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STUDIES ON AMARANTHUS CRUENTUS'S ETHANOLIC HERBAL EXTRACT'S ANTIANEMIC PROPERTIES AGAINST RATS' PHENYLHYDRAZINE-INDUCED ANEMIA

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Abstract:
Blood is an essential fluid that sustains life, self-possessed of plasma, erythroid cells , leukocyte cells ,and platelets, each performing crucial physiological functions. RBCs, containing hemoglobin, are vital for oxygen transport, while WBCs defend against infections, and platelets facilitate clotting. Anemia, a condition characterized by decreased hemoglobin levels or RBCs, impairs oxygen delivery and can arise from various causes, including nutritional deficiencies and genetic disorders. This study investigates the antianemic properties of Amaranthus cruentus, commonly known as blood amaranth, using a phenylhydrazine-induced rat model of anemia. The plant, rich in nutrients and traditionally used for its health benefits, was subjected to Soxhlet extraction with ethanol, yielding a 14% extract. Anemia was induce in albino rats through ip route by using phenylhydrazine injections (40 mg/kg) over two repeated days. Parameter such as RBC count, hemoglobin (Hb) concentration, and hematocrit (HCT) levels were monitored alongside histopathological analyses of key organs. Results demonstrated that Amaranthus cruentus extract significantly improved RBC count, Hb levels, and HCT values compare to the anemic control group, suggesting its potential to mitigate oxidative stress-induced hemolysis. The extract's rich phytochemical profile, including flavonoids, tannins, and phenolic compounds, likely contributes to its antioxidant activity and therapeutic efficacy against anemia. Furthermore, the presence of iron in the extract supports its application in treating iron-deficiency anemia. This research highlights the promising antianemic effects of Amaranthus cruentus, suggesting its potential as a natural remedy for anemia and reinforcing its significance in both nutritional and medicinal contexts.

Keywords: Blood, haemoglobin, anemia, Amaranthus cruentus.

Introduction

Blood is a vital fluid that supports life, composed of several key components and performing essential functions in the body. The liquid portion, plasma, makes up about 55% of blood and consists mainly of water, electrolytes, proteins (such as albumin and fibrinogen), hormones, gases, and waste products. Erythrocytes (RBCs), which account for 30-55% of blood which, carry oxygen throughout body. White blood cells (WBCs), construction up less than 1.2% of blood, are integral to the immune system, protecting against infections. Platelets are crucial for blood clotting and the body's response to injury. Blood performs several critical functions, including transporting oxygen from the lungs to body tissues, delivering nutrients from digestion to cells for energy and growth, and carrying metabolic waste to excretory organs for elimination. It also plays a key role in the immune response, with WBCs defending the body against pathogens and infections, and helps maintain body temperature by regulating heat distribution. Beyond its physiological role, blood holds significant cultural and symbolic meanings, often representing life, kinship, and connection. It is depicted in literature as a symbol of passion, violence, and sacrifice. Understanding blood's complex composition and functions deepens our appreciation for its essential role in sustaining life and the intricate connections it fosters among

individuals and cultures. However, disorders like anemia, characterized by a decrease in RBCs or hemoglobin leading to fatigue and weakness, highlight its importance; causes of anemia can include nutritional deficiencies and genetic conditions, with treatment options ranging from dietary supplements to blood transfusions. In summary, blood is essential not only for maintaining bodily functions but also for its profound cultural significance throughout history.

Anemia is a condition marked by reduced hemoglobin levels or a decrease in red blood cells (RBCs), impairing oxygen transport to tissues. Common types comprise iron deficiency anemia, cause by inadequate iron for hemoglobin; vitamin B12 deficiency anemia, leading to macrocytic anemia and neurological symptoms; folate deficiency anemia, resulting from inadequate intake; hemolytic anemia, characterized by premature RBC destruction; aplastic anemia, a rare bone marrow failure condition; thalassemia, an inherited disorder affecting hemoglobin synthesis; and sickle cell disease, which causes sickled RBCs and complications like vaso-occlusive crises. Diagnosis involves clinical evaluation and laboratory tests, with treatments tailored to the underlying cause.

Amaranthus cruentus, commonly known as blood amaranth or crimson amaranth, is an annual flowering plant belonging to the Amaranthaceae family. Native to Central and South America, it is cultivated worldwide for its edible leaves and seeds, as well as its ornamental appeal. This herbaceous plant can grow up to two meters tall and features lanceolate to ovate leaves that vary in color from green to reddish-purple, topped with small, clustered green flowers. Amaranthus cruentus thrives in warm climates, requiring well-drained soil and full sun. The young leaves are nutritious and can be used in salads or cooked dishes, while the seeds, gluten-free and rich in protein, are often prepared like grains. Additionally, its striking red flowers enhance garden aesthetics, and some parts are used in traditional medicine, though scientific support is limited. Overall, Amaranthus cruentus is a versatile plant valued for its culinary, nutritional, and decorative benefits.

Method and Methodology

Collection and Authentication

Dried leaves of Amaranthus cruentus were wash, desiccated in dark, and ground hooked on a coarse fine particles using a mechanical chopper.

Preparation of Plant Extract

The crushed leaves were extract using a Soxhlet extractor with ethanol as the solvent. After extraction, the ethanol was evaporated to concentrate the extract, which was then stored in the refrigerator for future use.

Animal Study

Wistar albino rats (200–250 g, either sex) be obtained from the NIBS, Pune, India. The rats be acclimatized for ten days in an approved animal home under normal conditions, house in polypropylene cage by way of a 12-hour light/dark cycle, and maintained at 25 ± 0.5 °C and 50 ± 5% humidity. They were feed a pattern pellet diet from Nutrivet Life Sciences, Pune, and had unrestricted access to water.

Preliminary Phytochemical Examination and Iron

Estimation

Phytochemical screening was performed using conventional techniques to identify various phytoconstituents. The iron content of the extract was measured using ICP-OES.

Induction of Anemia

Anemia was induce in the rats in the course of IP route injections of phenylhydrazine at 40 mg/kg for 2 consecutive day, characterized with a 30% reduction in RBC cell count and HG concentration.

Experimental Design

The rats were divided into five groups of six:

- Group I: Normal control (no treatment).
- Group II: Anemic control (phenylhydrazine).
- Group III & IV: the extract at low doses and high dose of 200 and 400 mg/kg, respectively, twice daily.
- Group V: Received Livogen XT (9 mg iron/kg, twice daily) as a standard treatment.

Blood samples were collected on days 0, 3, 7, 10, and 15 to measure RBC count, Hb concentration, and HCT levels. Histopathological analysis of vital organs was conducted on the last day of the study.

Results and Discussions

Extraction of Plant Material

Leaves of Amaranthus cruentus were collect, washed, and shade-dried. The dried Rhizomes were weighed out after being crumpled into a powder. Soxhlet extraction with ethanol was used to remove coarse particles. It was discovered that the product's yield was 14% w/w.

PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF AMARANTHUS CRUENTUS

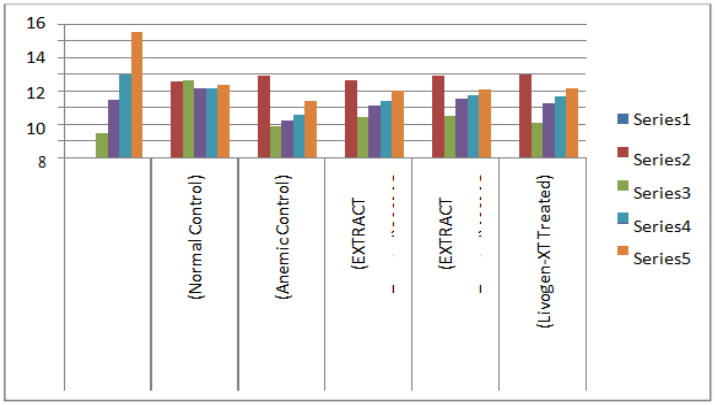
Table 01: Chemical Constituents Presence/absence

Sl.No:	Constituents	Presence/absence
1	Phenol	+
2	Alkaloids	-
3	Flavonoids	+
4	Tannins	+
5	Carbohydrate	+
6	Saponin	+
7	Cardiacglycosides	+
8	Proteins	-
9	Fatsandoils	-
10	Steroids	+
11	Aminoacids	-

Table 02: Effect of EXTRACT on blood levels of RBCs

Days	Group-I	Group-II	Group-III	Group-III	Group-IV
			200MG	400MG	liv-xt
RBCs count (10^6/µl)					
0	9.28 ± 0.26	9.96 ± 0.17	9.31 ± 0.19	9.81 ± 0.19	9.93 ± 0.38
3	9.35 ± 0.50	3.80 ± 0.17a***	4.87 ± 0.16	5.07 ± 0.16	4.15 ± 0.16
7	8.30 ± 0.32	4.48 ± 0.29a***	6.23 ± 0.27b**	7.03 ± 0.37b**	6.44 ± 0.34b***
10	8.43 ± 0.33	5.12 ± 0.48a***	6.95 ± 0.33b**	7.50 ± 0.23b**	7.35 ± 0.34b***
15	8.75 ± 0.22	6.95 ± 0.38a***	8.13 ± 0.28b**	8.13± 0.37b**	8.26 ± 0.19b***

Values are expressed as mean±SEM, n=6, ?p<0.05, ??p<0.01,??? p<0.001. [a] vs. Normal control, [b]vs. Anemic control. SEM=Standard error of mean. RBCs-Reb blood cells,



Graph 01: Effect of EXTRACT on blood levels of RBCs

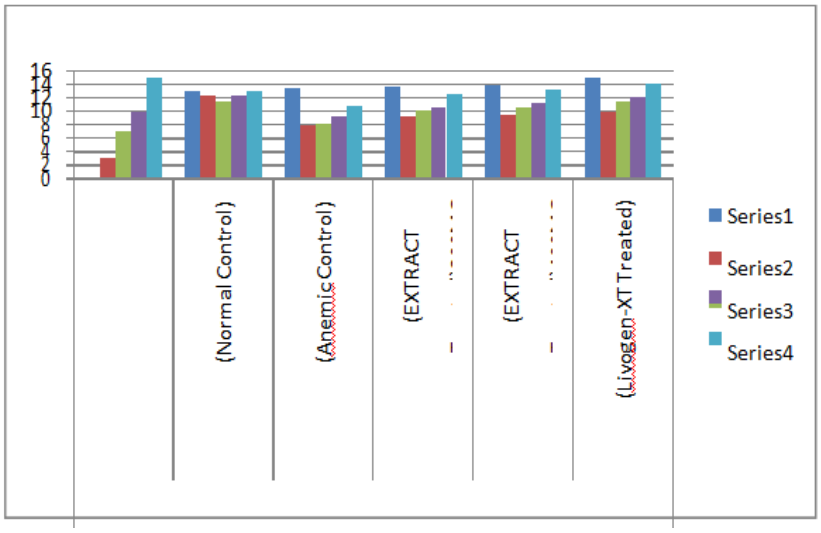
Table 03 shows that during the experimental period, the injection of phenylhydrazine, which induces anaemia, ($p < 0.001$) decrease the count of RBC. The administration of Livogen XT ($p < 0.001$) and both significantly restored this drop in RBC count ($p < 0.01$). In this context, it was discovered that Livogen XToutperformed extract.

Table 03: Effect of EXTRACT on blood levels of Haemoglobin Content

Days	Group-I	Group-II	Group-III	Group-III	Group-IV
			200 MG	400 MG	Livo-XT
Hg content (g/dl)					
0	12.13 ± 0.21	13.60 ± 0.22	13.71 ± 0.39	14.15 ± 0.38	15.05 ± 0.36

3	12.40 ± 0.95	8.01 ± 0.48a**	9.25 ± 0.48	9.52 ± 0.37	9.92 ± 0.37
7	11.59 ± 0.26	8.22 ± 0.33a***	10.15 ± 0.44b**	10.56 ± 0.37b**	11.56 ± 0.37b**
10	13.43 ± 0.24	9.36 ± 0.28a***	10.70 ± 0.18b**	11.35 ± 0.20b***	13.35 ± 0.20b***
15	14.13 ± 0.26	10.75 ± 0.21a**	12.87 ± 0.40b**	13.45 ± 0.29b***	14.25 ± 0.28b***

Values are expressed as mean±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001. [a] vs. Normal control, [b]vs. Anemic control. SEM=Standard error of mean.



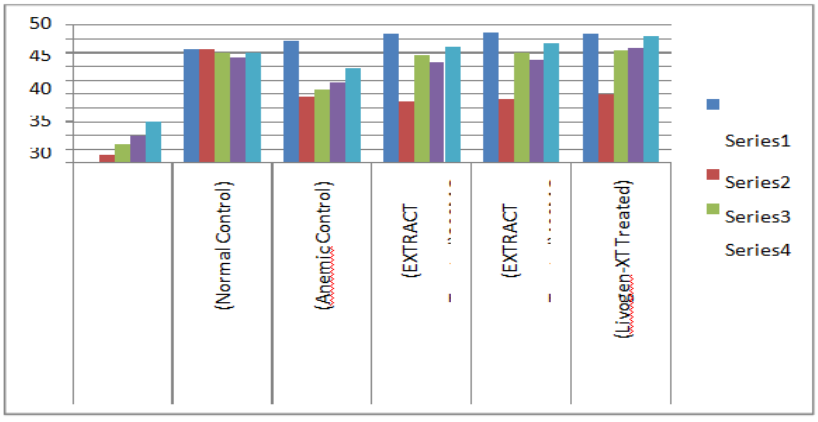
Graph 02: Effect of EXTRACT on blood levels of Haemoglobin Content

During the study period, the Hb content significantly decreased ($p < 0.001$) due to the administration of phenylhydrazine (Table 02). Both extract ($p < 0.01$) and Livogen XT ($p < 0.001$) therapy effectively restored this reduction in Hb content. When it came to raising the Hb content of anaemic rats, Livogen XT was shown to be more efficient than Extract (Table 03).

Table 04: Effect of EXTRACT on blood levels of Haemoglobin Content:

Days	Group-I	Group-II	Group-III	Group-III	Group-IV
			200MG	400MG	Livo-XT
Hg content (g/dl)					
0	12.13 ± 0.21	13.60 ± 0.22	13.71 ± 0.39	14.15 ± 0.38	15.05 ± 0.36
3	12.40 ± 0.95	8.01 ± 0.48a**	9.25 ± 0.48	9.52 ± 0.37	9.92 ± 0.37
7	11.59 ± 0.26	8.22 ± 0.33a***	10.15 ± 0.44b**	10.56 ± 0.37b**	11.56 ± 0.37b**
10	13.43 ± 0.24	9.36 ± 0.28a***	10.70 ± 0.18b**	11.35 ± 0.20b***	13.35 ± 0.20b***
15	14.13 ± 0.26	10.75 ± 0.21a**	12.87 ± 0.40b**	13.45 ± 0.29b***	14.25 ± 0.28b***

Values are expressed as mean±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001. [a] vs. Normal control, [b] vs. Anemic control. SEM=Standard error of mean.



Graph 03: Effect of EXTRACT on blood levels of Haemoglobin Content

Histological Studies

Rats with anemia-induced liver tissue had small, localised hepatocyte degenerative alterations along the central vein (Fig. 1-B). Hepatocytes were found to have focal regions of cellular enlargement, larger nuclei, and granular cytoplasmic alterations. A small number of the hepatocytes surrounding the principal vein also showed focal vacuolar alterations. Extract and Livogen XT treatment stopped these histological alterations and returned the liver's normal histomorphological characteristics (Fig. 1-C and D).

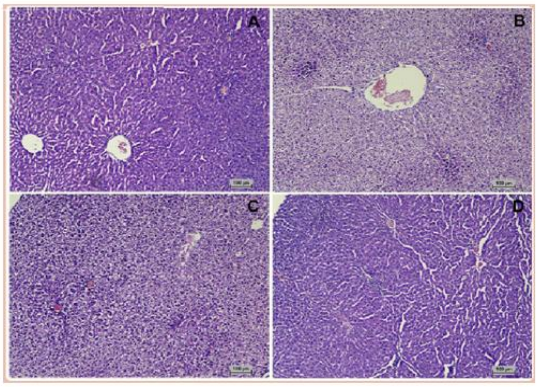


Fig 01

Representative liver slice pictures from animals in the (A) normal control group demonstrate the normal architecture of the liver tissue; in the (B) anaemic control group, the hepatocytes around the central vein have minor and focused alterations due to degeneration. Hepatocytes exhibited focal regions of cellular enlargement, accompanied by granular cytoplasmic alterations and an expanded nucleus in the (C) extract treated group and (D) Livogen XT treated group (H and E × 100). The injection of phenylhydrazine, which induces anaemia, results in modest degenerative alterations in the cardiac muscle that are focused and produce dilatation of the cardiac fibres (Fig. 2-B). Extract and Livogen XT treatment stopped these histological alterations and returned the cardiac muscles' normal histomorphological characteristics (Fig. 2-C and D).

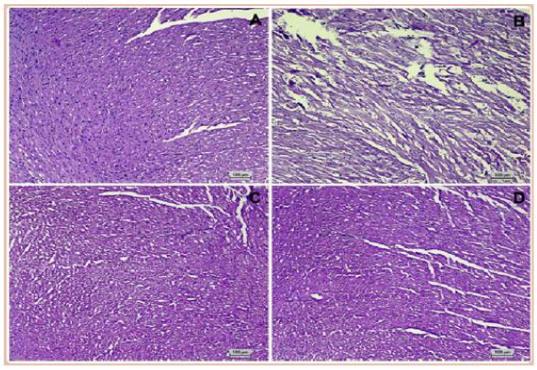


Fig 02

Figures A and B depict the representative heart section images from animals with normal control, anaemic control, extract treated, and Livogen XT treated hearts, respectively. The normal architecture of heart tissue with cardiac muscle fibres in the myocardium is shown in (A) the heart section images. (H and E × 100). In normal control animals, the spleen's proper architecture, red and white pulp characteristics, and sufficient lymphoid cell population were seen (Fig. 3-A). The splenic parenchyma's cellular population was focally depleted as a result of phenylhydrazine administration-induced anaemia (Fig. 3-B). Extract and Livogen XT therapy stopped this histological alteration (Fig. 3-C and D).

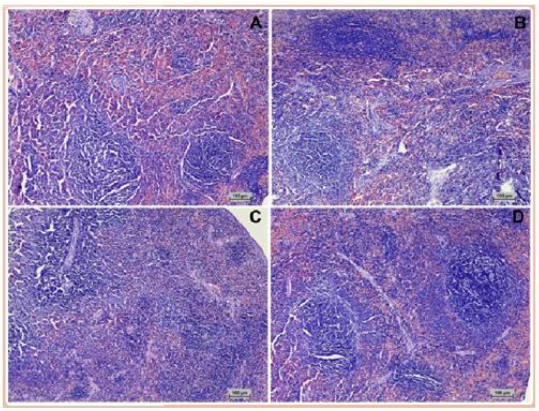


Fig 03

The phenylhydrazine rat model is a commonly usedapproach due to its rapidity, reliability, and ease of use. One of the species that is most frequently employed in biological research is the wistar albino rat. Rats have long been employed in experimental research to help learn more about illnesses, genetics, pharmacological effects, and other relevant areas of medicine and health.

Therefore, in order to test the antianemic efficacy of extract, we have used the rat model of phenylhydrazine-induced anaemia.

Erythropenia, decreased haemoglobin, and decreased HCT levels inthe blood are the hallmarks of phenylhydrazine-induced anaemia .According to earlier studies, rats given intraperitoneal injections of phenylhydrazine at a dosage of 40 mg/kg for two days in a row may become anaemic. In order to induce anaemia in rats, we thus chose to administer a 40 mg/kg dosage of phenylhydrazine intraperitoneally for two days in a row [10].

For eight to twelve days, the erythropenia brought on by phenylhydrazine decreased Hb and HCT levels. Moreover, phenylhydrazine causes pathological alterations in the kidney, liver, spleen, and heart. Thus, we assessed RBC, Hb, and HCT

levels during the experimental period as well as histological analyses of the main organs at the conclusion of the experimental period in order to screen the antianemic potential of extract.

The cellular makeup of blood is made up of RBCs. RBCs are primarily responsible for delivering oxygen to the body's tissues. The function of RBCs is altered in some pathological diseases, such as anaemia, which may be detrimental to the body's regular functioning. The administration of phenylhydrazine during the experimental period in this investigation resulted in a decrease in the number of red blood cells, is in line with previous results. This declinetherapy with extract resulted in an improvement in RBC count. Through oxidative stress, phenylhydrazine selectively destroys developed RBCs. Therefore, extract capacity to stop phenylhydrazine- induced hemolysis may be the reason for its positive effect on improving RBC count.

Because low haemoglobin levels reduce blood's ability to transport oxygen, haemoglobin is a key screening measure for antianemic medications. According to previous publications, the administration of phenylhydrazine during the experimental period resulted in a reduction in the Hb level of the blood in the current investigation. extract therapy improved this decrease in blood Hb concentration. Anaemia is said to be caused by phenolhydrazine by oxidative denaturation of haemoglobin, which is sparked by free radicals. Consequently, the observed impact of extract to increase blood haemoglobin concentration may be due to its antioxidant capability.

The ratio of the volume of packed red blood cells to the amount of total blood is called the packed cell volume, or HCT. A low HCT suggests that a patient is anaemic. This study's findings are in line with previous research as it found that phenylhydrazine-induced hemolysis significantly reduced HCT in anaemic control rats. RK therapy was able to reverse this drop in HCT. This could be the result of extract ability to defend against hemolysis brought on by phenylhydrazine.

According to a histopathological analysis, the liver, heart, spleen, and bone marrow tissues all had moderately graded pathological alterations as a result of the phenylhydrazine-induced anaemia. Extract therapy reversed these pathological alterations and brought these tissues' histomorphological characteristics back to a nearly normal state. The powerful antianemic potential of extract might be the cause of the observed effect.

According to reports, phenylhydrazine causes reactive oxygen species to develop more often, which in turn damages red blood cells. On the other hand, flavonoids can either reverse or prevent this damage to red blood cells due to their strong antioxidant properties. RK's phytochemical analysis revealed the presence of tannins, phenolic compounds, flavonoids, steroids, and saponins. Therefore, it would seem that the reported antianemic effect of extract might be due to the presence of flavonoids or other active principles.

The presence of iron in the extract was shown by quantitative iron quantification. As a result, extract is helpful in treating anaemia of the iron-deficiency kind. Extract iron content and its capacity to stop hemolytic anaemia caused by phenylhydrazine indicate that it is effective against both iron deficiency and hemolytic anaemia.

Conclusion

It has been determined that rats given intraperitoneal phenylhydrazine injections at a dosage of 40 mg/kg for 2 consecutive days experience anaemia. Extract countered the reduction in RBCs, Hb, and HCT levels brought on by phenylhydrazine. Moreover, it undid pathogenic alterations in the bone marrow, liver, heart, and spleen tissues. As a result, Extract significantly inhibits anaemia caused by phenylhydrazine in rats. An iron estimate revealed that Extract has iron, which is helpful in preventing iron-deficiency anaemia. Extract hence has a protective effect against hemolytic anaemia and iron deficient anaemia. To clarify the precise mechanism of action of the reported antianemic activity, more research on the quantitative measurement of phytoconstituents and in vivo assessments are required.

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