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Evaluation of Anti-Anemic and Hemopoietic Effects of *Drynaria quercifolia* Rhizome Extract in Phenylhydrazine Induced Anemic Wistar Albino Rats

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ABSTRACT

This study aims to evaluate the in-*vivo* anti-anemic activity and antioxidant potential of the ethanolic rhizome extract of *Drynaria quercifolia* in a phenylhydrazine-induced anemia model using male Wistar albino rats. The rats were divided into five groups (n= 6). Group I (normal control) received regular feed and water. Anemia was induced in all groups except Group I through intraperitoneal administration of phenylhydrazine (40 mg/kg for 2 days). Group II (negative control) received phenylhydrazine only. Group III (positive control) received hematinic syrup (0.68 ml/kg). Group IV and V received the test extract at 200 mg/kg and 400 mg/kg, respectively, for 12 days. Hematological and serum biochemical parameters were evaluated to assess the anti-anemic effect, while enzymatic antioxidant potential was measured by serum level of superoxide dismutase and glutathione peroxidase. Results showed that treatment with EEDQ significantly improved hematological parameters, serum biochemical parameters and reversed oxidative stress markers, indicating its efficacy in reducing anemia and enhancing antioxidant defense in erythrocytes.

1. INTRODUCTION

Anemia is one of the most common health problems in the world. It affects more than one-third of the world's population. In almost all developing countries, one-third to one-half of women and children are anemic[1].Anemia is characterised by a lower than normal plasma hemoglobin level due to a decrease in the number of circulating red blood cells or an abnormally low total hemoglobin concentration per unit volume of blood[2].The consequences of anemia were general weakness of the body, constant fatigue and lowered resistance to diseases. [3]

Anemia can result from a variety of factors, such as inadequate iron consumption or absorption, low folic acid or vitamin B12 intake, red bone marrow deterioration, genetic abnormalities, etc. [4]

Hemolytic anemia is a form of inherited or acquired anemia resulting from either intravascular or extravascular RBC destruction [3]. Hemolytic anemia is associated with several situations such as heavy bleeding, nutritional deficiencies, genetic defects, infectious diseases, prolonged use of non-steroids drugs and exposure to toxic chemicals as phenylhydrazine which reduce in quality and quantity of red blood cells and hemoglobin. Various treatments are carried out according to hemolytic anemia type. Such as oral intake of iron, vitamin B12 or B9, treatment with immunosuppressors or corticosteroids, erythropoietin injection, blood transfusion, or bone marrow transplantation. [5]

Long-term usage of these oral iron supplements may result in adverse effects like nausea, discomfort in the epigastrium, abdominal cramps, constipation and diarrhoea. Parenteral iron therapy can also cause local adverse effects including discomfort at the injection site, as well as systemic side effects such anaphylactoid reaction, which can occasionally result in vascular collapse and death. [6]

We strive to ensure the therapeutic value of herbal medicine and secure the necessary approval for their commercialization. More clinical trials involving these drugs for various diseases are currently being conducted. Some phytochemicals or medicinal plants have a direct effect on the improvement of anemia by increasing resistance to oxidative stress through their antioxidant activity. [7]

Drynaria quercifolia linn., belonging to Polypodiaceae well known as Mudavattukkal kizhangu in Tamil. It is used as traditional remedy for many diseases and has

been revealed in several studies and has its importance in Ayurveda in the treatment of various diseases such as typhoid, chronic jaundice, headache, cough, cholera and skin diseases. Some tribes of the Eastern Ghats of Tamil Nadu often use a decoction made from the rhizomes of *Drynaria quercifolia* to get relief from rheumatic diseases.

Consumption of *Drynaria quercifolia* can help to heal, strengthen bones and can also be used to promote healing of fractures. The leaves are astringent and have been found to strengthen and promote healing of tendons, muscles and bones. [8] The objective of this study aims at investigating the therapeutic benefit of the *Drynaria quercifolia* in the treatment of anemia.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant material

The rhizome of *Drynaria quercifolia* was collected from the local market in the month of October 2023 at Chennai district, Tamilnadu. The plant was identified and authenticated by Prof.Dr.S. Sankaranarayanan Msc, M.Phil., Ph.D., HOD, Department of Medicinal Botany and Pharmacognosy, Government Siddha Medical College, Arumbakkam, Chennai-600106.The freshly collected rhizome were sliced and dried under shade. The dried material was powdered and passed through a 40-mesh sieve. Then stored in air tight container.

2.2 Preparation of ethanolic extract

The rhizome powder was extracted with ethanol (99.9%) by hot percolation method using Soxhlet extractor. The extract was concentrated by using a rotary evaporator. The semisolid residue obtained were weighed and stored in desiccator. The Percentage yield of the ethanolic extract of *Drynaria quercifolia* rhizome was calculated using the following formula.[9]

$$\text{Yield value} = \frac{\text{Weight of the residue}}{\text{Weight of the dried plant}} \times 100$$

2.3 Experimental animals

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA/IAEC approval no: 1917/GO/ReBi/S/16/CPCSEA/25.10.2016 and 1/AEL/

IAEC/MMC, Date: 26.12.2023. For this investigation, 30 male Wistar albino rats were purchased from the Madras Medical College Animal House in Chennai, India. In a quarantine period, animals were kept apart from those already housed in the facility while their health as well as their microbiological condition are being assessed. The newly procured male Wistar albino rats were quarantined for a period of one week to minimize the chance of introduction of pathogens into established animals and allowed to develop psychological, physiological and nutritional stabilization before their use. The animals were housed in a well-ventilated condition which was maintained at a constant temperature and relative humidity of 55 to 60%. The animals were housed in spacious polypropylene cages and paddy husk was utilized as bedding material. The bed material was changed twice a week. The animals were maintained on standard pellets and purified water. The animals were provided with food ad libitum except during fasting. All animal cages used in the study had proper identification i.e., labels. Each animal in the cage was marked on the tail with picric acid for their appropriate identification.

2.4 Acute toxicity studies (as per the OECD guidelines no. 423):

Acute toxicity study has been carried out already and the animals did not show any toxic effects up to dose of 2000 mg/kg-5000 mg/kg and hence $1/10^{th}$ and $1/5^{th}$ of the maximum dose administrated (i.e., 200 mg/kg and 400 mg/kg) was selected for the present study. [10]

2.5 Experimental design

All the animals were weighed and separated into 5 groups each consisting of n= 6. Anemia was induced by intraperitoneal administration of phenylhydrazine (40 mg/kg) in all groups except normal control group rats on day 1 & day 2.

Group I: Normal control received normal feed and water

Group II: Negative control received normal feed and water from day 3 to day 14.

Group III: Positive control received hematinic syrup (0.68 ml/kg) from day 3 to day 14.

Group IV: Test group received ethanolic extract of *Drynaria quercifolia* (EEDQ) 200 mg/kg from day 3 to day 14.

Group V: Test group received ethanolic extract of *Drynaria quercifolia* (EEDQ) 400 mg/kg from day 3 to day 14

After 14 days of treatment, whole blood sample were collected from Lateral tail vein. Samples were added to tube containing ethylenediaminetetraacetic acid after 2 weeks of treatment. Blood samples were subjected to analyse hematological parameters [10-17], serum biochemical parameters [5,18] and Antioxidant potential such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) was also analysed using the standard procedure. [20]

2.6 STATISTICAL ANALYSIS

The results were expressed as mean ± SEM.The data was statistically analyzed by means of one-way ANOVA followed by Dunnett' s multiple comparison test using Graphic pad prism software version 8.0.2. One-way ANOVA was used to correlate the statistical difference between the variables. P value ($P< 0.05$) , ($P<0.01$), ($P< 0.001$) was considered statistically significant.

3. RESULTS

3.1 Percentage yield of ethanolic extraction of *Drynaria quercifolia*

The Percentage yield of the ethanolic extract of *Drynaria quercifolia* rhizome was found to be 7.2 % w/w.

3.2 Effect of EEDQ on Hematological parameters

Induction of anemia after the administration of phenylhydrazine (I.P) at the dose of 40 mg/kg for 2 days caused significant ($P< 0.0001$) reduction of RBC Count, hemoglobin, hematocrit in disease control rats when compared with normal control rats. It was due to the presence of oxidative stress within RBC due to formation of ROS, subsequent hemolysis and premature splenic destruction. The RBC count, hemoglobin and hematocrit were increased significantly ($P<0.0001$) after treatment with low dose (200 mg/kg) and high dose (400 mg/kg) as well as in standard drug (hematinic syrup, 0.68 ml/kg) treated rats and EEDQ treated rats as shown in the table 1.

There was significant increase ($P< 0.0001$) of mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration in anemia induced rats when compared with normal control rats. while on treatment with low dose (200 mg/kg), high dose (400 mg/kg), as well as standard drug (hematinic syrup, 0.68 ml/kg) showed significant reduction of mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration in standard treated rats and EEDQ treated rats as shown in the table 1.

Table 1: Effect of EEDQ on hematological parameters at the end of 14th day

S. NO	PARAMETERS	GROUP I (Normal control)	GROUP II (Disease control)	GROUP III(standard control)	GROUP IV (lowdose)	GROUP V (highdose)
1.	**RBC Count ($X10^{12}$/L)**	9.43±0.04	6.45±0.13****	8.4±0.14####	7.39±0.06##	8.12±0.18####
2.	**Hemoglobin(g/DL)**	16.41±0.14	11.18±0.14****	15.61±0.14####	14.28±0.32####	15.18±0.12### #
3.	**Hematocrit (%)**	50.51±0.40	35.41±2.64****	47.45±0.56####	42.39±1.13##	45.52±0.71### #
4.	**Mean Cell Volume (fL)**	61.03±0.30	70.01±0.20****	63.13±0.24####	67.05±0.59####	65.98±0.49### #
5.	**Mean Cell Hemoglobin(pg)**	18.01±0.20	26.31±0.51****	20.47±0.07####	24.38±0.33###	22.24±0.21### #
6..	**Mean Cell Hemoglobin Concentration (g/DL)**	26.09±0.53	35.23±0.22****	28.63±0.10####	32.16±0.30####	30.14±0.48### #

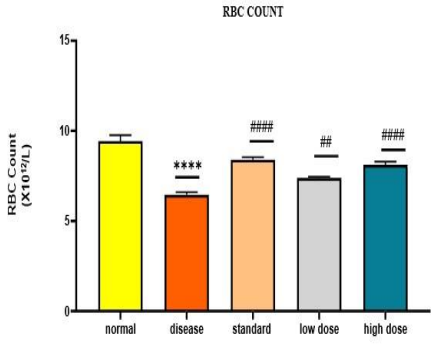

Figure 1a. RBC Count

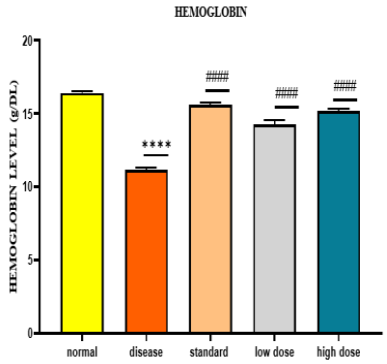

Figure 1b. Hemoglobin

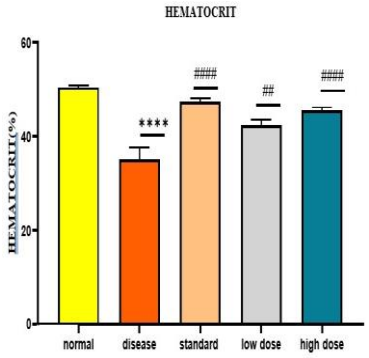

Figure 1c. Hematocrit

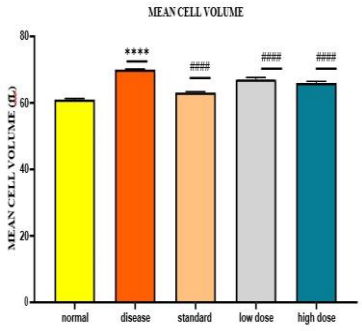

Figure 1d. Mean cell volume

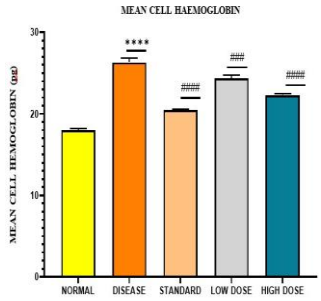

Figure 1e. Mean cell hemoglobin

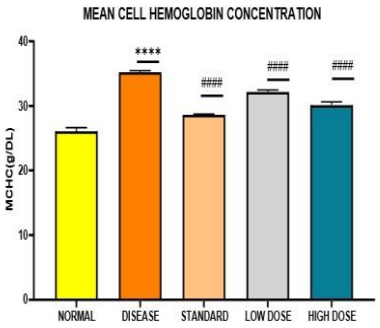

Figure 1f. Mean cell hemoglobin Concentration

Figure 1: Effect of EEDQ on hematogical parameters

All the values are plotted as mean ± sem (n=6). Analysed by One-way analysis of variance (ANOVA) followed by multiple comparison Dunnet t'test.

P<0.0001 compared with disease control group; **** P<0.0001 compared with normal control.

3.3 Effect of EEDQ on serum biochemical parameters

Induction of anemia with phenylhydrazine (I.P) at the dose of 40 mg/kg for 2 days caused significant (P<0.0001) increase of serum iron level in disease treated rats when compared with normal control rats. This was due to alteration of iron metabolism by phenylhydrazine administration which results in increased expression of DMT1 transporter in the spleen, duodenum and liver. DMT1 promotes the expression of genes related to iron metabolism such as ferric reductase DCytb, Ireg1 and DMT1 in humans and rats. Increased mRNA expression of DCytb, DMT1, Ireg1 and IFR1 in spleen and liver would increase the iron

demand. On treatment with low dose (200 mg/kg) and high dose (400 mg/kg) as well as standard drug (hematinic syrup, 0.68 ml/kg) showed significant decrease (P<0.0001) of serum iron level in standard drug rats and EEDQ treated rats as shown in the table 3.

After the induction of anemia by intraperitoneal administration of phenylhydrazine (40 mg/kg) at day 1 and day 2, there is significant (P<0.0001) increase of serum ferritin level in disease treated rats when compared with normal control rats. While on treatment with standard drug (hematinic syrup, 0.68 ml/kg) and EEDQ at both the doses had showed significant decrease (P<0.0001) of serum ferritin level in low dose and high dose group and Hematinic syrup treated rats as shown in the table 3.

There was significant (P<0.0001) increase of serum erythropoietin level in disease treated rats when compared with normal control rats after the intraperitoneal administration of phenylhydrazine (40 mg/kg). On treatment with standard drug (hematinic syrup, 0.68ml/kg), low dose (200 mg/kg) and high dose (400 mg/kg) of EEDQ showed significant decrease (P<0.0001) of serum erythropoietin level in hematinic syrup treated rats and EEDQ treated rats as shown in the table 3.

Table 2: Effect of EEDQ on serum biochemical parameters at the end of 14th day

Groups	**Iron**	**Ferritin**	**Erythropoietin**
Normal control	144.93±0.14	18.82±0.15	20.21±0.16
Disease control (Phenylhydrazine 40 mg/kg for 2 days)	275.45±1.75****	30.06±0.36****	30.00±0.09****
Standard control (hematinic syrup 0.68 ml/kg)	154.38±0.40####	20.15±0.09####	23.08±0.24 ####
Low dose (200 mg of *Drynaria quercifolia*)	168.50±0.85####	27.62±0.46####	27.79±0.23 ####
High dose (400 mg of *Drynaria quercifolia*)	159.69±0.19####	24.98±0.22####	25.45±0.33####

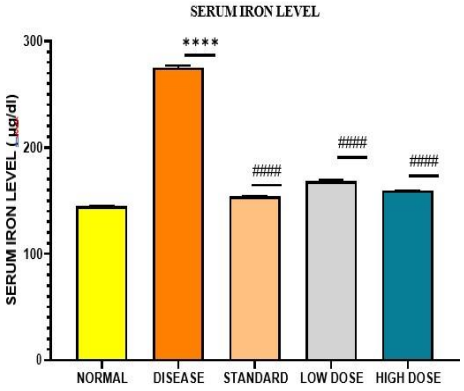

Figure 2a. Serum iron

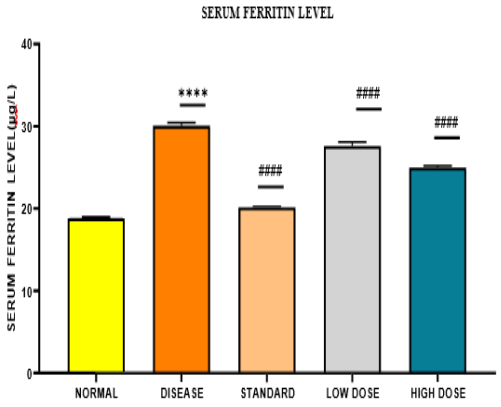

Figure 2b. Serum ferritin

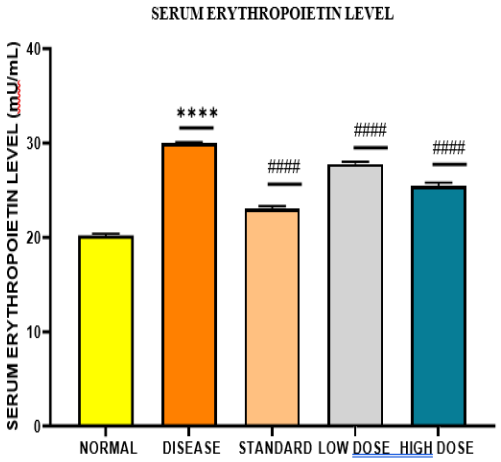

Figure 2c: Serum erythropoietin

Figure 2: Effect of EEDQ on serum biochemical parameters

All the values are plotted as mean ± sem (n=6). Analysed by One-way analysis of variance (ANOVA) followed bymultiple comparison Dunnet t'test.

P<0.0001 compared with disease control group; **** P<0.0001 compared withnormal control group.

3.4 Antioxidant enzyme parameters

After the administration of phenylhydrazine (40 mg/kg in I.P) at day 1 and day 2, there was significant (P<0.0001) reduction of the Superoxide dismutase concentration of disease control rats when compared with the normal control group. There was significant increase

(P<0.0001) of the Superoxide dismutase concentration in standard drug treated rats and EEDQ treated rats on treatment with hematinic syrup and EEDQ (low dose 200 mg/kg and high dose 400 mg/kg) at the end of 14th day as shown in table 3.

After the induction of anemia by intraperitoneal administration of phenylhydrazine (40 mg/kg) at day 1 and day 2, there is significant (P<0.0001) decrease Glutathione peroxidase in disease treated rats when compared with normal control rats. While on treatment with standard drug (hematinic syrup, 0.68 ml/kg) and EEDQ at both the doses had showed significant increase (P<0.0001) of Glutathione peroxidase in low dose (200 mg/kg), high dose (400mg/kg) group and Hematinic syrup treated rats as shown in the table 3.

Table 3: Antioxidant enzyme level at the end of 14th day

Groups	**Superoxide dismutase**	**Glutathione peroxidase**
Normal control	3.16±0.12	0.04±0.0041
Disease control (Phenylhydrazine 40 mg/kg for 2 days)	1.33±0.06****	0.015±0.001****
Standard control (hematinic syrup 0.68 ml/kg)	2.94±0.14 $^{####}$	0.04±0.0040$^{####}$
Low dose (200 mg of *Drynaria quercifolia*)	2.32±0.05 $^{####}$	0.03±0.001$^{####}$
High dose (400 mg of *Drynaria quercifolia*)	2.55±0.17 $^{####}$	0.03±0.003$^{####}$

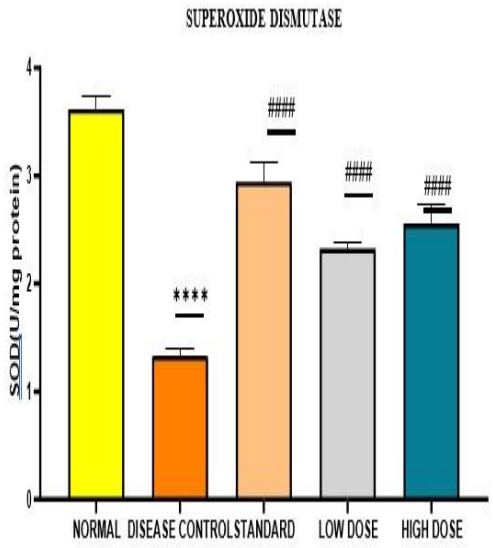

Figure 3a. Superoxide dismutase

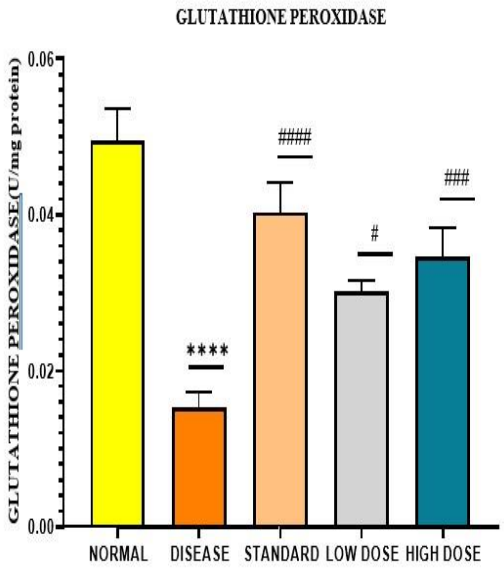

Figure 3b. Glutathione peroxidase

Figure 3*:* Antioxidant enzyme level

All the values are plotted as mean ± sem (n=6). Analyzed by One-way analysis of variance (ANOVA) followed bymultiple comparison Dunnet t'test.

P<0.0001 compared with disease control group; **** P<0.0001 compared withnormal control group.

4. CONCLUSION

From the study it was concluded that the rhizome extract of *Drynaria quercifolia* possess beneficial effect against phenylhydrazine induced anemia by significant improvement in hematological indices, serum biochemical parameters (iron, ferritin and erythropoietin) and Antioxidant potential was confirmed by protective effect of *Drynaria quercifolia* on affected RBC's by increased concentration level of superoxide dismutase and glutathione peroxidase.

The present study provided only basic evidence that the rhizome extract of *Drynaria quercifolia* has the beneficial effect for the treatment of anemia. Further studies are required for the isolation of the bioactive compound and to develop suitable formulations to ensure maximum bioavailability and therapeutic efficacy. Further clinical trial can also be performed to include *Drynaria quercifolia* in the treatment of anemia.

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Conflict of interest statement: The authors declared no conflict of interest.

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