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Research Article

Effect of Poly-Herbal Extract in Treatment of Experimentally Induced Hemolytic Anemia in Rabbits

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ABSTRACT

Anemia is a condition in which the body's oxygen transport capacity is reduced due to a drop in Hemoglobin (Hb) levels in the blood. Considering the beneficial effects of moringa, garlic, and coriander, the present study was proposed to determine the beneficial effect of feeding polyherbal extract in treating induced hemolytic anemia in the rabbit model. These herbs contain Iron, Vitamin C, Folate and B12 for the production of hemoglobin in the body. Anemia was induced by giving Phenyl hydrazine injections intra-peritoneally (20 mg/kg B.wt). After induction of hemolytic anemia in 24 rabbits, three equal groups were made. Group A was given iron sucrose (Inj. Monofer® 5 ml/kg B.wt) as it helps to recover the anemia immediately. Group B rabbits were given orally polyherbal extract (5 ml/kg B.wt). Group C was left untreated. The efficacy of the treatments was measured by analyzing the concentrations of biochemical parameters, hematological parameters, and iron on the 1st, 7th, 15th, and 21st days of treatment. A two-way analysis of variance was used to analyze the collected data. Results revealed that the mean ± SE values of hematological parameters like Hb, RBCs, MCH, MCV, and Fe were significantly decreased ($P<0.01$) at Day 0 while the values of all these parameters were significantly increased ($P<0.01$) at Day 21, after treatment with polyherbal extract. Based on the results of this study, it was concluded that polyherbal extracts of *Moringa*, coriander, and garlic have excellent anti-anemic properties and significantly improved the hematological and biochemical parameters.

Keywords: Polyherbal extract; Anemia; Anti-anemic effects; Medicinal plants; *Moringa oleifera*

INTRODUCTION

Blood disorders are the most common systemic diseases now a day, and these kill a lot of people every year. Erythrocytes are destroyed prematurely and cause hemolytic anemia. Hereditary hemolytic anemias are caused by problems with red blood cell enzymes, membranes, and hemoglobin. Red Blood Cells (RBCs) are destructed before they reach their normal lifespan of 120 days, resulting in hemolytic anemia. Hemolytic anemia can be chronic or life-threatening, thus this is a very dangerous type of anemia. Direct cell death is caused by toxins, trauma, and lysis. Extrinsic factors cause RBCs to shear and rupture, leading to hemolysis fragmentation. When the cells' defense mechanisms become overworked, oxidative hemolysis occurs [1]. Blood transfusions have been the first-line treatment for anemia brought on by serious illness. As a result, RBCs are frequently given to critically ill animals who are in a life-threatening condition or who have already been infected. Transfusion, on the other hand, is linked to a number of well-known acute and chronic risks, which increase as the number of red blood cell units that have been given rises [2].

The cost of treating anemia with drugs is usually very high [3]. Nature has provided medicinal treatments and plant-based

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systems, which include a combination of more than one plant extract (poly-herbs), which play a vital role in improving health in 80 percent of the world's developing countries [4]. Garlic (*Allium sativum*) is an Alliaceae plant that is used as a medicinal plant, additive, and spice [5]. The antioxidant effects of Aged Garlic Extract (AGE) can decrease the risk of disease related to the cardiovascular system that's why it is helpful for treating anemia if related to cardiovascular dysfunction [6]. Non-heme iron can be present in both animal and plant sources, and it is used to treat anemia. 85% of the iron content in the diet comes from non-heme sources. Cereals, iron-fortified breads, coriander leaves, and spinach are all good sources of non-heme iron contents and can be used in the treatment of skin inflammation, menstrual disorders, anemia, high cholesterol levels, smallpox, blood sugar disorders, hemorrhoids and joint pain [7]. *Moringa oleifera* is high in nutrients such as carbohydrate, protein, beta carotene, fiber, vitamin C, and minerals such as iron, calcium, potassium, and phosphorous, strengthen the red blood cells in the body and are thus beneficial in the treatment of anemia [8].

The Commercial anti-anemic therapies and blood transfusions accompany with them many risks and poor outcomes. Herbal medicines are now attaining their place in the battle against different diseases. Considering all this scenario, the proposed study was planned with the objective to: Evaluate and compare the anti-anemic potential of a polyherbal preparation with commercially available iron preparations.

MATERIALS AND METHODS

Selection of experimental animals

Twenty-four adult and healthy rabbits were housed in the experimental animal house of department of clinical medicine and surgery at the University of Agriculture, Faisalabad. An appropriate environment, including light and humidity, was provided throughout the duration of the study. Water and food were supplied in accordance with standard nutritional requirements three equal groups of eight animals were formed after the rabbits had become accustomed to their surroundings

Poly-herbal extract preparation

Moringa, coriander, and garlic leaves were collected, then washed with distilled water, kept in the shade to dry, and grounded with the aid of pastel and mortar to make a powdered form. The powdered form of 700 mg moringa, 4 g garlic, and 3 g coriander [9-11] were mixed into 25 ml of ethanol at 100°C to perform soxhlet extraction. The method was run for six hours, and then the solvent was evaporated in a hot air oven at 40°C to get the extract. The extract was then stored in a labeled sterile bottle and kept in the refrigerator.

Induction of anemia

Phenyl hydrazine injections were given through the intraperitoneal route, at the dose rate of 20 mg/kg three times at different days intervals (days 1,3, and 5) to induce anemia. Induction of anemia was confirmed before the start of treatment through a complete blood count analysis.

Experimental plan

Three groups were formed by allocating 8 rabbits in each group. Blood samples were c prior to the induction of anemia in rabbits. Following that, injection Monofer® (ferric derisomaltose) as administered to the 1st group once daily at a dose rate of 5 ml per kg. Second group received 5 ml polyherbal extract/kg on daily basis. Third group was kept as control group and didn't receive any treatment.

Blood sampling

Antiseptic gauze was used to clean the area and 5 ml blood samples were collected from the jugular vein in EDTA tube. Samples were kept at room temperature, to induce clotting. Then it was centrifuged at 25000 RPM for 10 minutes to separate serum. Serum samples were then stored in the refrigerator at -20°C till further analysis.

Methodology of serum and blood analysis

The following parameters were used to assess the efficacy of treatment.

- Biochemical analysis (ALT, AST, Total Bilirubin and GGT)
- Hematological Parameters (RBCs, HB concentration, MCH and MCV)
- Iron Test

Sample collection

- Collected blood from rabbit ear vein.
- Used EDTA tubes for whole blood and serum separator tubes for serum.
- Labelled samples with rabbit ID and date.

Preparation and handling

- Gently mixed EDTA tubes.
- Allowed serum separator tubes to clot for 30 mins, then centrifuge.
- Transferred serum to labeled tubes.
- Stored samples at -20°C.

Basic measurements

- Measured hemoglobin using hematology analyzer.
- Assessed hematocrit for packed red blood cells.
- Performed CBC for blood components.

Serum analysis

- Used clinical chemistry analyzer for electrolytes, enzymes, lipids, glucose, etc.
- Employed ELISA for protein or hormone quantification.

Blood and serum analysis

- Analyzed results against reference ranges.
- Compared to previous data.
- Generate comprehensive report.

Statistical analysis

Analysis of Variance (ANOVA): ANOVA is a statistical technique used to compare the means of two or more groups to determine if there are any statistically significant differences among them. It assesses the variation between group means and within-group variability. ANOVA calculates an F-statistic, which is the ratio of between-group variability to within-group variability. If the F-statistic is significant, it suggests that at least one group mean differs significantly from the others.

Least Significant Difference (LSD): LSD is a post hoc test often used after performing an ANOVA to determine which specific group means are significantly different from each other. When the ANOVA indicates a significant difference, the LSD test helps identify which groups are responsible for that difference. It calculates the minimum difference required between means for them to be considered significantly different.

Data was analyzed by using the Analysis of Variance (ANOVA) technique to assess the potential differences among multiple groups under observation. Post hoc testing was performed using the Least Significant Difference (LSD) method. The software used for these analyses was SPSS version 20.0 (Heinisch, 1962). This technique was applied for the purpose of creating graphs illustrating the statistical comparisons

RESULTS

The blood samples were collected at different intervals to evaluate and compare the anti-anemic potential of a polyherbal preparation with the commercially available iron preparations.

Biochemical analysis

Alanine Aminotransferase (ALT) (IU/L): In terms of ALT, the values significantly ($p<0.05$) increased after induction of anemia than baseline (Figure1). There was a decreasing trend in the values of ALT after administration of allocated treatments to the respective groups throughout the duration of study. The values of ALT were statistically significant ($p<0.05$) at each sampling point compared to previous in all three groups (Figure1). After completion of the study period, provision of allocated treatments led to a decrease of these values towards baseline *via* 67.6 ± 0.754, 72.2 ± 0.583, and 118.9 ± 0.984 for groups A, B, and C respectively.

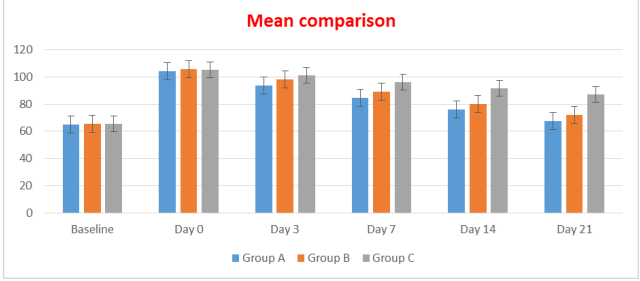

Figure 1: Effect of poly-herbal extract administration at dose (5 ml/kg) on serum ALT (IU/L; Mean ± S.E) on different days in rabbits suffering from phenyl-hydrazine induced anemia. **Note:** ( ): Group A treated with Inj. Monofer®; ( ): Group B treated with poly-herbal extract; ( ): Group C was without any treatment.

Aspartate Aminotransferase (AST) (IU/L): In terms of AST, the values significantly ($p<0.05$) increased after induction of anemia than baseline (Figure 2).

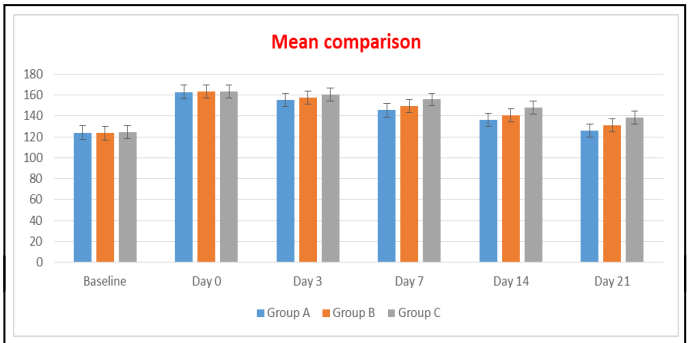

Figure 2: Effect of poly-herbal extract administration at dose (5 ml/kg) on serum AST (IU/L; Mean ± S.E) on different days in rabbits suffering from phenyl-hydrazine induced anemia. **Note:** ( ): Group A treated with Inj. Monofer®; ( ): Group B treated with poly-herbal extract; ( ): Group C was without any treatment.

There was decreasing trend in the values of AST after administration of allocated treatments to the respective groups throughout the duration of study. The values of AST were statistically significant ($p<0.05$) at the end of study period in treatment groups when compared with start of treatment (Figure2). After completion of the study period, provision of allocated treatments led to a decrease of these values towards baseline *via* 126.1 ± 0.892, 130.9 ± 1.042, and 159.7 ± 1.053 for groups A, B, and C respectively (Table 1).

S.no	Groups name	Treatment
1	Group A	(Inj. Monofer®) 5 ml for 1 kg body weight, single dose a day.
2	Group B	Treated with poly-herbal extract 5 ml for 1 kg body weight, single dose a day.
3	Group C	No treatment.

Table 1: Treatment protocol for each group.

Hematological parameters

Hemoglobin concentration (Hb) (g/dL): In terms of Hb, the values significantly ($p<0.05$) decreased after induction of anemia than baseline (Figure 3). There was an increasing trend in the values of Hb after administration of allocated treatments to the respective groups throughout the duration of study. The values of Hb were statistically significant ($p<0.05$) at the end of the study period in the treated groups when compared with the start of treatment (Figure 3). After completion of the study period, provision of allocated treatments led to an increase of these values towards baseline *via* 9.1 ± 0.432, 8.5 ± 0.873, and 6.4 ± 1.422 for groups A, B, and C respectively.

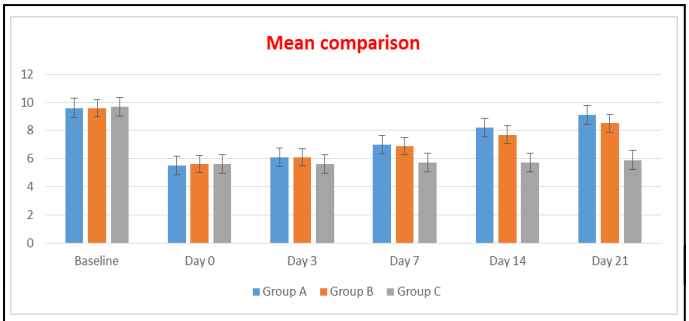

Figure 3: Effect of poly-herbal extract administration at dose (5 ml/kg) on blood Hb (g/dL; Mean ± S.E) on different days in rabbits suffering from phenyl-hydrazine induced anemia. **Note:** (■): Group A treated with Inj. Monofer®; (■): Group B treated with poly-herbal extract; (■): Group C was without any treatment.

Red Blood Cells concentration (RBC) (×10^6): In terms of RBC's, the values significantly ($p<0.05$) decreased after induction of anemia than baseline (Figure 4). There was an increasing trend in the values of RBC's after administration of allocated treatments to the respective groups throughout the duration of study. The values of RBC's were statistically significant ($p<0.05$) at the end of the study period in the treated groups when compared with the start of treatment (Figure 4). After completion of the study period, provision of allocated treatments led to an increase of these values towards baseline, *via* 4.1 ± 0.892, 3.9 ± 0.743, and 2.9 ± 0.583 for groups A, B, and C respectively.

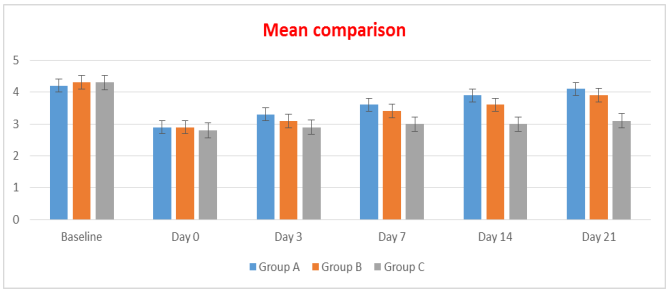

Figure 4: Effect of poly-herbal extract administration at dose (5ml/kg) on blood RBC (x106; Mean ± S.E) on different days in rabbits suffering from phenyl-hydrazine induced anemia. **Note:** (■): Group A treated with Inj. Monofer®; (■): Group B treated with poly-herbal extract; (■): Group C was without any treatment.

Iron concentration (Fe) (ug/dL)

In terms of Fe, the values significantly ($p<0.05$) decreased after induction of anemia than baseline (Figure 5). There was an increasing trend in the values of Fe after administration of allocated treatments to the respective groups throughout the duration of study. The values of Fe were statistically significant ($p<0.05$) at the end of the study period in the treated groups (Figure 5). After completion of the study period, provision of allocated treatments led to an increase of these values towards baseline, *via* 28.9 ± 0.872, 27.2 ± 0.872, and 13.3 ± 0.743 for groups A, B, and C respectively.

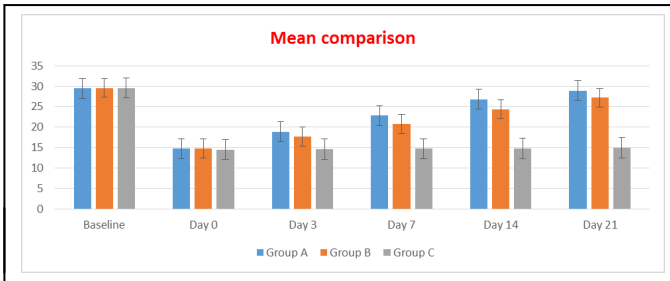

Figure 5: Effect of poly-herbal extract administration at dose (5ml/kg) on serum Fe (ug/dL; Mean ± S.E) on different days in rabbits suffering from phenyl-hydrazine induced anemia. **Note:** (■): Group A treated with Inj. Monofer®; (■): Group B treated with poly-herbal extract; (■): Group C was without any treatment.

DISCUSSION

Blood disorders are the most common systemic diseases now a day, and kill a lot of people every year. Chronic or acute anemia, reticulocytosis, or jaundice is all symptoms of hemolysis. Infection, autoimmunity, and microangiopathy are all commonly acquired causes of hemolytic anemia. Hereditary hemolytic anemias are caused by problems with red blood cell enzymes, membranes, and hemoglobin [12]. Antibody-mediated hemolysis can happen intra-vascularly or extra-vascularly, resulting in phagocytosis or complement-mediated destruction. Direct cell death is caused by toxins, trauma, and lysis. Extrinsic factors cause RBCs to shear and rupture, leading to hemolysis fragmentation. When the cells' defense mechanisms become overworked, oxidative hemolysis occurs [1].

Blood transfusions have been the first-line treatment for anemia, but they have been always costly for poor people [2]. Poly-herbs play essential role in the improvement of health in 80% of the world's population, especially in developing countries [4]. The antioxidant effects of Aged Garlic Extract (AGE) and other components that is present in AGE, such as fructosyl arginine and S-Allylcysteine (SAC). The leaves of the M. *olifera* tree strengthen the red blood cells in the body and are thus beneficial in the treatment of anemia [8].

The present study showed that the values of ALT were significantly ($p<0.05$) increased after the induction of anemia than baseline. There was a decreasing trend in the values of ALT after administration of allocated treatments to the respective groups throughout the duration of study. Similar results were reported by [1] in which anemia was induced by phenyl-hydrazine injection. Four groups of rats received A. Saralicum at a concentration of 25, 50, 100, and 200 mg/kg. After the completion of trial, samples of blood and liver were collected to evaluate the parameters. Results showed that there was a clear decrease in the total conjugated bilirubin, ALT, and other serum biochemical markers. This study justified that A. sacralicum leaf aqueous extract has anti-anemic properties. Another similar research was elaborated by [13] in which *Solanum aethiopicum* extract decreased the level of Alanine Transaminase (ALT), the findings of this study revealed that the use of *Solanum aethiopicum* leaves was a very safe anti-anemic tonic with the ability to protect the liver and kidneys.

In terms of the values of Hemoglobin (Hb), a significant ($p<0.05$) decrease after induction of anemia than baseline was observed. There was an increasing trend in the values of Hb after administration of allocated treatments to the respective groups throughout the duration of study. These findings are in broad agreement with the findings of [14] that used Raktavardhak Kadha (RK) as medicine in the treatment of induced anemia. Anemia was induced by phenyl-hydrazine. After induction of anemia, polyherbal preparation of RK was given to the animal models, which significantly improved the values of Hb altered by the induction of anemia.

In the present study, the values of iron significantly ($p<0.05$) decreased after induction of anemia than baseline. There was an increasing trend in the values of Fe after administration of allocated treatments to the respective groups throughout the duration of study. These findings are in line with the reports of [15] who have reported that sobo is a medicinal plant and contains a high amount of iron. It contains 30-35 mg/kg iron and that is very soluble. Due to high solubility its availability is maximum in the body. A similar study was designed by [16] in which they elaborated that iron deficiency anemia is the most common form of malnutrition-related anemia worldwide. Supplements containing *Moringa* leaf water extract can help to cure anemia due to iron shortage. Another similar research was done in which a diet supplemented with 5% tested herbs and 1% iron gluconate was given to five experimental groups for 4 weeks. The results showed a 5% improvement in the case of tested herbs. Results showed that if chamomile and mint powder are given after ginger powder, then iron absorption in the body can increase. Ultimately it increases the total bioavailability of iron in the daily diet thus it could be very beneficial for the treatment of anemic patients [13].

CONCLUSION

Based on the findings of the current study, one can very confidently conclude that poly-herbal extract of *Moringa*, coriander and garlic have excellent anti-anemic properties. Keeping in view the seriousness of the problem, the widespread issue of anemia and the scarcity of resources in developing countries like Pakistan, this extract can be used as effective therapeutic agent against anemia. External validation of the result by reciprocating the trials on a large scale and different spp. including the human population is first desired.

DECLARATIONS

Author contributions

- Conceptualization: Misbah ijaz
- Data curation: Muhammad ali haider
- Formal analysis: Haider ali
- Funding acquisition: Misbah ijaz
- Investigation: Muhammad ali haider
- Methodology: Muhammad ali haider
- Project administration: Misbah ijaz
- Resources: Misbah ijaz
- Software: Haider ali
- Supervision: name; Misbah ijaz
- Validation: Muhammad ali haider
- Visualization: Misbah ijaz
- Writing - original draft: Haider ali
- Writing - review & editing: Misbah ijaz.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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