ (40 mg/kg p.o. daily for 2 days) on hematological parameters

Parameters	Group 1 control	Group 2 anemic	Group 3 standard	Group 4 NB 100 mg/kg	Group 5 NB 200 mg/kg	Group 6 NB 400 mg/kg
Hb (g/dl)	17.6±0.29	10.68±0.19**	11.14±0.19**	10.88±0.21**	10.86±0.11**	10.92±0.10**
RBC (×10^6/µl)	7.6±0.05	4.42±0.12**	3.94±0.10**	4.02±0.07**	3.9±0.12**	3.98±0.14**
MCV (fl)	75.22±0.34	95.52±1.81**	99.72±2.05**	98.96±1.10**	99.14±0.59**	99.4±1.80**
MCH (pg)	25.26±0.28	40.12±0.70**	38.56±0.52**	39.66±0.77**	38.5±0.50*	36.5±0.98**
MCHC (g/dl)	31.24±0.40	30±0.44	30.58±0.19	30.12±0.45	30.22±0.53	30.1±0.43

Values are mean±SEM for six rats per group *$p<0.05$, **$p<0.01$. SEM: Standard error of the mean, Hb: Hemoglobin, RBCs: Red blood cells, MCV: Mean cell volume, MCH: Mean cell hemoglobin

Table 2: Hematological parameters of rats after week treatment with NB

Parameters	Group 1 control	Group 2 anemic	Group 3 standard	Group 4 NB 100 mg/kg	Group 5 NB 200 mg/kg	Group 6 NB 400 mg/kg
Hb (g/dl)	18.5±0.14	11.02±0.13**	14.16±0.57**	13.86±0.13**	13.52±0.15**	13.26±0.12**
RBC (×10^6/ul)	7.71±0.03	4.3±0.04**	5.6±0.2**	4.92±0.23**	4.72±0.12**	4.85±0.20**
MCV (fl)	75.34±0.36	90.08±1.06**	84.6±0.5**	87±0.7**	90.62±0.04**	91.02±0.44**
MCH (pg)	24.3±0.76	41.32±0.39**	28.4±0.24**	28.64±0.26**	30.66±1.1**	27.9±0.5**
MCHC (g/dl)	32.66±0.70	30.7±0.48	30.5±0.40	30.8±0.2	31.06±0.1	31.42±0.33

Values are mean±SEM for six rats per group *$p<0.05$, **$p<0.01$. SEM: Standard error of the mean, Hb: Hemoglobin, RBCs: Red blood cells, MCV: Mean cell volume, MCH: Mean cell hemoglobin

Table 3: Hematological parameters of rats after 2 weeks treatment with NB

Parameters	Group 1 control	Group 2 anemic	Group 3 standard	Group 4 NB 100 mg/kg	Group 5 NB 200 mg/kg	Group 6 NB 400 mg/kg
Hb (g/dl)	18.67±0.25	12.24±0.25**	16.74±0.31**	15.18±0.28**	14.58±0.22**	14.44±0.28**
RBC (×10^6/ul)	7.76±0.02	4.54±0.05**	6.24±0.11**	5.9±0.11**	5.02±0.11**	4.96±0.12**
MCV (fl)	75.02±0.31	93.46±0.55**	73.8±0.80**	76.6±0.50**	78.6±1.32**	79.2±1.15**
MCH (pg)	24.5±1.76	40.68±0.5**	25.24±0.61**	26.2±0.37*	28.3±0.20**	27.2±0.37**
MCHC (g/dl)	32.6±0.92	32.9±0.48	31.6±1.48	30.6±1.03	30±0.44	31.2±0.66

Values are mean±SEM for six rats per group *$p<0.05$, **$p<0.01$. SEM: Standard error of the mean, Hb: Hemoglobin, RBCs: Red blood cells, MCV: Mean cell volume, MCH: Mean cell hemoglobin

DISCUSSION

Anemia occurs when the body's iron stores become depleted accompanied by restricted supply of iron in various tissues. This results in depletion of Hb and iron-dependent intracellular enzymes that participate in many metabolic pathways. Thus, there is a need for proper management of iron deficiency [11]. PHZ derivatives were used first as antipyretics, but the toxic action on RBCs made their use dangerous. PHZ decreases Hb level, RBC concentration, and packed cell volume and impairs erythrocyte deformability. It induces reticulocytosis, increased osmotic resistance, free plasma Hb, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and erythropoietin levels, and extramedullary hematopoiesis in the spleen and liver [12]. Ayurveda and other Indian systems of medicine use metals, but their use is also amply described in Chinese and Egyptian civilization in 2500 B.C [13]. Art of preparing formulation requires certain expertise and very less ion formation is available about the likely impact of changes in the manufacturing techniques [14]. Herbomineral formulations are prepared by addition of *bhasmas* (ashes)*/rasayogas* (mineral preparations)*/*purified minerals and herbal drugs, and finally, they get triturated in *Khalva yantra,* that is, mortar and pestle. They are popular for quick action, low dosage, good palatability, and long-lasting stability. All these qualities of herbomineral preparations show significant importance from other dosage forms [15]. PHZ is recognized for its capacity to cause hemolysis both *in vitro* and *in vivo* by the formation of aryl and hydroxyl radicals. Oxidative stress in erythrocytes is considered as an important mechanism of hemolysis. Disruption of membrane integrity arises from fragility, dehydration, as well as increased production of reactive oxygen species. Chronic hemolysis leads to loss of Hb. These metabolic changes lead to the depletion of essential nutrients and micronutrients which are required for proper cell function. The accumulation of hydrogen peroxide in addition to the detoxifying capacity of the red cell may lead to the oxidation of essential cellular constituents including membrane phospholipids. Such alterations presumably contribute to the eventual hemolysis of affected cells [16]. Administration of rats with PHZ (40 mg/kg/day for 2 days) resulted in a marked hemolytic anemia characterized by decreased RBC and Hb.

This study was intended to evaluate the effect of NB on the hemolytic anemia induced by PHZ. Biomarkers of anemia are reduced Hb concentration (Hb), RBC count, and WBC. Mean corpuscular hemoglobin concentration and mean corpuscular volume are also increased in hemolytic anemia as the result of reticulocytosis. Mean corpuscular Hb is also increased in hemolytic anemia [17]. In our study, PHZ caused a decrease in Hb levels, RBC, and WBC whereas it increased MCV, MCH, and MCHC. Treatment with NB (100, 200, and 400 mg/kg) restored this altered hematological parameters.

CONCLUSION

The progressive recovery of anemic rats responding to the treatment of NB may be due to increased erythropoiesis. However, the mechanism of action by which NB produced its effect on increasing RBC and Hb in experimental animals needs to be investigated.

AUTHORS' CONTRIBUTIONS

Both the authors contributed equally in preparing, editing, and reviewing the article.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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