Journal of Ayurvedic and Herbal Medicine

Research Article

ISSN: 2454-5023
J. Ayu. Herb. Med.
2024; 10(3): 81-88
Received: 28-05-2024
Accepted: 15-09-2024

www.ayurvedjournal.com
DOI: 10.31254/jahm.2024.10303

Evaluation of anti-anemic activity of ferrous sulphate and copper sulphate in phenylhydrazine induced anemia in wistar rats

Sujay Chaphekar[1], Nitin D Jadhav[1], S. R. Rajurkar[1], G. M. Chigure[1], P. R. Rathod[1], M. D. Jadhav[2]

[1] Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani-431402, Maharashtra, India

[2] Siddharth Arts, Commerce and Science College Jafrabad, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, Maharashtra, India

ABSTRACT

The anemia is worldwide problem and generally defined as decrease in the haematological parameters such as haemoglobin, haematocrit percentage and erythrocyte count. Iron deficiency is the most common nutritional deficiency in the world related to changes in trace elements (iron, zinc, copper, and cobalt) as well as vitamins like Vitamin B12 and Vitamin B9 (Folate), which are essential for erythropoiesis. Phenylhydrazine acts through production of reactive oxygen species that contribute for oxidative stress on the animal body leading to development of haemolytic anemia. In the present research 56 wistar rats of 8-10 weeks of age were selected and divided into 7 different groups. Group 1 was kept as healthy control and group 2 to 7 were induced anemia with phenylhydrazine. The efficacy of ferrous sulphate and copper sulphate and their combination were evaluated by studying haematological, biochemical and histopathological parameters. The group III and VII had highest improvement in all the haematological parameters as compared with other control and treatment groups, whereas group VII had highest levels of TEC on 14th day of the experiment itself suggesting role of copper in erythropoiesis mechanism.

Keywords: Anemia, anti-anemic activity, Ferrous sulphate, Copper sulphate, Phenylhydrazine.

INTRODUCTION

Anemia is characterised by reduction in haemoglobin, haematocrit, total erythrocyte count that affects blood's ability to supply tissues with adequate oxygen, thereafter metabolic processes depended on oxygen gets hampered as well. Anemia is one of the most important clinical manifestations of diseases seen in livestock as well as in companion animals. The major clinical infection involving Ehrlichiosis, Anaplasmosis, Theileriosis, Babesiosis, Trypanosomiasis have wide range of impact on animals [1]. The leptospirosis, clostridium infections, equine infectious anemia have serious contribution in development of anemia. Iron deficiency is the most common nutritional deficiency in the world often related to changes in trace elements (iron, zinc, copper, and cobalt) as well as vitamins like Vitamin B12 and Vitamin B9 (Folate), which are essential for erythropoiesis. "Iron deficiency" and "anemia" are two different conditions Although they are frequently linked, people can be iron deficient without being anemic [2]. The term "iron deficiency" refers to a lack of iron in the body's stores, whereas "anaemia" to a lack of iron in the red blood cells.

The 5th National family health survey conducted in 2019-2021 across India revealed that anemia was major concern among the women and young children. About 64.2% of urban and 68.3% rural children who aged between 6-59 months were found to be anemic. The data concerned with women aged between 15-19 years showed about 56.5% of the urban women and 60.2% rural women were found to be anemic. All women age 15-49 years who are anemic accounted for 53.8% of urban and 58.5% rural population.

Phenylhydrazine is drug discovered by Fisher H.E in 1895, which was mainly used as antipyretic drug, as an intermediate constituent in agrochemical, pharmaceutical and chemical industries, besides it was earlier being used to treat conditions like polycythemia vera. Phenylhydrazine is known for producing acute haemolytic anemia models. It is known to decrease the haematological parameters such as haemoglobin, packed cell volume, total erythrocyte count. It mainly acts through production of reactive oxygen species that contribute for oxidative stress on the animal body leading to development of haemolytic anemia.Considering the scenario of anemia as global problem among humans and animals, the present research was planned to evaluate the efficacy of ferrous sulphate and copper sulphate on anemic rat induced with phenylhydrazine.

***Corresponding author:**
Dr. Nitin D Jadhav
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani-431402, Maharashtra, India
Email: nitinjadhav@mafsu.in

MATERIALS AND METHODS

Animals and Experimental Design

The research was carried out in small animal laboratory house of college of veterinary and animal sciences, Parbhani, Maharashtra. During the experiment 56 rats of 8-10 weeks of age weighing 180-200 gm body weight were selected. The acclimatization period of one week was followed before start of the experiment. The selected animals were grouped into 7 different groups, viz one healthy control (without induction and treatment), group II served as anaemic control (induced with phenylhydrazine but without treatment), group III to VII were induced with phenylhydrazine @ 20 mg/kg intraperitoneal to develop anemia and followed by the treatment as mentioned below. The drop in the level of haemoglobin below 8 g/dl was observed in the treated groups and rats were said to be anaemic.

Groups	Name of the group	Treatment	Dose & Route
I	Healthy control	Distilled water	1 ml/kg P.0
II	Anaemic control	Distilled water	1 ml/kg P.0
III	Ferrous sulphate low dose	Ferrous sulphate	@ 50 mg/kg P.0
IV	Ferrous sulphate high dose	Ferrous sulphate	@ 100 mg/kg P.0
V	Copper sulphate low dose	Copper sulphate	@ 50 mg/kg P.0
VI	Copper sulphate high dose	Copper sulphate	@ 100 mg/kg P.0
VII	Combination Group	Ferrous sulphate & Copper sulphate	Both @ 50 mg/kg

Sample collection and analysis

Blood sample was collected on days 0, 14th and 28th day of the experiment for haematological and biochemical Parameters. In haematological, total erythrocyte count, Packed cell volume, total leucocyte count and differential leucocyte count was evaluated and in biochemical Parameters, SGOT, SGPT, Blood urea nitrogen, creatinine and total protein values were recorded.

Histopathological examination

At the end of experiment, the rats were sacrificed animals and liver, kidney, lung, spleen and bone marrow were collected in 10% formalin for histopathological examination.

Statistical Analysis

The data obtained from various parameters from all the groups was analyzed as per method suggested by Snedecor and Cochran (1994) using factorial randomised block design (FRBD) and completely randomised block design (CRD) using WASP

RESULTS AND DISCUSSION

After induction of anemia in the rats there was decreased in activity was noted, the rats were lethargic along with decreased in the appetite was seen. The rats in the control group appeared active, bright, alert and healthy throughout the experiment. Though the weights in the treatment group were increased as compared with the positive group, no decreased in weight was observed after administration of phenylhydrazine in those treatment groups. So, no effect of drugs/ treatment was imposed on the mean body weight gain of the experimental animals, similar pattern of results was obtained by He [3] and Urso [4] where after treatment with ferrous sulphate increase in the body weight was noted. However, Lee [5], Elaby and Ali [6], Allahmoradi [7] and Parvaz [8] found significant decrease in the mean body weights of rats treated with phenylhydrazine which was contrasting with the current study may be because dose and duration of exposure of phenylhydrazine used in their respective research.

The haematological values

The mean haemoglobin, total erythrocyte, total leucocytes, packed cell volume and platelet count were analysed on 0. 14 and 28th day of experiment and represented in table 1. The decrease in the mean haemoglobin levels after treatment with phenylhydrazine was observed in II to VII group on 0 day of experiment confirmed that the anaemia has been induced in the rats. Ssimilar observation were obtained Kamble[9] and Beshel[10]. Then after the treatment in te II to VII group the haemoglobin values were increased from 14 to 28th day of treatment and was dose and duration dependant. Beshel[10], Srivastava[11], Mandal[12], Goklaney[13], Drowdy and Matrone[14] also reported the same observation in their research.

Mean erythrocyte count in groups II to VII had significantly lower on 0 day of the experiment as compared with control group of non-anaemic rats. All the treatment groups showed increase in total erythrocyte count values from day 14 and 28 with increase in dose and duration of the treatment. On 14th day the groups IV, VI and VII achieved the total erythrocyte values within the normal range. On 28th day of treatment groups the total erythrocyte values were in the normal physiological limits. The study concludes that the ferrous sulphate @ 100 mg shown increase in total erythrocyte values on 28th day of experiment when compared with the copper sulphate. But the combined dose of ferrous sulphate and copper sulphate enhance the total erythrocyte values from 14 day onwards similar results were procured by the researchers Suva and Tirgar[15], Chaudhary[16], Tang[17], Ghada[1], after treatment with ferrous sulphate.

There was significant increase in total leucocytes count in group II to VII on 0 day of experiment was observed. The increased values were higher than normal physiological limits in the respective groups. On 14th day of experiment the total leucocytes values were gradually come down to its normal physiological levels in the treatment groups and on 28th day the total leucocytes values were reached within their normal limits but were at higher side as compared with the control group. Ousaaid[18], Abdo[19] Elaby and Ali[6]. and Gheith and El-Mahmoudy[20] found similar rise in the TLC levels after treatment with phenylhydrazine. The decreasing trend of total leucocyte count after treatment with minerals was seen based on dose and duration manner similar findings were procured by Khandaker[21] and Rahman[22].

On day 0 of the experiment there was reduction in packed cell volume of groups from II to VII. On day 14 treatment groups had significantly lower packed cell values as compared with the non-anaemic control group and found lower than the normal physiological range. On 28th day, groups III, IV, V, VII have no significant difference in packed cell values and were significantly higher than II group, these increase in the levels of packed cell volume was dose and duration dependent. Lee, *et al* (2012), Gheith, and Mahmoudy[20], and Ousaaid[18], Even some of researcher, Aduwamai[23], Elaby and Ali[6], Agbor[24] and Ashour[25]

(2014) Sheth[26] and Ogbe[27] obtained similar results where the packed cell volume dropped after phenylhydrazine induction. Al-Shebli, and Ali[28], and Mandal[12] obtained similar results where there was increase in the packed cell volume was observed which was dose and duration dependent.

The mean platelet count there was significantly increased in the platelet count after the phenylhydrazine administration. Similar observation was also noted by Meena[29], and Ibe[30], where the platelet count found higher than the normal range after induction of anaemia in rats, at the end of the experiment there was linear decrease in these levels noticed in the treatment groups.

The biochemical values

SGOT, SGPT, Blood urea nitrogen, serum creatinine and total protein levels were analysed on 0. 14 and 28th day of experiment and represented in table 2. The significant increase in the levels of liver enzymes SGOT and SGPT was observed in the groups treated with phenylhydrazine on day 0 of the experiment which further dropped down to the normal physiological range during the course of the treatment on day 14 and 28th of the experiment. similar findings were obtained by Henneh[31], Allahmoradi[32], Abdo[19], Shen[33] and Ezeigwe[34].

There was no significant difference observed in levels of Blood urea nitrogen in treatment and control groups, all the values were within the normal physiological range. However, Nwauche[35] and Ezugwu[36] reported that after induction of anaemia with phenylhydrazine the blood urea nitrogen levels were significantly increased in the anaemic rats when compared with control groups, may be because of use of higher dose rate for induction of anemia but all the values were within normal physiological limits.

The levels of serum creatinine were evaluated; there was non-significant increase in the mean serum creatinine levels in the groups treated with phenylhydrazine. During the course of the experiment on days 14 and day 28these values dropped down and stayed within normal physiological range, similar observations were obtained by Ezugwu[36], and Henneh[31].

No significant difference observed in the values of mean total protein levels in control as well as the treatment groups during the course of experiment on day 0, 14th and 28th and all values identified were within the normal physiological range. Similar findings were obtained by Mohammed[37], Kale[38] as after induction of anemia with phenylhydrazine non-significant difference was observed.

Histopathological examinations

After completion of the experiment animals from each group were scarified on 29th day. The vital organs such as heart, liver, spleen, lung, kidney, and bone marrow were collected and preserved in 10% formalin solution until processing for microscopic examination. The control group animal had no significant alteration of histoarchitecture in studied organs. Heart of the control group reveals normal histoarchitecture *i.e.,* skeletal, cardiac muscle fibre, intercalated disc, normal striation of muscle. The section of liver from control group revealed normal hepatic parenchyma *i.e.,* normal hepatocytes, central vein, bile duct, hepatic triad. The rats from the control group showed normal splenic parenchyma composed of red and white pulp. The rats from the control group revealed no abnormal alteration in the histoarchitecture of the lung. The kidney form control rats revealed structure involving normal glomeruli, proximal and distal convoluted tubule. Control group of animals showed no abnormal alterations in the architecture of the bone marrow.

As far as anaemic control group was concerned there was mild to moderate intensity changes were observed. The heart from anaemic control group revealed separation of cardiacmusclefibre with mild to moderate dilatation of muscle fibre. Some of the sections revealed severe degeneration, congestion; focal areas of necrosis. The microscopical examination of liver revealed moderate congestion, degeneration of hepatocytes, moderate to severe dilatation of sinusoidal space, focal necrotic patches at some places, and along with mononuclear cell infiltration. The spleen of the rats from the positive control group treated with phenylhydrazine showed moderate depletion of cellular contents specially lymphocytes. Lung from anaemic control group rats revealed moderate congestion, degeneration; rupture of bronchioles and thickened alveolar septa along with enlarged alveolar space. mild vacuolar degeneration, focal areas of necrosis, and mild congestion in glomerular tufts along with variation in the glomeruli was noted in histoarchitecture of kidney in anaemic control group. Further depletion of the cellular contents and presence of small amount of the adipose tissue was revealed in sections of bone marrow from rats treated with phenylhydrazine.

Acknowledgments

The authors are thankful to the Associate Dean, College of Veterinary and Animal Sciences, Parbhani a constituent college under Maharashtra Animal and Fishery Sciences, University Nagpur Maharashtra for providing facilities to carry out the research work.

Conflict of interest

There is no conflict of interest.

Funding

None declared.

ORCID ID

Sujay Chaphekar: https://orcid.org/0009-0008-7298-8296
Nitin D Jadhav: https://orcid.org/0009-0000-9357-1790
G. M. Chigure: https://orcid.org/0000-0002-6067-0423
P. R. Rathod: https://orcid.org/0000-0001-9233-9738
M. D. Jadhav: https://orcid.org/0009-0007-1226-8011

Table 1: Hematological values

Group	Hb g/dl			TEC 10^6/µl			PCV %			TLC $\times10^3$/µl			Mean Platelets level Mean ± SE × 10^3/mm^3		
Days	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day
Group I	13.87^{a} ±0.26	13.93^{a} ±0.31	15.12^{p} ±0.22	8.45^{ak} ±0.25	8.31^{k} ±0.22	8.46^{pk} ±0.19	42.46^{a} ±0.81	41.95^{a} ±1.13	46.56^{p} ±0.56	11.98^{c} ±0.74	$11.6l^{c}$ ±1.0	10.65^{qc} ±1.29	1035^{cp} ±45.82	898.75^{l} ±64.59	853.75^{q} ±72.38
Group II	7.37^{b} ±0.18	9.81^{m} ±0.16	10.68^{sml} ±0.20	3.76^{c} ±0.14	5.15^{n} ±0.22	6.88^{sm} ±0.21	20.67c ±0.51	29.35^{m} ±0.45	32.05^{sm} ±0.78	21.22^{ab} ±0.56	17.42^{m} ±0.62	15.32^{p} ±0.48	1725^{a} ±50.63	1275^{k} ±48.29	1121.2^{pk} ±60.25
Group III	6.98^{b} ±0.21	11.56^{l} ±0.31	14.41^{pa} ±0.50	4.34^{bc} ±0.19	6.93^{m} ±0.21	8.36^{pk} ±0.26	21.73c ±0.60	35.43^{ls} ±0.76	42.41^{qt} ±0.94	20.27^{b} ±0.76	17^{m} ±0.59	12.08^{q} ±0.74	1702.5^{b} ±94.12	1277.5^{k} ±39.31	906.25^{q} ±19.90
Group IV	8.35^{b} ±0.52	11.52^{l} ±0.49	15.02^{pa} ±0.62	4.73^{b} ±0.15	7.8^{lq} ±0.48	8.91^{pk} ±0.40	26.23b ±1.23	35.57^{l} ±1.28	44.9^{pta} ±0.92	21.12^{ab} ±0.38	15.98^{k} ±0.62	11.41^{q} ±1.2	1687.5^{b} ±56.49	1137.5^{k} ±52.39	912.5^{q} ±53.24
Group V	8.45^{b} ±0.46	11.52^{l} ±0.32	13.98^{pa} ±0.54	4.64^{b} ±0.20	6.49^{m} ±0.14	7.92^{q} ±0.27	26.56b ±1.18	35.85^{l} ±0.54	42.37^{qt} ±1.09	21.93^{ab} ±0.89	17.45^{km} ±0.6	11.12^{qc} ±0.66	1743.75^{a}± 41.65	1196.2^{k} ±74.49	818.75^{q} ±45.25
Group VI	7.22^{b} ±0.48	11^{l} ±0.35	12.02^{rl} ±0.66	4.66^{b} ±0.25	7.14^{l} ±0.21	7.86^{q} ±0.28	22.86^{c} ±1.27	35.12^{ls} ±0.75	37.75^{rl} ±1.97	23.11^{a} ±1.05	16.96^{km} ±0.32	12.27^{q} ±1.27.	1868.75^{a}± 79.58	1196.2^{k} ±64.33	787.5^{q} ±54.07
Group VII	7.01^{b} ±0.50	12.05^{l} ±0.36	13.76^{qa} ±0.48	4.53^{b} ±0.25	8.56^{k} ±0.21	8.51^{pk} ±0.28	22.01^{c} ±1.53	37.3^{l} ±1.16	42.73^{qta} ±1.61	22.17^{ab} ±0.85	16.45^{km} ±0.48	11.73^{qc} ±0.96	1712.5^{a} ±30.98	1275^{k} ±38.95	887.5^{q} ±53.24

Hb: Superscript a, b, l, m p, q, r, s shows significant difference between the groups and within the groups on different days
CD: At 5% =1.151 At 1%=1.513.
TEC: Superscript a, b, c, k, l, m, n, p, q, r, s shows significant difference between the groups and within the groups on different days
CD: At 5% = 0.693; At 1%= 0.911
PCV: Superscript a, b, c, l, m, p, q, r, s, t shows significant difference between the groups and within the groups on different days
CD: At 5% = 3.001; At 1%= 3.944
TLC: Superscript a, b, c, k, l, m, p, q shows significant difference between the groups and within the groups on different days
CD: At 5% =0.693 At 1% = 0.911
Platelet: Superscript a, b, c, k, l, p, q shows significant difference between the groups and within the groups on different days
CD: At 5% =159.421; At 1% 209.524

Table 2: Biochemical values

Group	AST Mean ± SE, IU/L			ALT Mean ± SE, IU/L			BUN Mean ± SE, mg/dl			Creatinine Mean ± SE, mg/dl			Total Protein Mean ± SE, g/dl		
Days	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day
Group I	97.5^{b} ±17.20	107.37^{n} ±17.55	80.87^{t} ±12.1	55.75^{b} ±7.33	41.62^{l} ±5.64	39.75^{rl} ±5.84	21.25^{a} ±1.448	14.25^{kb} ±1.520	16.87^{pb} ±1.684	1.08^{a} ±0.11	0.93^{kb} ±0.08	0.90^{p} ±0.06	7.30^{a} ±0.353	6.74^{kb} ± 0.343	6.92^{pb} ±0.242
Group II	202.7^{ak} ±20.68	194.12^{k} ±6.97	185.37^{p} ±6.64	103.25^{a} ±11.43	88.25^{kp} ±4.94	97.62^{pa} ±4.34	18^{a} ±1.085	17.12^{akb} ±1.826	17.5^{ap} ±1.782	1.11^{a} ±0.07	1.00^{ka} ±0.10	1.04^{pa} ±0.08	7.86^{a} ±0.166	7.27^{ka} ±0.448	7.18^{pa} ±0.266
Group III	195.1^{ak} ±1.09	185.6^{l} ±1.96	165.12^{q} ±1.65	94.25^{ak} ±1.47	85.75^{k} ±1.34	73.25^{q} ±0.94	15.25^{b} ±1.555	15.5^{kb} ±1.414	16^{bq} ±1.52	1.01^{a} ±0.05	0.70^{l} ±0.02	0.53^{q} ±0.02	6.87^{b} ±0.438	7.18^{ka} ± 0.204	6.80^{pb} ±0.404
Group IV	195.8^{ak} ±1.25	183.87^{l} ±2.89	147.75^{s} ±2.52	97.75^{ak} ±1.14	83.37^{k} ±3.6	69.37^{q} ±0.77	16.87^{b} ±1.287	14.12^{kb} ±1.076	15^{bq} ±1.36	1.01^{a} ±0.07	0.7^{l} ±0.03	0.53^{q} ±0.02	6.82^{b} ±0.159	6.94^{kb} ±0.280	6.85^{pb} ±0.165
Group V	201.5^{ak} ±2.93	181.2^{l} ±1.63	162.12^{q} ±2.54	98.62^{ak} ±1.23	79.62^{k} ±2.92	66.87^{q} ±4.02	17.5^{a} ± 1.295	15.37^{kb} ± 1.308	15.37^{bq} ±1.721	1.06^{a} ±0.07	0.69^{l} ±0.01	0.52^{qs} ±0.02	6.66^{b} ±0.340	7.32^{ka} ± 0.310	6.72^{pb} ±0.191
Group VI	190^{ak} ±2.54	175.6^{m} ±1.83	158.37^{r} ±1.95	98.12^{ak} ±1.44	84.87^{k} ±1.02	73.62^{q} ±0.99	14.75^{b} ±1.622	14.12^{kb} ± 1.407	17.37^{ap} ± 1.148	0.92^{b} ±0.05	0.70^{l} ±0.03	0.51^{rs} ±0.01	6.91^{b} ±0.218	7.51^{pa} ± 0.241	6.91^{pb} ± 0.268
Group VII	203.37^{a} ±1.98	179.2^{l} ±1.75	146.5^{s} ±3.54	97.75^{ak} ±1.69	83^{k} ±1.26	65.37^{q} ±2.92	16.25^{b}± 1.176	16.75^{kb} ± 1.305	14.25^{bq} ± 1.161	1.02^{a} ±0.06	0.62^{m} ±0.02	0.44^{r} ±0.02	6.24^{b} ±0.304	6.87^{kb} ± 0.269	6.58^{pb} ±0.130

AST: Superscript a, b, k, l, m, n, p, q, r, s shows significant difference between the groups and within the group on different days
CD: At 5% =5.218; at 1% 6.858
ALT: Superscript a, b, k, l, p, q, r shows significant difference within the groups and between the groups on different days
CD: At 5% = 3.99; At 1% = 5.247
BUN: Superscript a, b, k, p, q shows significant difference between the groups and within the groups on different days
CD: At 5% = 3.99 At 1% = 5.247
Creatinine: Superscript a, b, k, l, m, p, q, r, s shows significant difference between the groups and within the groups on different days
CD: At 5%0.165; At 1%0.217
Total Protein: Superscript a, b, k, p shows significant difference between the groups and within the groups on different days
CD: At 5%=0.78; 1%=1.03

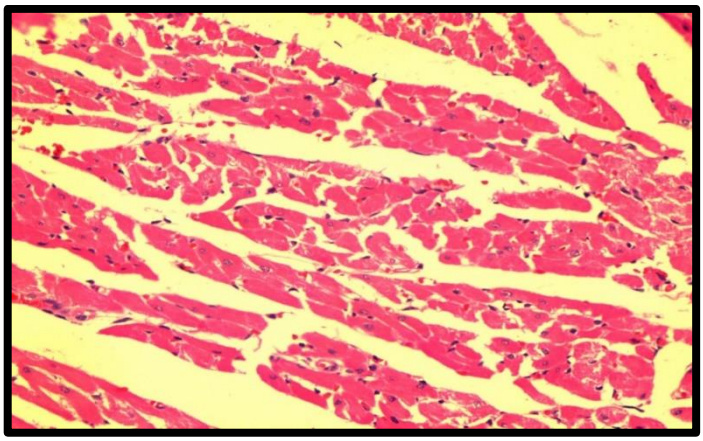

Plate 1: Heart showing dilation and separation of cardiac muscle fibre along with degenerative changes.(H&E 400x)

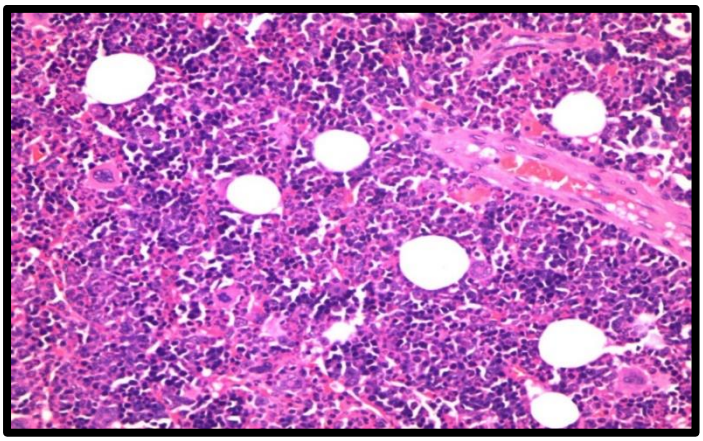

Plate 4: bone marrow showing minimal adipose tissue, megakaryocytes (H&E400x)

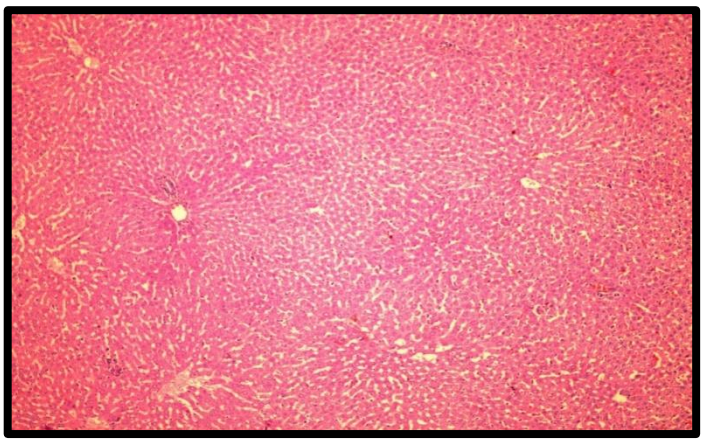

Plate 2: Liver showing mild to moderate lesion of dilatation of sinusoidal space and degeneration along with congestion at some places H&E 100X

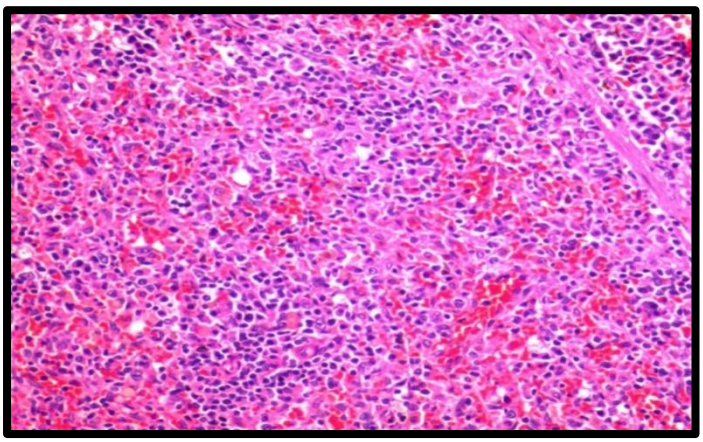

Plate 5: Spleen showing mild congestion and degeneration in splenic parenchyma (H&E400x)

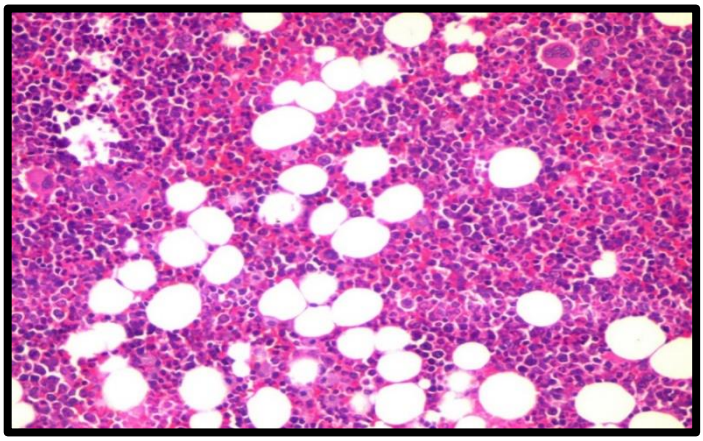

Plate 3: Bone marrow showing depletion in cells along with adipose tissue(H&E 400x)

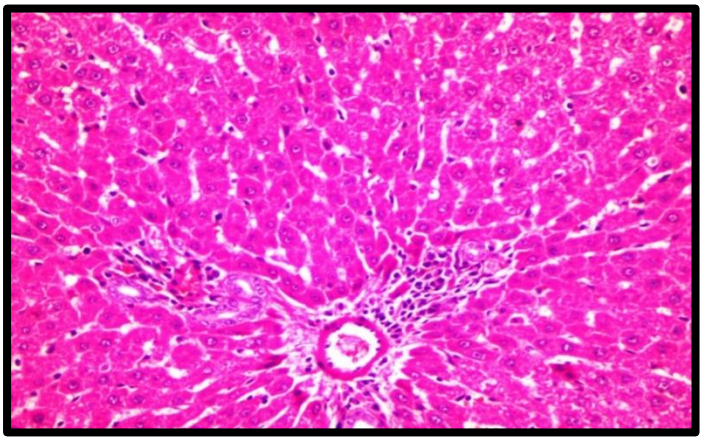

Plate 6: Liver showing degeneration, congestion, focal areas of necrosis, mononuclear cell infiltration and dilatation of sinusoidal space (H&E 100x)

REFERENCES

1. Ghada ZA, Mahfouz MH, Emara IA. Effect of different types of oral iron therapy used for the treatment of iron deficiency anemia and their effects on some hormones and minerals in anemic rats. J Am Sci. 2010;6:109-8.
2. Pandey K, Meena AK, Jain A, Singh RK. Molecular mechanism of phenylhydrazine induced haematotoxicity: a review. Ame J Phytomed Clin Therapeut. 2014;2(3):390-4.
3. He H, Huang Q, Liu C, Jia S, Wang Y, An F, Song H. Effectiveness of AOS–iron on iron deficiency anemia in rats. RSC advances. 2019;9(9):5053-63.
4. Urso K, Leal Martínez-Bujanda J, Del Prado JM. Iron protein succinylate in the management of iron deficiency anemia: A comparative study with ferrous sulphate at low and high therapeutic doses. Nutrients. 2021 Mar 17;13(3):968.
5. Lee SH, Suh HJ, Lee HS, Park Y, Park JW, Jung EY. Hematopoietic effect of Bacillus subtilis–fermented antler extract on phenylhydrazine-induced hemolytic anemia in Sprague–Dawley rats. Journal of Medicinal Food. 2012 Sep 1;15(9):774-80.
6. Elaby SM, Ali JB. The anti-anemic effect of dried beet green in phenylhydrazine treated rats. Archives of Pharmaceutical Sciences Ain Shams University. 2018 Dec 1;2(2):54-69.
7. Allahmoradi M, Alimohammadi S, Cheraghi H. Protective effect of Cynara scolymus L. on blood biochemical parameters and liver histopathological changes in phenylhydrazine-induced hemolytic anemia in rats. Pharmaceutical and Biomedical Research. 2019
8. Parvaz N, Amin F, Askari N, Khademalhosseini M, Khanamani Falahati-pour S, Khanamani FP. The protective effect of Pistacia vera pericarp on kidney function in rats with hemolytic anemia. Research journal of pharmacognosy. 2022 Jan 1;9(1):17-28.
9. Kamble SS, Killedar SG, Jarag RJ, Dol HS. Screening and evaluation of Sonchus asper n-hexane extract against Phenylhydrazine induced anemia. International Journal for Advance Research and Development. 2019;4(1):68-74.
10. Beshel FN, Eyo HE, Beshel JA. Ferrous sulphate improves electrolyte levels in phenylhydrazine induced hemolytic anaemia in Wistar rats. Sch Int J Anat Physiol. 2019;2:209-14.
11. Srivastava V, Sarkar S, Pattanayak C, Mishra M, Jena J. Comparative Analysis Of Haematinic Effect Of Dhatryarista With Standard Ferrous Sulphate In Tannic Acid Induced Iron Deficiency Anaemia In Albino Wistar Rats.
12. Mandal S, Dutta GK, Thakur KK, Nath S. Effect of Hygrophila spinosaExtract on Induced Anemic Wistar Rats and It's Comparison with Conventional Hematinics. Environment & Ecology. 2015 Apr;33(2A):910-3.
13. Goklaney D, Singh AP, Dhuria RK, Ahuja A. Therapeutic evaluation of mineral preparation for the amelioration of anaemia in goats of arid zone of Rajasthan. Iranian Journal of Applied Animal Science. 2012 Jun 1;2(2):137-41.
14. Dowdy RP, Matrone G. Copper-molybdenum interaction in sheep and chicks. The Journal of Nutrition. 1968 Jun 1;95(2):191-6.
15. Suva MA, Tirgar PR. Comparative assessment of anti-anemic effect of Sucrosomial iron in haloperidol-induced iron deficiency anemia in Wistar rats. Journal of Integrated Science and Technology. 2022 Aug 31;10(3):204-8.
16. Chaudhary B, Jyothi Y, Rabbani SI. Potential effect of Asparagus racemosus root extract on experimental anemic and thrombocytopenic conditions in rats. Journal of Pharmaceutical Research. 2016 Mar 1:15-9.
17. Tang N, Chen LQ, Zhuang H. Effects of heme iron enriched peptide on iron deficiency anemia in rats. Food & function. 2014;5(2):390-9.
18. Ousaaid D, Ghouizi AE, Laaroussi H, Bakour M, Mechchate H, Es-Safi I, Kamaly OA, Saleh A, Conte R, Lyoussi B, El Arabi I. Anti-anemic effect of antioxidant-rich apple vinegar against phenylhydrazine-induced hemolytic anemia in rats. Life. 2022 Feb 4;12(2):239.
19. Abdo EM, Shaltout OE, El-Sohaimy S, Abdalla AE, Zeitoun AM. Effect of functional beetroot pomace biscuit on phenylhydrazine induced anemia in albino rats: Hematological and blood biochemical analysis. Journal of Functional Foods. 2021 Mar 1;78:104385.
20. Gheith I, El-Mahmoudy A. Laboratory evidence for the hematopoietic potential of Beta vulgaris leaf and stalk extract in a phenylhydrazine model of anemia. Brazilian Journal of Medical and Biological Research. 2018 Oct 11;51:e7722.
21. Khandaker RK, Awal MA, Mostofa M, Haider MG, Khair A. Effects of Ferrous Sulphate and Spinach in Prevention of Lead Toxicity in Mice.
22. Rahman MM, Miah MA, Majumder S, Alam MM, Hosain MZ. Hematology of anorectic, anemic and hematinic treated cattle. Journal of the Bangladesh Agricultural University. 2005;3(1):95-7
23. Aduwamai UH, Abimbola MM, Ahmed ZH. Effect of Solanum nigrum methanol leaf extract on phenylhydrazine induced anemia in rats. Jordan J Biol Sci. 2018 Mar 21;11(1):65-71.
24. Agbor GA, Oben JE, Ngogang JY. Haematinic activity of Hibiscus cannabinus. African journal of Biotechnology. 2005 Sep 1;4(8):833-7.
25. Ashour TH. Hematinic and anti-anemic effect of thymoquinone against phenylhydrazine-induced hemolytic anemia in rats.
26. Sheth PA, Pawar AT, Mote CS, More C. Antianemic activity of polyherbal formulation, Raktavardhak Kadha, against phenylhydrazine-induced anemia in rats. Journal of Ayurveda and Integrative Medicine. 2021 Apr 1;12(2):340-5.
27. Ogbe RJ, Adoga GI, Abu AH. Antianaemic potentials of some plant extracts on phenyl hydrazine-induced anaemia in rabbits. Journal of Medicinal Plants Research. 2010 Apr 18;4(8):680-4.
28. Al-Shebli KS, Ali AA. Effect of different doses of ferrous sulfate drug on some haematological parameters in male albino rat. Al-Kufa University Journal for Biology. 2013 Apr 10;5(1).
29. Meena AK, Patidar D, Singh RK. Ameliorative effect of Hibiscus rosa-sinensis on phenylhydrazine induced haematotoxicity. International Journal of Innovative Research in Science, Engineering and Technology. 2014;3(2):8678-83.
30. Ibe OE, Akuodor GC, Elom MO, Chukwurah EF, Ibe CE, Nworie A. Protective effects of an ethanolic leaf extract from Ficus capensis against phenylhydrazine induced anaemia in Wistar

rats. Journal of Herbmed Pharmacology. 2022 Sep 19;11(4):483-9.
31. Henneh IT, Owusu Agyei PE, Obese E, Biney RP, Antwi-Adjei M, Yahaya ES, Asiamah E, Acquah-Mills J, Boateng DO, Ekor M. Leaf and root bark extracts of Ziziphus abyssinica Hochst ex. A. Rich (Rhamnaceae) ameliorate hepatic, renal and splenic injuries induced by phenylhydrazine in rats. Journal of basic and clinical physiology and pharmacology. 2021 Jan 29;32(1):20200111.
32. Allahmoradi M, Alimohammadi S, Cheraghi H. Protective effect of Cynara scolymus L. on blood biochemical parameters and liver histopathological changes in phenylhydrazine-induced hemolytic anemia in rats. Pharmaceutical and Biomedical Research. 2019.
33. Shen X, Song C, Wu T. Effects of nano-copper on antioxidant function in copper-deprived Guizhou black goats. Biological Trace Element Research. 2021 Jun;199:2201-7.
34. Ezeigwe OC, Nzekwe FA, Nworji OF, Ezennaya CF, Iloanya EL, Asogwa KK. Effect of aqueous extract of F. capensis leaves and its combination with C. aconitifolius leaves on essential biochemical parameters of phenylhydrazine-induced anemic rats. Journal of Experimental Pharmacology. 2020 Jul 1:191-201.
35. Nwauche KT, Anacletus FC, Ugwu GM. Hepatoprotective and Nephroprotective Potentials of Aqueous Leaves ExtractofLimoniaacidissimain Phenylhydrazine-Induced AnaemicWistar Rats. Asian Journal of Pharmaceutical Research and Development. 2019 Feb 15;7(1):27-33.
36. Ezugwu HC, Jankada PA, Ipav SS, Dasofunjo K. Anti-Anaemic And Hepato-Renal Activities Of Ethanol Leaf Extract Of Alchornea Cordifolia In Phenyl Hydrazine Induced-Anaemic Wistar Rats. Global journal of pure and applied sciences. 2022 Apr 1;28(2):221-9.
37. Mohammed O, Dyab N, Kheadr E, Dabour N. Effectiveness of inulin-type on the iron bioavailability in anemic female rats fed bio-yogurt. RSC advances. 2021;11(4):1928-38.
38. Kale OE, Awodele O, Akindele AJ. Protective effects of acridocarpus smeathmannii (DC.) guill. & perr. Root extract against phenylhydrazine-induced haematotoxicity, biochemical changes, and oxidative stress in rats. Biochemistry Insights. 2019 Nov;12:1178626419883243.

HOW TO CITE THIS ARTICLE
Chaphekar S, Jadhav ND, Rajurkar SR, Chigure GM, Rathod PR, Jadhav MD. Evaluation of anti-anemic activity of ferrous sulphate and copper sulphate in phenylhydrazine induced anemia in wistar rats. J Ayu Herb Med 2024;10(3):81-88. DOI: 10.31254/jahm.2024.10303