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Histoarchitectural investigation of herbo mineral formulation on phenylhydrazine induced anaemic wistar rats

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Abstract

The present study was planned to evaluate histoarchitectural changes of herbo mineral formulation on phenylhydrazine induced anaemic wistar rats for a period of 28 days. 56 Wistar rats of either sex were divided into 7 groups of 8 animals in each group. The rats in Group-I kept as healthy control while Group-II were kept as positive control and remaining 5 groups were kept as treatment groups. Group-II and remaining 5 treatment group were treated with phenylhydrazine @ 20 mg/kg body weight for 2 days to induce anaemia. Group-III was kept as standard control and treated with standard drug RBC rakt (vetoquinol) @ 0.5 ml/rat, orally. Group-IV and V treated with aqueous extract of Herbo-Mineral Formulation (*Hibiscus rosa sinensis* and Ferrous sulphate) @ 110 and 180 mg/kg b. wt. respectively, while Group-VI and VII treated with aqueous extract of Herbo-Mineral Formulation (*Boerhavia diffusa* and Copper sulphate) @ 150 and 300 mg/kg b. wt. respectively. Histopathological study of liver revealed focal necrosis, degenerative changes in hepatic parenchyma, dilatation of central vein. There was mild to moderate congestion, MNC infiltration in kidneys. Histopathological study of bone marrow revealed focal necrotic changes and adiposity. The section of spleen and heart revealed moderate congestion. All the treatment groups showed positive results in hematological and biochemical parameters.

Keywords: Histoarchitectural, anaemia, aqueous extract, wistar rats

Introduction

Anaemia is one of the most common dietary deficits of the blood, it affects people of all ages around the world, with children and teenagers having a far higher risk of developing the disorder. Anaemia is the second cause of death and the fourth most common reason for hospital admissions. The term "anaemia" refers to a broad range of illnesses where there is a decrease in the blood's ability to carry oxygen. It can result from a shortage of blood, red blood cells, or haemoglobin as it carries oxygen. Numerous other disorders may contribute to these diseases. Anaemia can be brought on by blood loss following an injury. It can also result from nutritional deficiencies, including a lack of certain vitamins and minerals necessary for the formation of haemoglobin. Because haemoglobin is the pigment that gives blood cells their red colour, a deficiency in this pigment will result in paler blood cells, giving rise to the term hypochromic, or deficient in colour. Iron deficiency is the most typical cause of anaemia in adults [1]. The WHO has recognized iron deficiency anaemia (IDA) as the most common nutritional deficiency in the world, with 30% of the population being affected with this condition [2]. Phenylhydrazine has long been known as a haemolytic agent and historically it was used as a therapeutic agent in the treatment of polycythaemia vera [3].

A well-known medicinal plant *Hibiscus rosa-sinensis* and *Boerhavia diffusa* have been used as analgesic, antipyretics, antiasthmatic, anti inflammatory, anti cancer, anti oxidant, antifungal, antibacterial and gastro- intestinal, hepatoprotective, gynecological purposes respectively. *Boerhavia diffusa*. Copper is a required element for the utilization of iron and the synthesis of haemoglobin. Anaemia, growth problems, bone deformity, loss of hair pigmentation, diarrhoea, myelinization problems, and cardiac lesion all are symptoms of copper deficiency in animals. Haemoglobin has iron as a component. The end result of iron deficiency is characterized by microcytic hypochromic anaemia. Because of iron shortage, microcytic hypochromic anaemia

Develops. Vitamin B₁₂ contains cobalt, which is required for erythrocyte development. Recent research has found that supplementing with haematinics (Cu, Fe, Co, and vitamin B₁₂) removed the fundamental causes of nutritional anaemia, and increased erythropoiesis ^[4].

Material and Methods

Drugs:

Phenylhydrazine- Phenylhydrazine was used to produce anaemia in Wistar rats.

RBC Rakt (Vetoquinol): RBC Rakta a product available in market manufactured and marketed by Vetoquinol, Pvt. Ltd, Thane. Batch no. FVRT22003 manufacturing date Jan/2022 Expiry date Jan/2024. At the dose rate of 0.5 ml per rat per day was used as standard drug in the present investigation.

Copper sulphate- Copper sulphate used to treat anaemia in Wistar rats

Methods

Location

The research was conducted in the Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra.

Experimental animals

The present study was conducted on 56 Wistar rats of either sex, 8- 10 weeks age and 200-300 g body weight. Animals were randomly selected after physical and behavioural examination; the live body weight range was within ± 20 percent of the mean body weight. The treatment was given once daily per orally for 28 consecutive days. In the experimental room of Laboratory Animal House, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, all the rats were maintained as per specified laboratory conditions with ad-libitum water and feed throughout the experimental period. The Institutional Animals Ethical Committee (IAEC) approved the experimental protocol, which met the national guidelines as per the guidelines of Committee for Control and Supervision of Experiments on Animals (CCSEA) vide Resolution no. IAEC/93/22 dated 05/07/2022. The experimental animals were maintained under continual observation for at least five days before the experiment.

Husbandry

Housing	Cage system
Temperature	22 $\pm$ 3°C
Relative Humidity	30-70%
Photoperiod	12 hours light and 12 hours dark period
Light intensity	130-400 Lux
Feed	Pelleted diet
Water	Clean water purified by aqua guard water system

Animal housing

The animals were kept in laboratory animal house, College of Veterinary and Animal Sciences, Parbhani, Maharashtra. Sterilised bedding material and cages were replaced at least three times a week.

Environmental conditions

Wistar rats were kept in an environmentally controlled room

With 22 $\pm$ 3 °C temperature and 30-70 % relative humidity. Floor was swept and mopped with a disinfectant solution every day.

Drinking water and diet

Throughout the trial, the animals were given purified ad-libitum drinking water and laboratory animal pellet feed (VRK pellet feed, Sangli, Maharashtra).

Acclimatization and grouping

Wistar rats were subjected to a five-day acclimatisation period in an experimental environment. After acclimating to the experimental circumstances, the 56 Wistar rats were separated into seven groups, each having 8 rats of either sex. All the animals were kept for the 28 days experimental period.

Animal numbering and identification

Four Wistar rats were housed in an individual cage. Each cage included a label containing the animal's details; cage number, group details, treatment given and marking.

Induction of Anaemia

Anaemia was induced using phenylhydrazine @ 20 mg/kg, 2 days, IP in Wistar rats as per the guidelines, and blood collection in an anaemia induced groups is done after induction of anaemia & that day is considered as 0 day.

Technical programme

The present study was conducted to study the effects of aqueous extract Herbo-Mineral Formulation on phenylhydrazine induced anaemic Wistar rats, as per the guidelines.

The Herbo-Mineral Formulation were synthesised for use in the current study. The treatment was given once daily per orally for 28 consecutive days.

Collection and authentication of plant material

Department of Botany, Vasantrao Naik Marathwada Agricultural University, Parbhani, recognised the *Hibiscus rosa sinensis* and *Boerhavia diffusa* roots extract plant material. The material was collected in bulk from the same farm and transported in batches to the laboratory, where it was processed for further research.

Hibiscus Rosa sinensis leaves extract and Boerhavia diffusa roots extract

Aqueous extract of *Hibiscus Rosa sinensis* leaves extract was prepared by cold extraction method. They were washed and allowed to dry completely under shade. Dried plant material were ground to powder with the help of an electrical grinder. Then, 20% of aqueous solution was made by dissolving 200 grams of powder in double distilled water and the final quantity was made to 1 liter. It was mixed thoroughly and allowed to soak for 48 hours at room temperature. It was shaken intermittently with an electrically operated flask shaker. Thus, resulting solutions were first filtered by using muslin cloth and then by ordinary filter paper onto glass plates. They were allowed to dry under shade and aqueous extract of plant material obtained. The weight of empty glass plates was noted and final weight after drying was obtained. The increase in weight was considered as the amount of aqueous extract obtained. Resulting extracts were scraped off the glass plate and stored in an air tight container at 40 % & used whenever necessary (Plate No. 1).



A) Hibiscus rosa sinensis whole plant



B) Powder of Hibiscus rosa sinensis leaves after grinding of dried leaves



C) Aqueous extract of Hibiscus rosa sinensis leaves on glass plate prepared from its powder

Plate 1: Preparation of Hibiscus rosa sinensis leaves extract



A) Boerhavia diffusa whole plant



B) Boerhavia diffusa roots



C) Powder of Boerhavia diffusa roots after grinding of dried roots



D) Aqueous extract of Boerhavia diffusa roots on glass plate prepared from its powder

Plate 2: Preparation of *Boerhavia diffusa* roots extract

Pathological observations

All the rats of seven groups were sacrificed on the termination of the experiment and the organs were subjected to the gross and microscopic examinations.

Organ weight

The absolute organ weights of liver, kidneys, spleen, heart, and lung tissues were noted in control and treatment groups at the end of the experimental study.

Gross pathology

The systematic and scientific examination of each and every organ was made with naked eye and the gross findings were recorded.

Histopathological changes

The visceral organs like liver, kidneys, heart, spleen, lungs and bone marrow were collected immediately after sacrificing the rats by inhalation anaesthesia using carbon monoxide and processed separately for histopathological examination. The bone marrow is collected from femur or tibia by cutting both the epiphysis of bone and extraction is performed with cannula. The tissue samples were fixed in 10% Neutral Buffer Formalin

as per the time schedule (Singh and Sulochana., 1997) [5]. The fixed tissues of visceral organs were cut into proper size pieces of small thickness and processed routinely to obtain paraffin sections of about 3-5 μ . The sections of tissues were stained by Mayers Haematoxyline and Eosin staining method [5] [5] for histopathological studies.

Statistical analysis

The data from various parameters from all groups was evaluated and interpreted using Panse and Sukhatme [6] method of factorial randomised block design (FRBD) and completely randomized block design (CRD).

Results and Discussion

The purpose of present study was to evaluate effects of Herbo-Mineral Formulation (*Hibiscus rosa sinensis* & Ferrous sulphate) and (*Boerhavia diffusa* & Copper sulphate) on phenylhydrazine induced anaemic Wistar rats under experimental conditions.

During acclimatisation period, all the experimental rats were alert, active, and healthy, with no adverse effects, indicating proper acclimatisation. The animals in the control group were active and alive throughout the study. After induction of anaemia, rats in all the groups were found to be sluggish, restlessness and lethargic for 2-3 days. Significant increase observed in absolute organ weight of liver and lung in treatment groups when compared with healthy control group-I.

Gross pathology of organs

The experimental trial was evaluated through gross and histopathological examination of visceral organs. The experimental rats of group-I to group-VII were sacrificed on 28th day of trial. The gross examination of liver shows dark colour, multifocal necrosis, dark colour recorded in positive control group-II. The size of liver and spleen was increased in all treatment group compared to positive control group-II. Kidney shows no morphological alterations on gross examinations. The size of lung increased in all treatment groups compared to positive control group-II.

Histopathological examination

The study was evaluated through histopathological examination of kidney, spleen, liver, lung, heart, and bone marrow. The experimental rats of group-I to group-VII were sacrificed on 28th day of trial. After sacrifice pieces of kidney, liver, heart, spleen, lung, and bone marrow were collected for further histopathological examination.

Kidney

On Histoarchitectural studies the sections of kidney from the rats of positive control group-II revealed mild to moderate congestion, coagulative necrosis, vacuolar degenerative changes in epithelial lining of PCT and mononuclear cells infiltration at corticomedullary junction (Plate 3, 4 and 5). Our results were in accordance with [7] who administered intraperitoneal injection of phenylhydrazine at the dose of 40 mg/kg b. wt. in rats for 2 days and recorded parallel histopathological changes. [8] who observed similar histopathological changes at the dose of 60 mg/kg b. wt. orally in rats. The histopathological examination of kidney from rats of all other treatment groups-IV, V, VI and VII did not show any appreciable changes (Plate 6, 7 and 8).

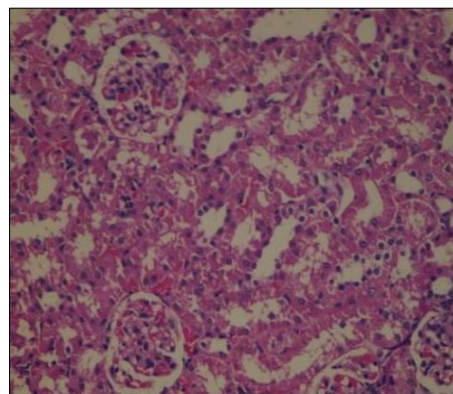


Plate 3: Microphotograph of kidney of rat of group-II showing mild congestion and vacuolar degenerative changes in epithelial lining of PCT (H and E x 400)

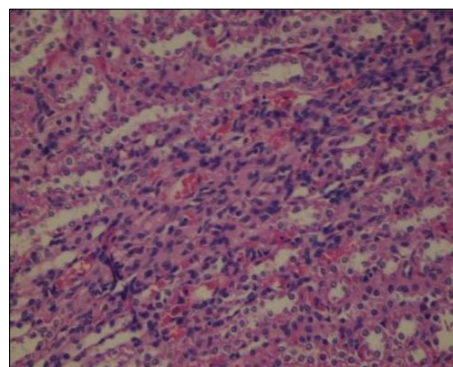


Plate 4: Microphotograph of kidney of rat of group-II showing mild congestion and vacuolar degenerative changes in epithelial lining of PCT (H and E x 400)

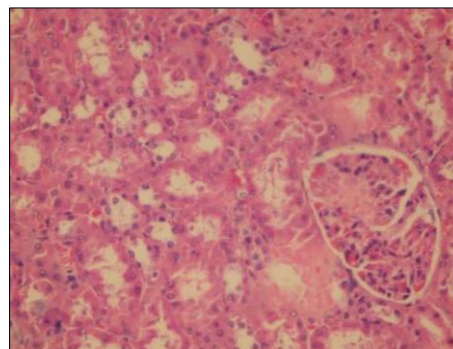


Plate 5: Microphotograph of kidney of rat of group-II showing focal coagulative necrotic changes (H and E x 400)

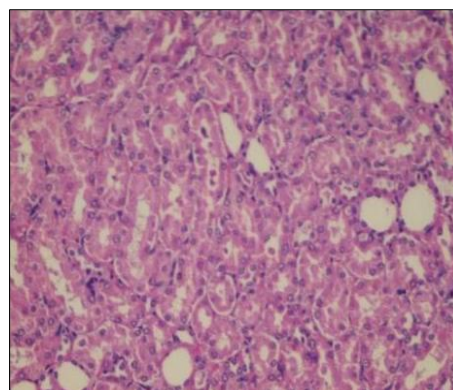


Plate 6: Microphotograph of kidney of rat of group-IV showing apparently normal histoarchitecture (H and E x 400)

Liver

The histomorphological study of liver from the positive control group-II showed focal necrosis, degenerative changes in hepatic parenchyma, multifocal congestion, and severe dilatation of central vein (Plate 9, 10 and 11). The histopathological examination of liver from rats of all treatment groups-IV, V, VI and VII did not show any appreciable changes (Plate 12, 13, 14 and 15). Our results obtained were in the agreement with ^[9] who administered intraperitoneal injection of phenylhydrazine @ 40 mg/kg b. wt. for 2 days in rats and recorded similar histopathological damage. ^[10] observed comparable changes in liver after administration of phenylhydrazine @ 40 mg/kg b. wt. intraperitoneally. ^[8] also observed similar histopathological changes after administration of phenylhydrazine @ 60 mg/kg b. wt. orally in rats.

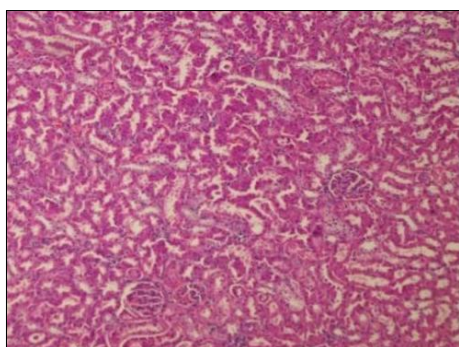


Plate 7: Microphotograph of kidney of rat of group-V showing apparently normal histoarchitecture (H and E x 400)

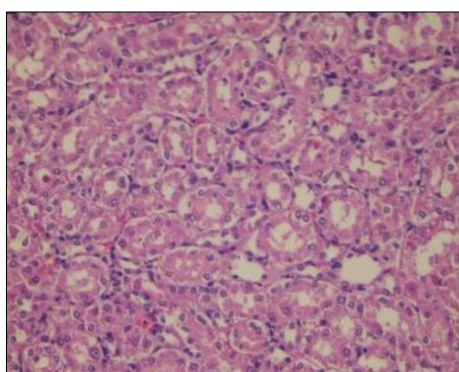


Plate 8: Microphotograph of kidney of rat of group-VI showing apparently normal histoarchitecture (H and E x 400)

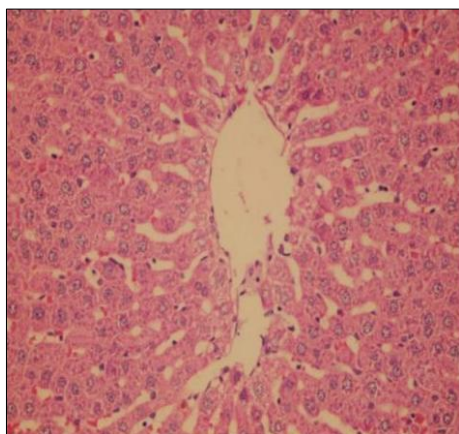


Plate 9: Microphotograph of liver of rat of group-II showing focal necrosis and degenerative changes in hepatic parenchyma (H and E x 400)

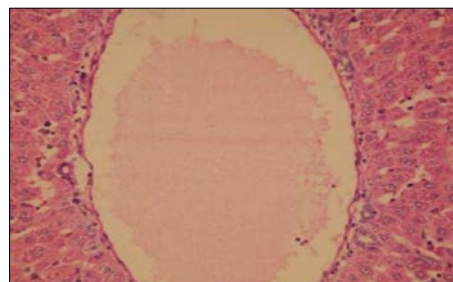


Plate 10: Microphotograph of liver of rat of group-II showing severe dilatation of central vein (H and E x 400)

Heart

The sections of heart from the rats of treatment groups did not show any appreciable changes (Plate 16). Section of heart from rats of positive control group-II show multifocal congestion (Plate 17 and 18). The similar findings observed by ^[7] who administered intraperitoneal injection of phenylhydrazine @ 40 mg/kg b. wt. for 2 days in rats.

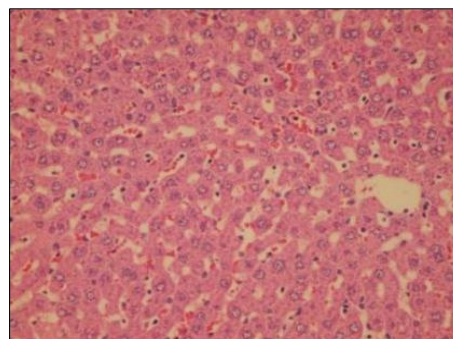


Plate 11: Microphotograph of liver of rat of group-II showing multifocal congestion and changes in parenchyma (H and E x 400)

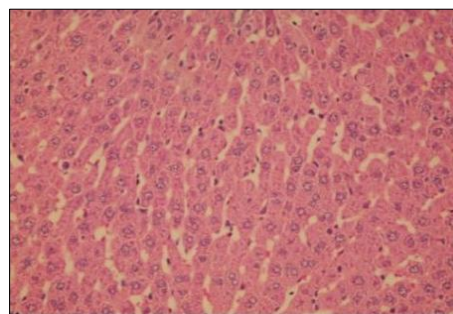


Plate 12: Microphotograph of liver of rat of group-V showing apparently normal histoarchitecture degenerative (H and E x 400)

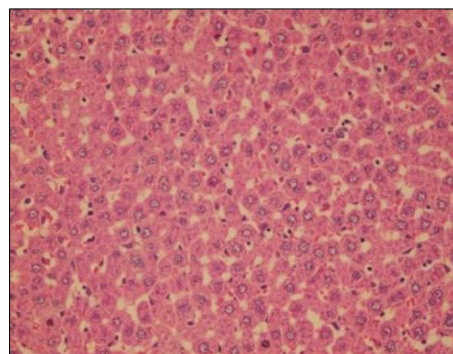


Plate 13: Microphotograph of liver of rat of group-IV showing apparently normal histoarchitecture (H and E x 400)

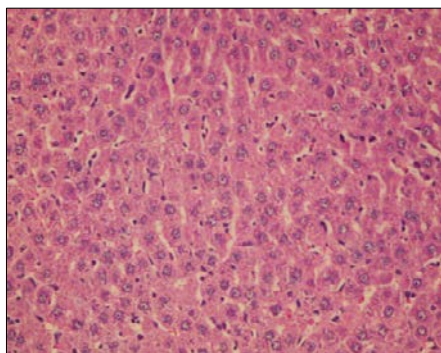


Plate 14: Microphotograph of liver of rat of group-VI showing apparently normal histoarchitecture (H and E x 400)

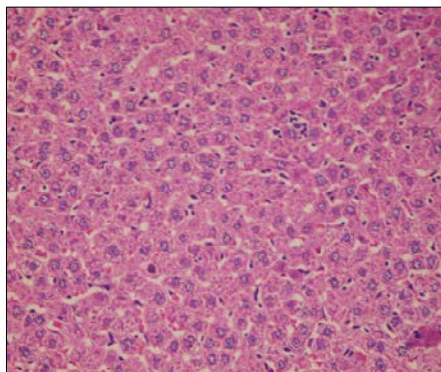


Plate 15: Microphotograph of liver of rat of group-VII showing apparently normal histoarchitecture (H and E x 400)

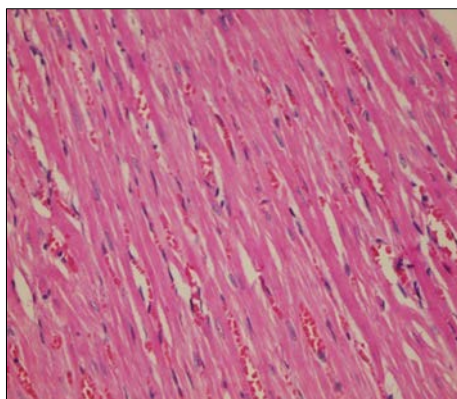


Plate 16: Microphotograph of heart of rat of group-II showing multifocal congestion (H and E x 400)

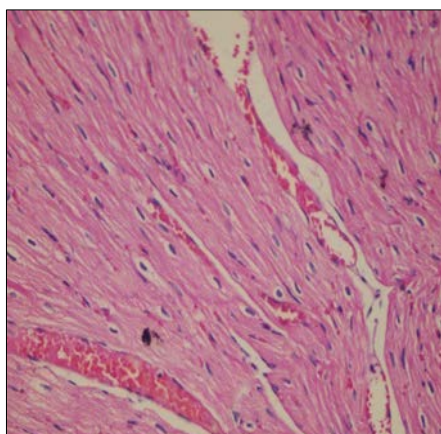


Plate 17: Microphotograph of heart of rat of group-IV showing apparently normal histoarchitecture (H and E x 400)

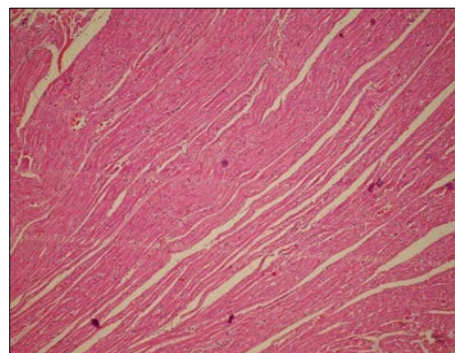


Plate 18: Microphotograph of heart of rat of group-VI showing apparently normal histoarchitecture (H and E x 400)

Spleen

On histoarchitectural studies the sections of spleen from the rats of group-II revealed congestion (Plate 19). Our histoarchitectural findings of spleen were in the agreement with [10] who administered intraperitoneal injection of phenylhydrazine @ 40 mg/kg b. wt. in rats. The spleen on its histoarchitectural studies did not show appreciable change in the rats of treatment groups (Plate 20).

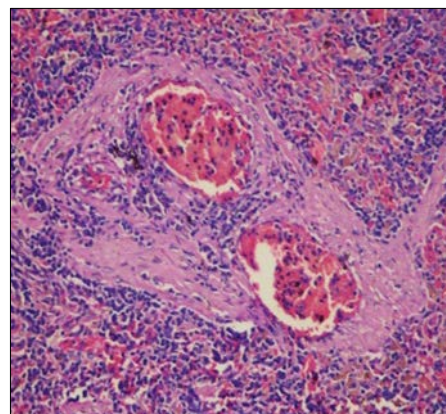


Plate 19: Microphotograph of spleen of rat of group-II showing congestion (H and E x 400)

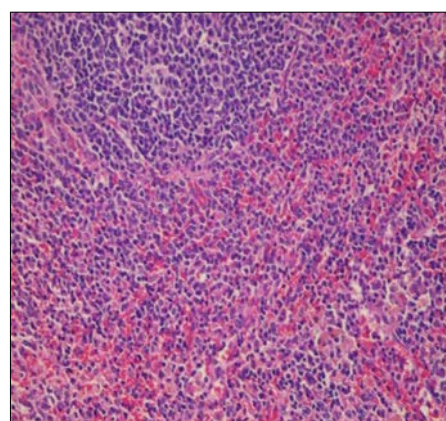


Plate 20: Microphotograph of spleen of rat of group-IV showing apparently normal histoarchitecture

Lung

The sections of lungs from the rats of control as well as other treatment related experimental groups did not show any histomorphological changes. The similar findings observed by [10] after intraperitoneal administration of phenylhydrazine @ 40 mg/kg b. wt. in rats.

Bone marrow

The sections of bone marrow from rats of positive control group-II revealed minimal focal necrotic changes and adiposity (Plate 21 and 22). The histopathological sections from control and treatment groups did not show any noticeable changes (Plate 23, 24, 25 and 26). Our results obtained were in accordance with the findings of [10] who administered phenylhydrazine @ 40 mg/kg b. wt. in rats intraperitoneally.

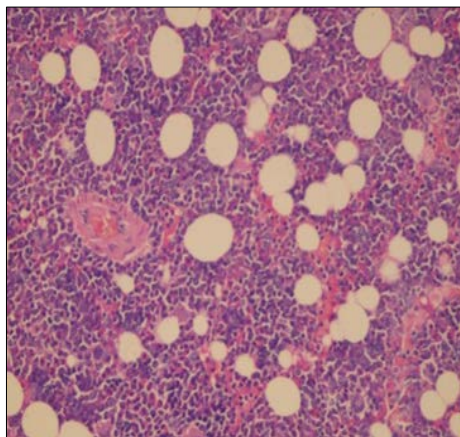


Plate 21: Microphotograph of bone marrow of rat of group-II showing focal adiposity (H and E x 400)

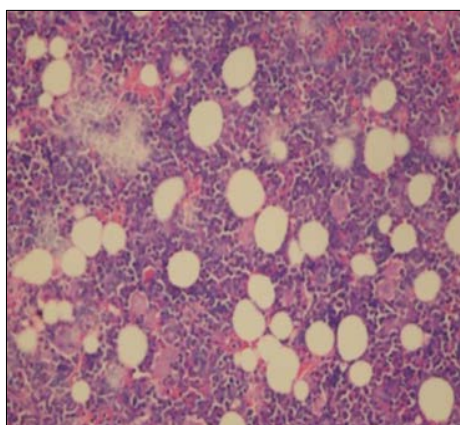


Plate 22: Microphotograph of bone marrow of rat of group-II showing necrotic changes and adiposity

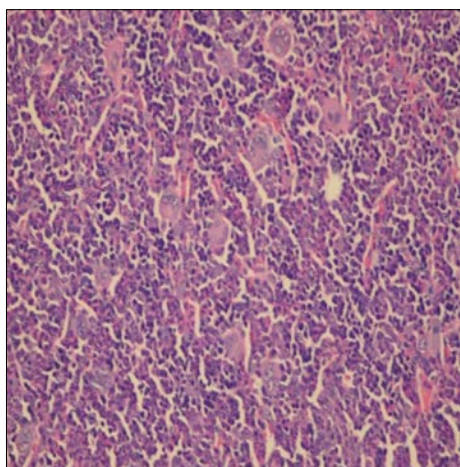


Plate 23: Microphotograph of bone marrow of rat of group-IV showing apparently normal histoarchitecture (H and E x 400)

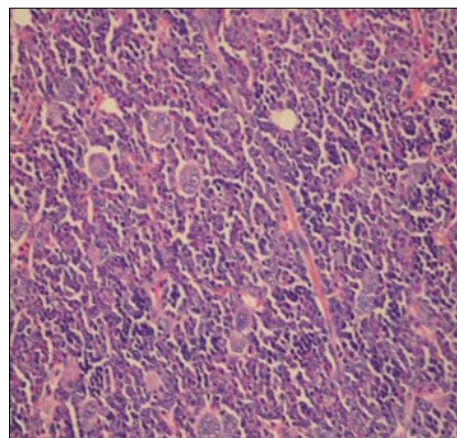


Plate 24: Microphotograph of bone marrow of rat of group-V showing apparently normal histoarchitecture (H and E x 400)

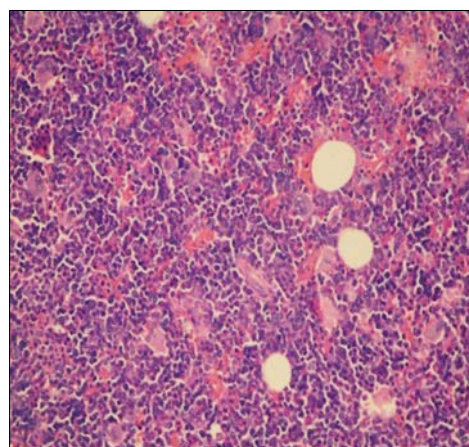


Plate 25: Microphotograph of bone marrow of rat of group-VI showing apparently normal histoarchitecture (H and E x 400)

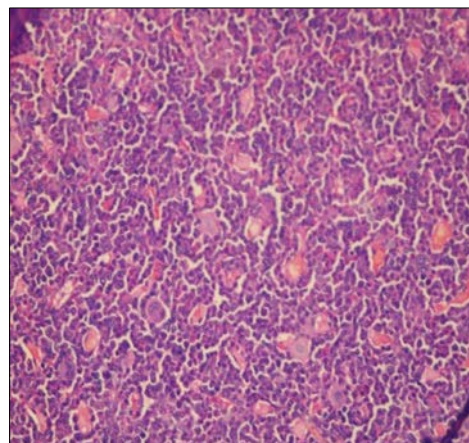


Plate 26: Microphotograph of bone marrow of rat of group-VII showing apparently normal histoarchitecture (H and E x 400)

Conclusion

From present investigation it can be concluded that significant histoarchitectural changes were observed in positive control group suggest oxidative effect of phenylhydrazine as no any supplementary treatment is given. In all other treatment groups and healthy control group no histoarchitectural changes seen indicating antioxidant properties of Herbo-Mineral Formulation.

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