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Evaluation of Anti-Anemic Activity of *Mangifera indica Linn.* Leaves against Phenyl Hydrazine Induced Anemia in Wistar Albino Rats

Sivaranjini.S*, Sakthi Abirami.M

Department of Pharmacology, College of Pharmacy, Madras Medical College, Chennai-03, Tamilnadu, India.

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ABSTRACT

BACKGROUND: Over 2 billion people suffer from anemia worldwide. Most people in developing countries use nutritional supplements and traditional therapies to cure their anemia. **AIM AND OBJECTIVE:** To perform live Rat models of anemia produced by phenyl hydrazine are sensitive to the anti-anemic effects of a leaf extract made from ethanol from Mangifera indica. **RESOURCES AND TECHNIQUES:** Thirty adult rats with albinism were selected, six of which were considered standard controls. PHZ was administered intraperitoneally to the remaining 24 rats, resulting in anemia. Four groups were randomly selected based on hemoglobin (Hb) estimate after 24 rats were discovered to be anemic following two days of PHZ administration. For 14 days, Groups IV and V were given an ethanolic extract from the leaves of Mangifera indica, whereas the positive and negative controls were Groups II and III. **RESULT:** The quantity of hemoglobin concentration, hemocrit and red blood cells (RBCs) percentage all significantly decreased in rats that received PHZ injection, indicating hemolytic anemia. Interestingly, treatment with an extract of Mangifera indica leaves in ethanol significantly reversed the declining effects of PHZ on RBCs, HGB, and HCT. Additionally, Mangifera indica leaves treatment significantly stopped the decreases in serum levels of superoxide dismutase activity and total glutathione. **CONCLUSION :**Thus, the study's findings suggested that the leaves of Mangifera indica is a useful treatment for human anemia.

KEYWORDS: Phenyl hydrazine, anemia, Mangifera indica, Hematological and antioxidant characteristics.

INTRODUCTION:

Anemia is a disorder where the body does not have enough red blood cells (RBCs) or their ability to carry oxygen to meet its needs. Every single one of the more than 400 types of anemia—many of which are rare-involve circulating RBS's that are below normal. Hemolytic anemia, sickle cell anemia, thalassaemia, aplastic anemia, sideroblastic anemia, pernicious anemia, megaloblastic anemia, and iron deficient anemia are only a few among the various forms of anemia. Globally, iron deficiency is believed to be the most prevalent cause of anemia.[1]

Anemia may be caused acquired or hereditary factors. Other problems includes hereditary diseases, chronic inflammation, vitamin A, folate and vitamin B12 deficiencies, in addition to lack of access to balanced diet can also cause anemia.[2]

When anemia develops, the body either generates too few red blood cells or destroys too many of them. Anemia caused by iron deficiency has a major impact on India's health, well-being, and social and economic impact.[3]

Anemia during pregnancy is the absence of enough healthy RBC's in the blood to provide oxygen to tissues and the growing fetus. A folate deficiency can be directly connected to birth deformities such low birth weight and spina bifida, which are neural tube abnormalities. Iron, folic acid, and vitamin deficient anemia can be treated with dietary changes and vitamin, folic acid, and iron supplements.

For thousands of years, people have used plants or their herbs as medicine to cure conditions including colds, fevers, and pains. The great majority of folks in rural areas rely largely on the usage of herbal treatments, and there are several benefits to utilizing these medications. Because herbal treatment is non-invasive and non-toxic, it is usable safely as a different kind of treatment or in combination with traditional therapies. Anemia has historically been treated with a variety of vegetation including dates, grapes, beetroot, broccoli, honey, and pomegranates.[4]

Many substances, such as flavonoids and phenols, are known to have haematinic properties.[5] The leaves of mangifera indica were used as an external remedy in baths or washes to prevent scabies and syphilis, in addition to genito-urinary inflammations, stomachaches, bronchitis, and asthma. The soft leaves were dried, ground into a powder, and used to treat diabetes. The leaves' smoke was also believed to aid in the treatment of some throat illnesses. Leave ashes were utilized for scalds and burns. In cases of aphonia, or voice loss, a leaf decoction with a small amount of honey was administered. [6]

Therefore, this study aims to determine the haematinic and antioxidant qualities of the foliage of the Mangifera indica Linn.

MATERIALS AND METHODS

Healthy rats were kept in the house of animals at Madras Medical College for three months before the study started.

COLLECTION OF PLANT MATERIAL

In May 2024, the plant Mangifera indica substance leaves were collected in Tamil Nadu's Chennai area.

PREPARATION OF ETHANOLIC EXTRACT

The extract of ethanol was made available using Soxhlet extraction. Approximately 450g of powdered plant materials were employed for the extraction procedure. Following the usage of petroleum ether for the defatting process, ethanol was utilized for the extraction of chemical ingredients. The goods were collected and dried for the purpose of the experiment.

EXPERIMENTAL ANIMALS

This study used thirty mature albino Wister rats of either sex and weighing between 100 to 200 g. The animals were allowed a week to acclimate to the research lab prior to the trial commencing. The standard conditions for the animals' housing were a temperature of 25°C±3, a humidity range of 35–60%, and a 12-hour light/dark cycle. Every animal had unlimited access to food and water. Madras Medical College in Chennai's Institutional Animal Ethics Committee formally approved the study's procedures. For the study, the CPCSEA guidelines were adhered.

ACUTE TOXICITY STUDIES

The acute toxicity studies have already been done by the author Sharma S.R *et.al* in the investigation of hypoglycaemic potential of *Mangifera indica* leaves under the OECD guidelines. The plant is safe up to 4640 mg/kg. Hence 1/20th and 1/10thof the maximum dose administered (i.e., 200 mg/kg and 400 mg/kg) is selected for the present study.[7]

EXPERIMENTAL DESIGN

Table 1: Grouping of animals

NUMBER OF GROUPS	GROUP NAME	INDUCING PERIOD	TREATMENT PERIOD	NO. OF ANIMALS
Group I	Vehicle Control	Treatment with vehicle (Normal saline)	Treatment with vehicle (Normal saline) from day 3 to day 14	6
Group II	Disease control	40 mg/kg phenyl hydrazine from day 1 to day 2	Treatment with vehicle (Normal saline) from day 3 to day 14	6
Group III	Standard control	40 mg/kg phenyl hydrazine from day 1 to day 2	Treatment with Iron supplements from day 3 to day 14	6
Group IV	Test group 1 - Low dose	40 mg/kg phenyl hydrazine from day 1 to day 2	Treatment with 200 mg/kg of extract P.O from day 3 to day 14 (test dose 1)	6
Group V	Test group 2 - High dose	40 mg/kg phenyl hydrazine from day 1 to day 2	Treatment with 400 mg/kg of extract P.O from day 3 to day 14 (test dose 2)	6
			Total no. of animals	30

ANALYSIS OF HEMATOLOGICAL PARAMETERS

A complete blood sample was collected from the lateral tail vein under anesthesia after a 14-day course of therapy. The number, shape, size, and color of the RBC's all reveal the blood's quality. Samples were placed in a tube containing ethylenediaminetetraacetic acid following 2 weeks of treatment. The red blood cells count, Hb, haematocrit (PCV), MVC, mean corpuscular Hb (MCH), and MCH concentration (MCHC) were measured at day 14 using an automated blood cell counter. Antioxidant potential, such as glutathione (GSH) and superoxide dismutase (SOD), was also tested using the traditional method.[8, 9]

STATISTICAL ANALYSIS

The SEM (standard error of mean) ± mean was utilized to express the results. Graph Pad Prism, version 10.3.1, will be utilized to analyze all data using the analysis of variance in one direction test (ANOVA) and Dunnett's multiple comparison test, with a significance threshold of $P < 0.05$, $P < 0.01$ and $P < 0.001$ vs control.

RESULT

The following tables and graphs show the outcomes of two different ethanolic extracts of leaves of Mangifera indica (200 mg/kg and 400 mg/kg) on the analysis of haematological parameters against the reference.

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Table 2: Haematological and antioxidant parameters after the treatment with leaf extracts of ethanol of Mangifera indica., 14 days later.

S. No.	Parameters	Normal control	Disease control	Standard	Treatment 1 (Low dose)	Treatment 2 (High dose)
1.	RBC Count (million/cu.mm)	8.40 ± 0.61	4.39 ± 0.87	8.09 ± 1.10	6.92 ± 0.98	7.59 ± 0.51
2.	Haemoglobin (g/dl)	15.82 ± 0.96	8.40 ± 0.59	14.83 ± 0.90	11.12 ± 1.50	13.21 ± 1.96
3.	Packed Cell Volume (%)	41.69 ± 3.12	30.18 ± 2.91	43.98 ± 3.12	37.91 ± 2.58	39.99 ± 2.64
4.	Mean Cell Volume (fl)	61.01 ± 1.63	56.96 ± 6.98	58.61 ± 6.91	57.21 ± 8.3	57.88 ± 1.32
5.	Mean Cell Haemoglobin (pg)	18.99 ± 2.13	17.02± 1.12	17.61 ± 1.23	17.30 ± 2.11	17.61 ± 1.01
6.	Mean Cell Haemoglobin Concentration (g/dL)	33.01 ± 3.4	29.91 ± 1.26	30.39 ± 1.39	30.11 ± 1.63	30.89 ± 3.03
7.	Superoxide Dismutase	9.48 ± 0.57	5.71 ± 0.61	7.68 ± 0.47	6.51 ± 0.85	7.32 ± 0.91
8.	Glutathione	15.31 ± 0.31	10.96 ± 0.38	13.33± 0.63	11.89 ± 0.37	13.12 ± 0.67

Standard deviation ± Mean is used to express values (n=6)

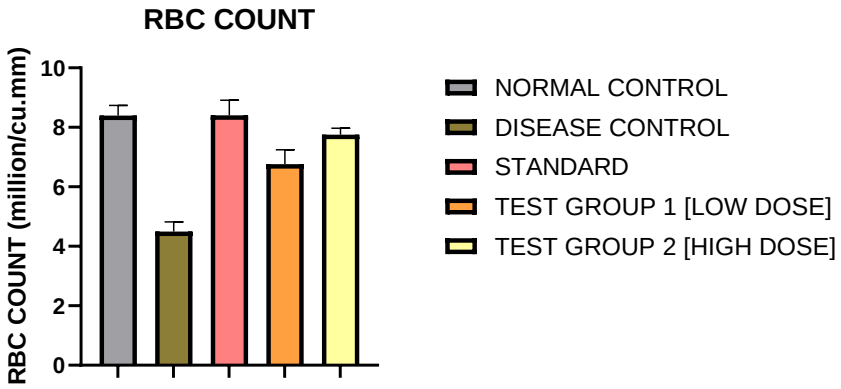

Fig 1: Graphical representation of changes in RBC count

The Standard deviation ± Mean is used to express values; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using One way Analysis of Variance (ANOVA) with Graph pad prism 10.3.1 software.

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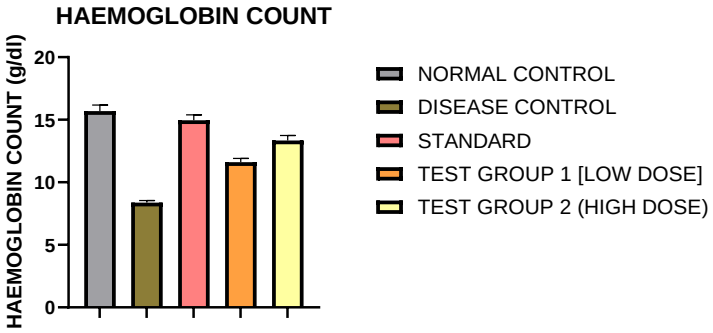

Fig 2: Graphical representation of changes in Haemoglobin

The Standard deviation ± Mean is used to express values; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using Analysis of Variance in One Way (ANOVA) with Graph pad prism 10.3.1 software.

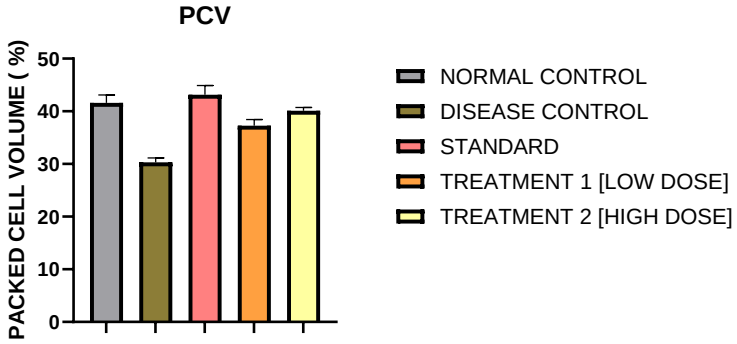

Fig 3: Graphical representation of changes in Packed Cell Volume (PCV)

The Standard deviation ± Mean is used to express values; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using Analysis of Variance in One Way (ANOVA) with Graph pad prism 10.3.1 software.

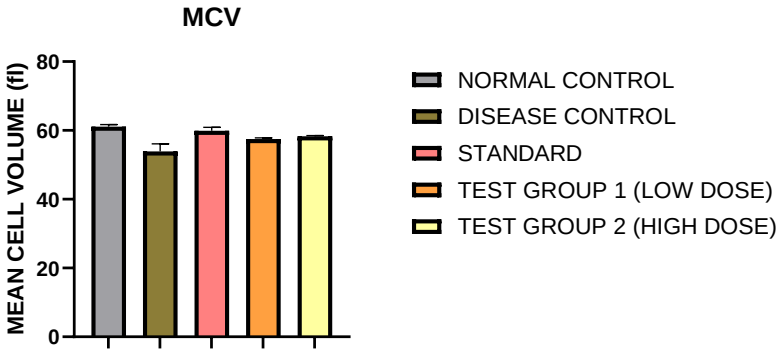

Fig 4: Graphical representation of changes in Mean Cell Volume (MCV)

The Standard deviation ± Mean is used to express values; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using Analysis of Variance in One Way (ANOVA) with Graph pad prism 10.3.1 software.

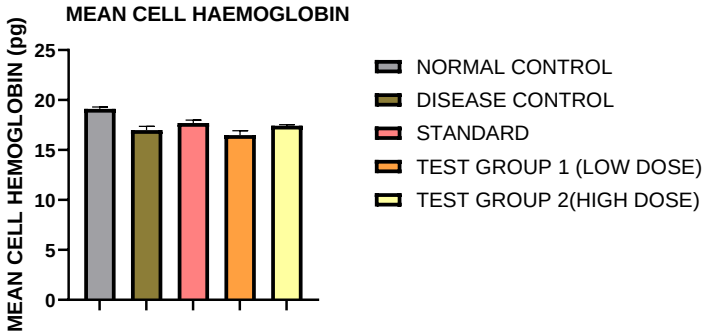

Fig 5: Graphical representation of changes in Mean Cell Haemoglobin

The Standard deviation ± Mean is used to express values; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using Analysis of Variance in One Way (ANOVA) with Graph pad prism 10.3.1 software.

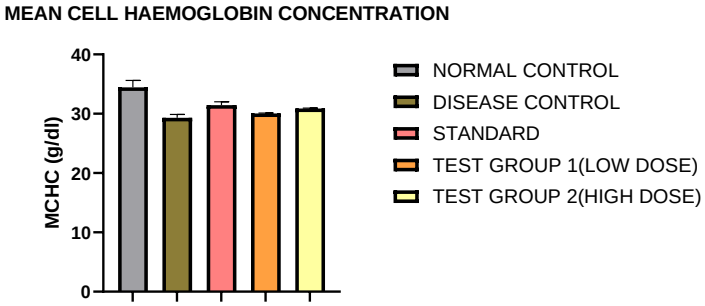

Fig 6: Graphical representation of changes in Mean Cell Haemoglobin Concentration (MCHC)

The Standard deviation ± Mean is used to express values; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using Analysis of Variance in One Way (ANOVA) with Graph pad prism 10.3.1 software.

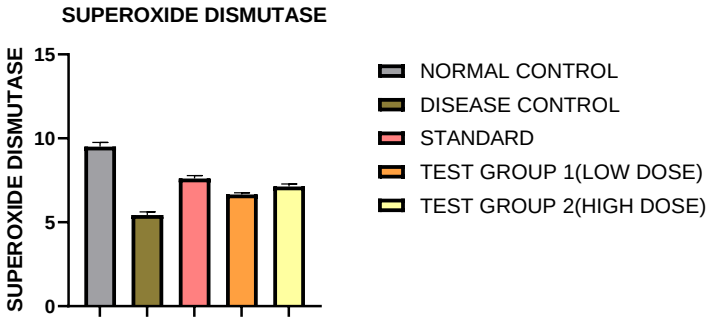

Fig 7: Graphical representation of changes in Superoxide Dismutase (SOD)

The Standard deviation ± Mean is used to express values; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using Analysis of Variance in One Way (ANOVA) with Graph pad prism 10.3.1 software.

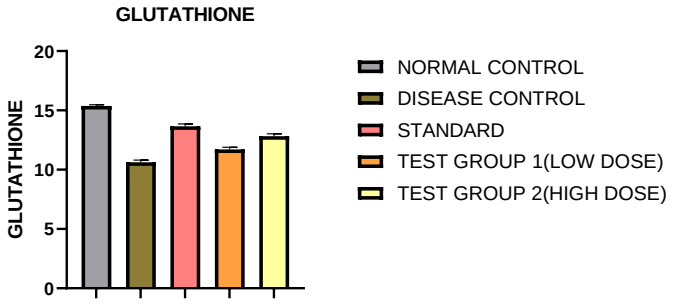

Fig 8: Graphical representation of changes in Glutathione (GTH)

The Standard deviation ± Mean is used to express values; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using Analysis of Variance in One Way (ANOVA) with Graph pad prism 10.3.1 software.

For the duration of the study, thirty mature, healthy albino Wister rats were employed. Twenty-four rats had anemia caused by injection of phenylhydrazine (PHZ) intraperitoneally, and six rats were considered normal controls. Twenty-four rats were discovered to be anemic based on Hb estimate after taking PHZ for two days. The tests haematological and antioxidant capabilities were contrasted with those of the control groups.

After taking PHZ for 2 days, the hemoglobin rates of rats from anaemic control, standard, test Group I, and test Group II were significantly lower ($P < 0.05$). After receiving the appropriate treatments for about fourteen days, the groups that received supplements with iron and those that received Mangifera indica leaves ethanolic extract showed an improvement in their hemoglobin levels. 400 mg of Mangifera indica leaves ethanolic extract per day can hasten recovery. For instance, in the negative control rats, the Hb increased gradually and organically. For instance, in the negative control rats, the Hb increased gradually and organically.

Rat's RBC counts dropped on the second day after receiving a PHZ injection. RBCs increased noticeably after 14 days of therapy. The findings showed in which the rats in Groups III–V had almost recovered by the second week.

Additionally, the PHZ therapy decreased the antioxidant indicators levels such as SOD and GSH on day two. Following two weeks of therapy, the impact of PHZ was reversed in anemic rats, resulting in a notable rise in GSH and SOD.

DISCUSSION

The current study set out to assess the anti-anemic effects of an ethanolic extracts of Mangifera indica leaves that PHZ prepared in albino rats. Anemia caused by iron deficiency anemia affects more than 30% of people worldwide, which hinders national development and has serious economic consequences.[10, 11]

Thus, it was essential to carry out this research in according to find an affordable drug to treat the anemia disease. The study shows that Mangifera indica effectively raises the amount of RBC, Hb, MCHC, MCV, PCV, and MCH in contrast to the negative control cohort. It is clearly seen that PHZ can induce both in vivo and in vitro hemolysis by generating aryl and hydroxyl radicals, which have been connected to its relationship to the erythrocytes. Intraperitoneal PHZ therapy has been proven to lower Hb, RBC, and PCV levels. Peter et al. claim that haematological parameters are lowered by an injection given through intraperitoneal route of 40 mg/kg PHZ for two days.[12]

Oxidative stress is one important hemolysis mechanism believed to exist in erythrocytes. The reasons for compromised membrane integrity are weakness, dehydration, and increased production of reactive oxygen species. Chronic hemolysis results in the loss of hemoglobin. Along with the red cell's capacity to detoxify, the accumulation of hydrogen peroxide may oxidize several essential

cell components, including membrane phospholipids. These alterations probably have an effect on the final hemolysis of affected cells.

The study's findings indicated that the increase in haematological markers that the Mangifera indica leaves extract showed might be secondary to the mineral and vitamin contents of the tree. The Mangifera indica treatment could suggest that the plant extract can activate erythropoietic factors, which have a direct effect on the generation of bone marrow blood. The percentage of committed, erythropoietin-sensitive stem cells in the bone marrow that eventually develop into mature erythrocytes and RBC' s is increased by erythropoietin, a maturation factor for red blood cell formation. When the lowest dosage of 200 mg/kg was given, the blood parameter recovery time was decreased.Furthermore, because the recovery was gradual, the Hb and PCV concentrations of the treated groups were higher than those of the control groups following 2 weeks of continuous medication. Additionally, it was demonstrated that the treated groups' recovery was dose-dependent, with the largest difference in recovery occurring at the highest dose of 400 mg/day.

It is demonstrated that in both people and rats, there is a substantial correlation between diagnostic outcomes and Hb, PCV, red cell indices (MCV, MCH, and MCHC) and RBC [13]. Animals also exhibit decreases in Hb, RBC, and PCV, which are suggestive of anemia, just like humans do.[14].

The quantity of hemoglobin per unit erythrocyte volume, or MCHC, is usually increased in situations of significant intravascular hemolysis and often decreased in cases of anemia caused by hemolysis. The average volume of the erythrocyte, or MCV, often rises in hemolytic anemia due to reticulocytosis. MCH, or the average amount of hemoglobin per cell, often rises in anemia caused by hemolysis.

Rats intoxicated with PHZ in the current investigation exhibited a substantial decrease in GSH content as contrast to control rats. After receiving extract from Mangifera indica L., rats intoxicated with PHZ show a notable increase in GSH levels. A notable decrease in SOD is also observed when contrasting the negative control with the normal control. SOD levels are also greater in treated groups than in negative controls.

CONCLUSION

Rats injected with PHZ developed hemolytic anemia, a condition marked by a decrease in haematological markers. When 200 mg and 400 mg of an ethanolic extract made from Mangifera indica leaves were administered orally, the Hb level was noticeably raised. Additionally, the findings demonstrated that Mangifera indica inhibits the generation of ROS in rats, which may have an antioxidant effect. This result indicates that Mangifera indica leaves have anti-anemic properties. Further research is needed to understand the mechanism behind the antianemic effects of Mangifera indica.

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