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AN EXPERIMENTAL STUDY TO EVALUATE THE HAEMATINIC EFFECT OF INDIGENEOUS DRUG FORMULATION IN PHENYLHYDRAZINE INDUCED ANEMIC PREGNANT WISTAR ALBINO RATS

Neethu[1*], Vidya Ballal[2], Mamatha K. V.[3] and Sudakar Bhat[4]

[1]PG Scholar, Dept. of Prasuthi Tantra and Stree Roga, Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital, Udupi, Karnataka.

[2]Associate Professor, Dept. of Prasuthi Tantra and Stree Roga, Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital, Udupi, Karnataka.

[3]Professor, Dept. of Prasuthi Tantra and Stree Roga, Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital, Udupi, Karnataka.

[4]Research Officer, Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka.

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***Corresponding Author**
Neethu
PG Scholar, Dept. of Prasuthi Tantra and Stree Roga, Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital, Udupi, Karnataka.

ABSTRACT

Pregnancy is a physiological process, associated with certain adaptation, which when not taken care in the antenatal period can lead to anemia in pregnancy. Iron deficiency anemia is the most common type of anemia met in obstetric practice. About 4 to 16% of maternal deaths are due to anemia. It also increases the maternal morbidity, fetal and neonatal mortality and morbidity significantly. In Ayurveda this condition is considered as *Garbhini Pandu*. The *rasa* and *rakta* of the mother are carried to the fetus for its proper growth and development. If not, it leads to *rasa dhatu kshaya* in *garbhini* which finally leads to *Garbhini Pandu*. Ayurveda highlights the contradiction of *shodana* in pregnant ladies, hence *shamana chikitsa* can be followed judiciously. Contemplating the essentiality to use a drug that is beneficial in *Garbhini pandu*, safe and conducive during pregnancy, certain indigeneous drugs like *Agasthya, Amalaki, Draksha, Musta, Pippali, Sigru, Shuddha Kasisa* and *bhavana dravyas* like *Dadima,*

Bhringaraja and *Mandukaparni* has taken. So an experimental study is essential prior to the clinical study inorder to understand the toxic effect and haematinic effect, owing to its properties like *raktavardhaka, yakrututtejaka, deepana, medhya, rasayana and krimigna.* The selected animal was grouped into 5 groups with 6 animals each. The animal experiment showed that the indigeneous drug formulation is found efficacious in increasing the hemoglobin concentration and serum iron, and no toxic effects have been observed throughout the duration of the study.

KEYWORDS: Anemia, Hemoglobin, Indigeneous drug formulation, Serum iron.

INTRODUCTION

Motherhood is the greatest gift of nature. A magnificent world full of excitement, fantasy, dread, fragility, and ultimate curiosity awaits pregnant women. Pregnancy is a state in which all the physiological functions are hyper stimulated in order to meet the demands of growing fetus**.** The continuous physical adaptation to meet and anticipate the demands of the growing foetus, to provide a stable environment in which its growth can take place for foetal needs is the aim of safe pregnancy. Good prenatal care is essential to ensure not only the health of the mother, but also the wellbeing of the baby. Nutrition requirements are very high in pregnancy. Among the various diet factors; iron is one which is in increased demand by the mother, fetus & placenta. Extra demand if not fulfilled will lead to disease Anemia.[1]

Anemia in pregnancy is one of the important public health problems. About 4 to 16% of maternal deaths are due to anemia. It also increases the maternal morbidity, fetal and neonatal mortality and morbidity significantly. Indeed, it is a known risk factor for many maternal fetal complications like preterm labor, increased risk of infection, PPH and fetal complications like low birth weight, growth retardation.[2]

According to Charaka, *pandu roga* is characterized by *pitta* dominance. When discussing *masanumasika garbha vruddhi*, it is mentioned that the *mamsa* and *shonita* increases during the 5th month and further intensifies in the 6th month. As a result, *balavarnahani* occurs during the 6th month of pregnancy.[3] Consequently, pregnant women may experience a decline in strength and skin radiance during this period, along with symptoms indicating primarily the loss of *rakta* and *mamsa* components. It can be considered as reference for *Garbhini Pandu*.

Thus *Garbhini Pandu* can be correlated with anemia in pregnancy. As said by Acharya Charaka, *Garbhini* should be treated like *poornameva tailapatram* and since *shodhana karma* is contraindicated in pregnancy, only *shamana oushadhas* can be formulated and advised.[4] So, considering the necessity to have a drug which is efficacious in *pandu roga* and without having any untoward effect on pregnancy. An indigeneous drug formulation owing to its properties like *raktavardhaka, yakrututtejaka, deepana, medhya, rasayana and krimigna* is considered for the present study.

MATERIALS AND METHOD

Experimental Design

Inclusion Criteria

- Healthy pregnant female albino rats with average weight of 225±50gm were selected.
- Pregnant rats were taken from the first day of gestational period.

Exclusion Criteria

- Diseased and injured rats.
- Rats which are under trials of other experiments.

Procedure- The animals were obtained from the animal house attached to the Pharmacology Laboratory, SDM Centre for Research in Ayurveda and Allied Science, Udyavara. The experiments were carried out in conformity with guidelines of the Institutional Animal Ethical Committee (IAEC) after obtaining its permission.

Ethical Committee Approval Number- SDMCRA/IAEC/SU-P-10.

Animal Grouping.

GROUP	DRUG USED	NUMBER OF RATS
Group 1 (normal group)	Food pellets and water	6
Group 2 (positive control)	Phenyl hydrazine	6
Group 3 (standard group)	PHZ+Ferrous fumarate	6
Group 4 (AOT group)	Indigeneous drugs	6
Group 5 (test group)	PHZ+Indigeneous drugs	6

Test drug: Indigeneous drug formulation as choorna is administered internally diluted with buttermilk.

Dose Selection: The dose determination of test drug will be carried out based on Acute Oral Toxicity test. AOT will be carried out following OECD Guidelines 425.LD50 is greater than 2000mg/kg.

ROUTE OF DRUG ADMINISTRATION: The test drug was administered by oral route with the help of No.8 infant feeding tube and Phenyl hydrazine was administered through Intra-Peritoneal Injection.

Duration of study: 21 Days.

Haematinic study procedure

30 pregnant rats were selected and grouped into 5 groups of 6 rats each. After overnight fasting, 6 pregnant rats were kept as normal control group, while 24 pregnant rats were made anaemic by intra-peritoneal injection of phenyl hydrazine (0.025 mg/g bodyweight of rat) dissolved in 1 ml of dimethyl sulphoxide on days- 1, 3 and 5. These anaemic rats were randomly divided into 4 groups and treated as follows; for 5th group, indigeneous drug formulation at the dose of 400mg/20ml buttermilk/ bodyweight was given orally once in a day for 17 days from day 5-day 21. For the standard group, Ferrous fumarate was administered in the dose of 210mg/g. The blood collection was performed on Day 1, Day 5, and Day 21 of the study by puncturing the retroorbital plexus.[5] The collected blood samples were evaluated for various parameters, including complete blood count and biochemical tests.

RESULT

The result of the experimental study on Indigeneous drug formulation have been enumerated as follows.

Table no 1: Effect of test drug (Indigeneous drug formulation) on complete blood count.

PARAMETERS	BEFORE TREATMENT	AFTER TRATMENT
HB	9.41±0.32	13.8±0.24**
WBC	14.85±1.90	11.94±1.01
RBC	3.85±0.17	5.85±0.07**
PCV	28.45±0.66	41.85±0.62**
MCV	74.15±2.05	71.46±0.76
MCH	24.48±0.38	23.55±0.25
MCHC	33.06±0.49	32.93±0.21
RDW-CV	27.16±0.09	18.35±0.69**

RDW-SD	82.68±2.40	52.88±2.21**
PLT	10.72±0.29	5.39±0.36**

Table no 2: Effect of Indigeneous drug formulation on Hemoglobin.

GROUPS	Hb(g/dl)	% CHANGE
NORMAL CONTROL	14.68±0.28	-
POSITIVE CONTROL	12.3±0.33*	16.21↓@
FERROUS FUMARATE	14.16±1.02	15.12↑#
INDIGENEOUS DRUG	13.8±0.24	12.19↑#

Table no 3: Effect of Indigeneous drug formulation on Total WBC.

GROUPS	WBC(10^3/uL)	% CHANGE
NORMAL CONTROL	9.74±0.43	-
POSITIVE CONTROL	11.30±1.49	16.09↑@
FERROUS FUMARATE	11.97±1.21	5.92↑#
INDIGENEOUS DRUG	11.94±1.01	5.66↑#

Table no 4: Effect of Indigeneous drug formulation on RBC.

GROUPS	RBC(10^6/uL)	% CHANGE
NORMAL CONTROL	7.10±0.11	-
POSITIVE CONTROL	6.48±0.19	8.75↓@
FERROUS FUMARATE	5.88±0.40	9.19↓#
INDIGENEOUS DRUG	5.85±0.07	9.77↓#

Table no 5: Effect of Indigeneous drug formulation on PCV.

GROUPS	PCV(%)	% CHANGE
NORMAL CONTROL	42.55±1.45	-
POSITIVE CONTROL	42.47±1.39	0.188↓@
FERROUS FUMARATE	41.33±2.94	2.684↓#
INDIGENEOUS DRUG	41.85±0.62	1.459↓#

Table no 6: Effect of Indigeneous drug formulation on MCV.

GROUPS	MCV(fL)	% CHANGE
NORMAL CONTROL	58.71±0.50	-
POSITIVE CONTROL	65.62±1.89**	16.5↓@
FERROUS FUMARATE	70.2±1.32	6.97↑#
INDIGENEOUS DRUG	71.46±0.76*	8.89↑#

Table no 7: Effect of Indigeneous drug formulation on MCHC.

GROUPS	MCHC(g/dl)	% CHANGE
NORMAL CONTROL	32.78±2.52	-
POSITIVE CONTROL	35.37±0.82	7.90↑@
FERROUS FUMARATE	34.26±0.18	3.13↓#
INDIGENEOUS DRUG	32.93±0.21	6.89↓#

Table no 8: Effect of Indigeneous drug formulation on MCH.

GROUPS	MCH(pg)	% CHANGE
NORMAL CONTROL	23.03±2.29	-
POSITIVE CONTROL	23.36±0.27	4.34↑@
FERROUS FUMARATE	24.05±0.36	2.95↑#
INDIGENEOUS DRUG	23.55±0.25	0.81↑#

Table no 9: Effect of Indigeneous drug formulation on RDW-SD.

GROUPS	RDW-SD(fL)	% CHANGE
NORMAL CONTROL	53.61±8.98	-
POSITIVE CONTROL	54.51±2.88	1.67↑@
FERROUS FUMARATE	61.61±4.82	13.02↑#
INDIGENEOUS DRUG	52.88±2.21	2.99↓#

Table no 10: Effect of Indigeneous drug formulation on RDW-CV.

GROUPS	RDW-CV(%)	% CHANGE
NORMAL CONTROL	19.33±0.76	-
POSITIVE CONTROL	23.45±0.69**	21.31↑@
FER1ROUS FUMARATE	21.45±1.42	8.52↓#

Table no 11: Effect of Indigeneous drug formulation on Platelet.

GROUPS	Platelet(10^3/uL)	% CHANGE
NORMAL CONTROL	5.57±0.92	-
POSITIVE CONTROL	5.11±0.45	8.93↓@
FERROUS FUMARATE	7.00±0.91	37.00↑#
INDIGENEOUS DRUG	5.39±0.36	5.41↑#

Table no 12: Effect of Indigeneous drug formulation on Serum iron.

GROUPS	SERUM IRON(mcg/dl)	% CHANGE
NORMAL CONTROL	299.38±10.28	-
POSITIVE CONTROL	154.09±19.82**	48.53↓
FERROUS FUMARATE	235.56±41.93	52.87↑
INDIGENEOUS DRUG	237.59±27.17	54.18↑

Data: MEAN ± SEM, *p<0.05, **p<0.01

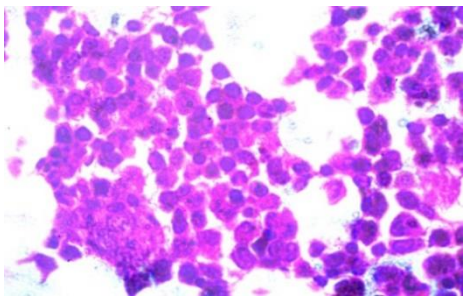
- compared with normal control group

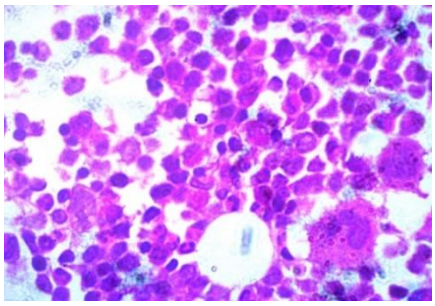
@- compared with positive control

Histopathology Report

1. BONE MARROW

NORMAL CONTROL





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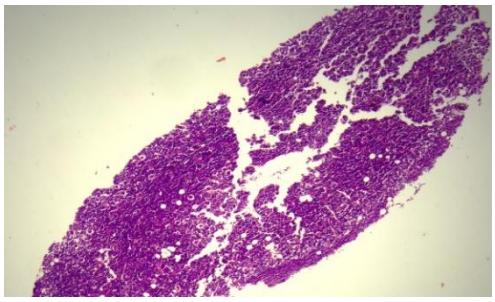

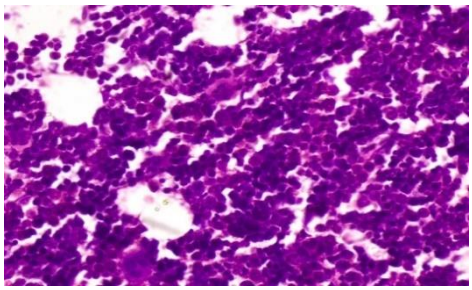

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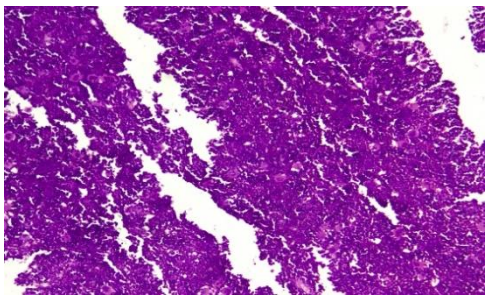

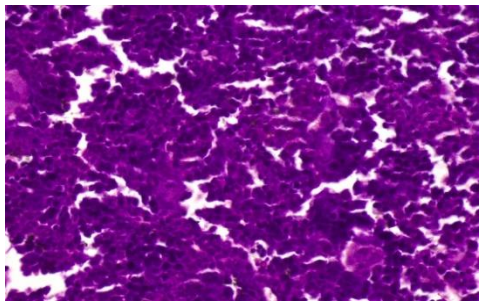

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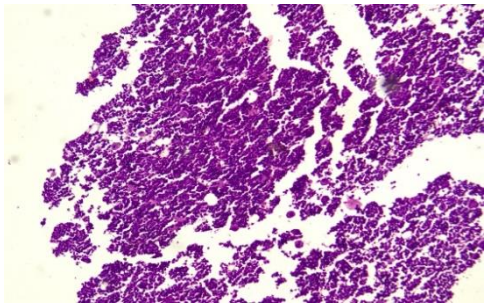
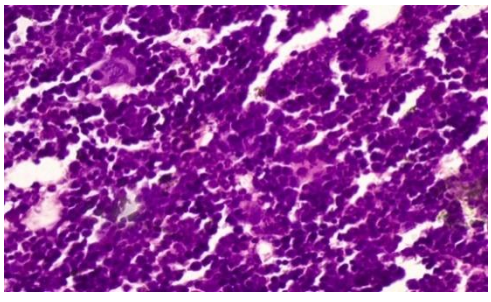
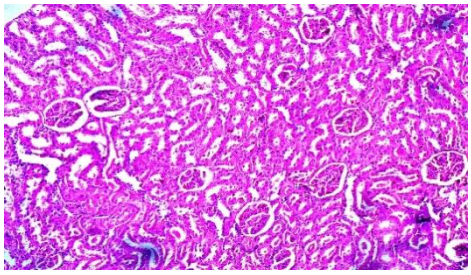
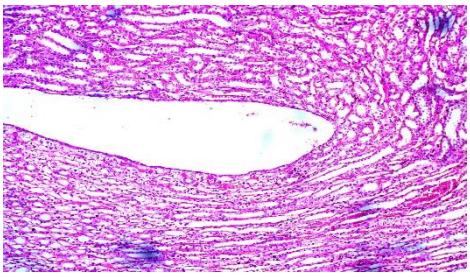




2. KIDNEY

NORMAL CONTROL

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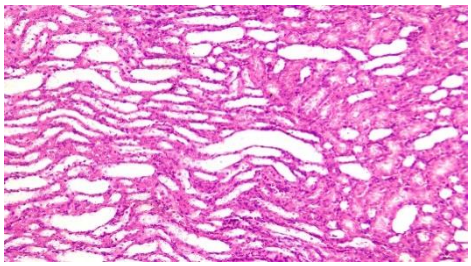
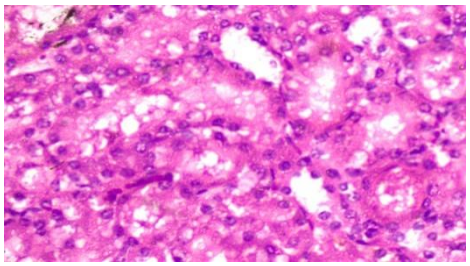

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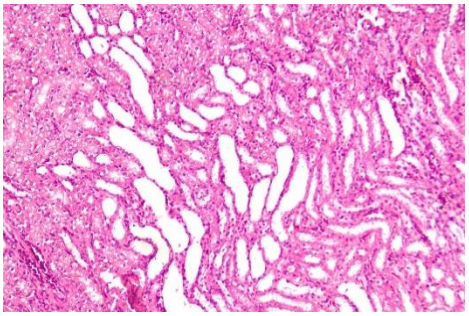
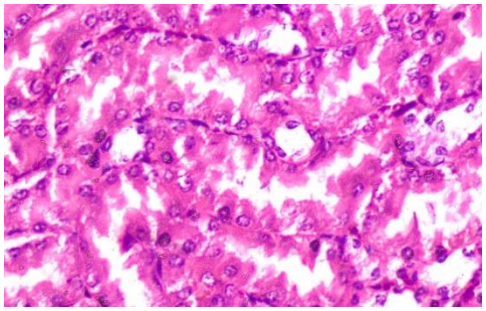

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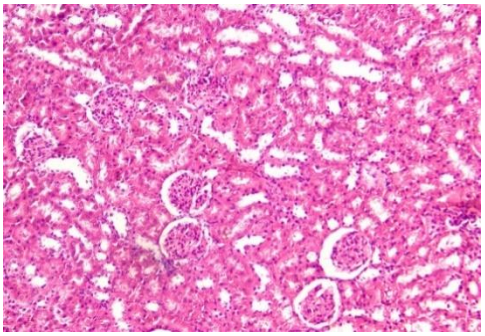
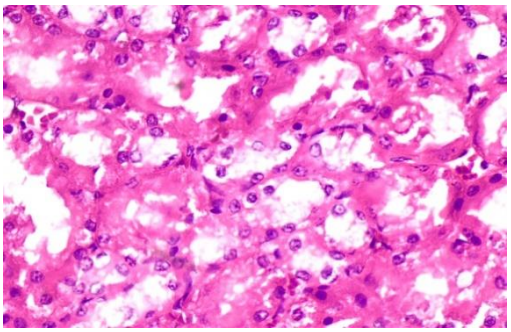
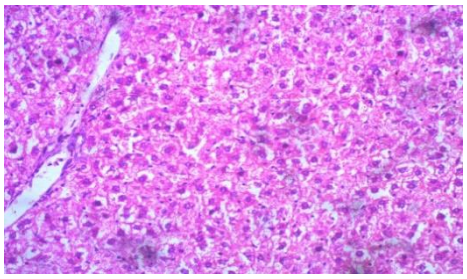
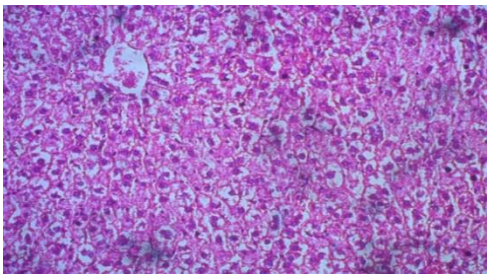



3. LIVER

NORMAL CONTROL

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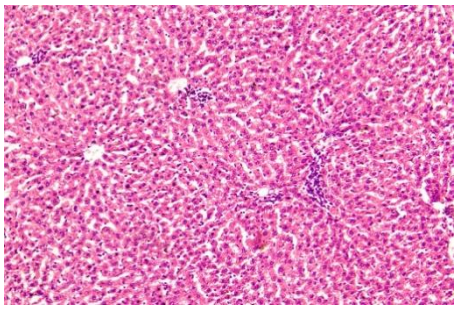
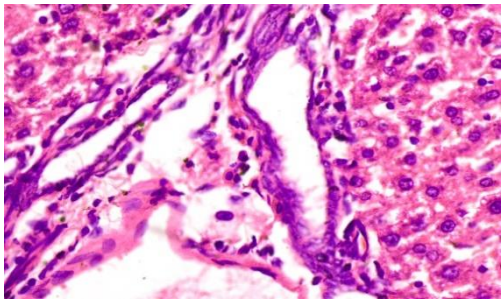

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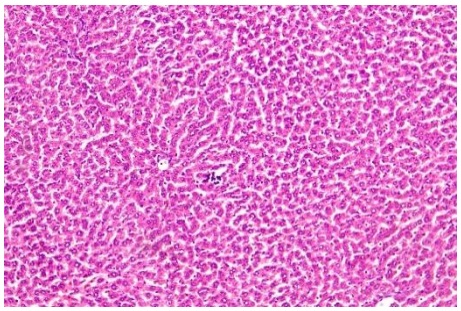
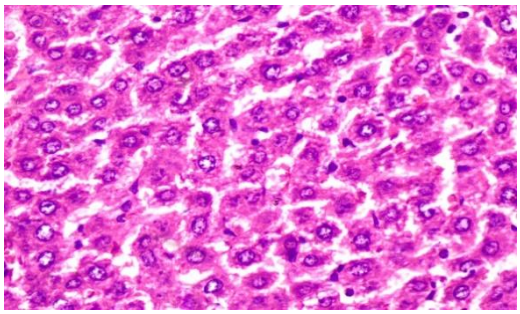

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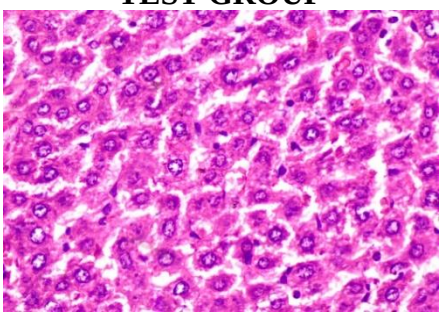
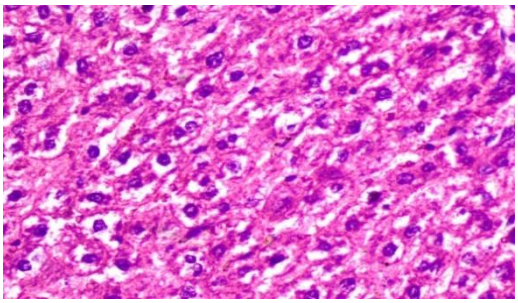

4. SPLEEN

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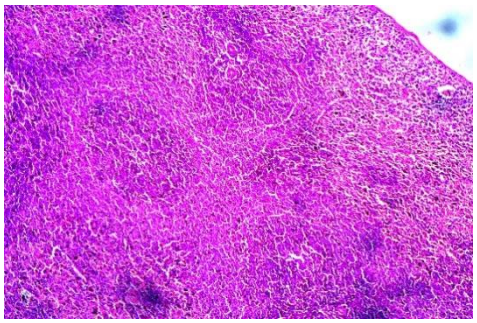

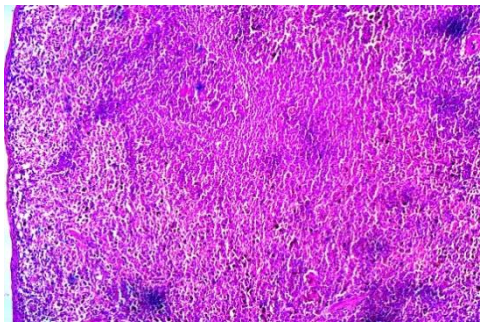

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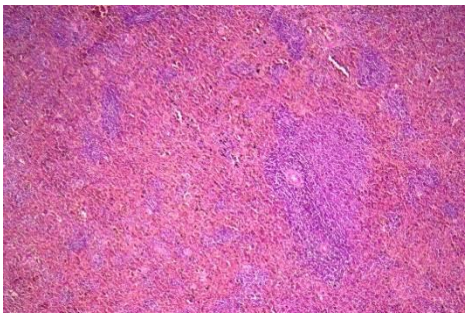

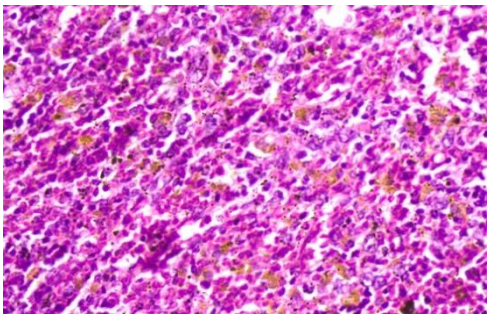

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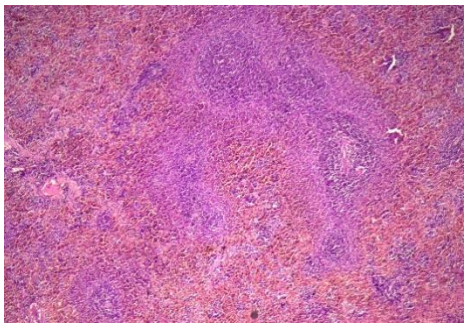

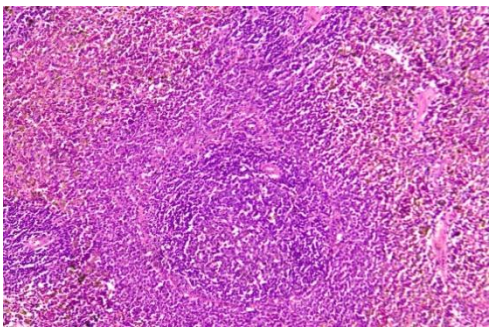

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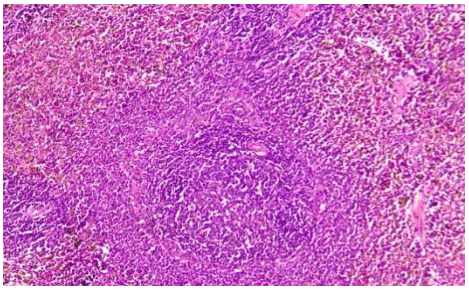

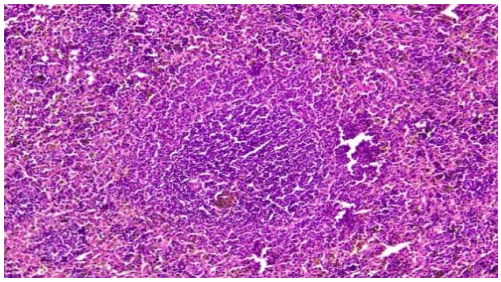

Table No. 13: Histopathology results seen in Bonemarrow.

	Megakaryocytes	Adipocytes	Myeloid	Erythroid
PC 1	++	+	+++	+
PC 2	++	+	++	++
PC3	++	+	+++	+
PC4	++	+	+++	+
S1	+++	-	++	++
S2	+++	-	+++	+
S3	++	-	++	++

S6	++	-	++	++
T1	++	-	+++	+
T2	+++	+	++	++
T3	+++	+	++	++
T4	+++	+	++	+

Table No. 14: Histopathology results seen in kidney.

Slide No	Necrosis	Inflammatory infiltrate	Degeneration	Dilated tubules
PC 1	-	-	++	+
PC 2	-	-	++	+
PC 3	-	-	-	++
PC 4	-	-	-	-
S1	-	-	++	+
S2	-	-	-	-
S3	-	-	-	-
S6	-	-	-	-
Test-Head	-	-	+++	-
Test-body	-	-	++	-
Test-Neck	-	-	++	-
Test-Tail	-	-	++	-

-Nil, + Mild, ++ Moderate, +++ severe

Grading for degeneration

- No degenerated tubules

+ <10% degenerated tubules

++10-25% degenerated tubules

+++25-50% degenerated tubules

++++ >50% degenerated tubules

Table No. 15: Histopathology results seen in liver.

Slide No	Necrosis	Inflammatory infiltrate	Degeneration	Dilated and congested sinusoids
PC 1	-	+	-	-
PC 2	-	++	-	-
PC 3	-	+	-	+
PC 4	-	-	-	-
S1	-	+	-	+
S2	-	+	-	-
S3	-	-	-	-
S6	-	-	-	-
Test-Head	-	+	-	-
Test-body	-	+	-	+
Test-Neck	-	-	-	-
Test-Tail	-	+	-	+

-Nil, + Mild, ++ Moderate, +++ severe

Table No. 16: Histopathology results seen in spleen.

	Changes observed
PC 1	No histological changes
PC2	No histological changes
PC3	Reduced cellularity
PC4	Reduced cellularity
S1	No histological changes
S2	No histological changes
S3	No histological changes
S6	No histological changes
Test-Head	No histological changes
Test-body	No histological changes
Test-Neck	No histological changes
Test-Tail	No histological changes

DISCUSSION

Analysis of the results showed moderate reversal in the Hb content as seen in both reference standard and test groups in comparison with the anaemia control group. In the ferrous fumarate administered group 15.12% and in indigeneous group 12.19% increase is seen in Hb content. Moderate increase is seen in total RBC count in both ferrous fumarate standard and the indigeneous group i.e. 9.19% increase in standard and 9.77% increase in the indigeneous group in comparison to the significantly decreased total RBC count in the PHz control group. There was non-significant decrease in the PCV % which signifies that there is no change in the haematocrit value as compared to the disease control. By observing these results, it clearly indicates that the indigeneous test drug has definite potential of increasing the Haemoglobin content.

The RBC indices are the parameters that denotes the size, weight, concentration of Hb with volume of RBC in the blood. These parameters are useful in the determination of the type of anaemia. In the present study, it may be useful to analyze the probable mode of action of the drug in the body. MCV denotes the size of the RBC, MCH denotes the average weight of the haemoglobin in a red blood cell, MCHC denotes the average concentration of haemoglobin in the RBC per unit volume. Both MCH and MCHC are indicative of the Hb content in the RBCs.

MCV, MCH, MCHC, RDW- The first indication of iron deficiency is an increase in RDW. RDW indicates variation in RBC size; therefore, it is a good indicator of the degree of

anisocytosis. Anisocytosis occurs when iron deficiency is present before the onset of anemia.[6] Individuals with early iron deficiency tend to have increased RDW with normal MCV levels; as the condition progresses, MCV levels also decrease.

In the present study there was very significant increase in RDW-CV and non significant increase in RDW-SD. Also, there was significant decrease seen in the values of MCV and non significant increase in MCH and MCHC values. This reflects that the observed anisocytosis seems to be due to iron deficiency. On administration of the indigeneous test drug, there was significant reversal seen in case of RDW, MCV, MCH and MCHC, suggesting that the variation in size and volume of RBCs and the Haemoglobin concentration in RBCs were reversed, supporting the fact that the drug is efficacious in iron deficiency anaemia.

The platelet count reflects the number of platelets present in the blood. In the study conducted, a non-significant decrease in platelet count was observed. However, after administering both the test drug and the standard drug, a non-significant reversal in the platelet count was observed. These findings suggest that the test drug has minimal impact on the platelet levels.

Serum Iron value represents the amount of iron in the blood that is bound to transferrin (90%) and serum ferritin (10%). Transferrin, produced by the liver, acts as a carrier for one or two iron ions (ferric, Fe3+).[7] This is crucial for the transportation and utilization of stored iron. Following the administration of the PHz injection, there will be change in the RBC membrane which leads to oxidative denaturation of hemoglobin and thereby a significant reduction in serum iron levels was observed. Both the standard ferrous fumarate and the test drug indigenous formulation showed an increase, although statistically non-significant, in serum iron level.

The histopathological slides of bone marrow showed that the PHz induction caused the changes in the cells of bone marrow suggesting iron deficiency anaemia. In test group, increased cellularity with increased erythroid cells compared with PC group were seen in few slides. Hence indigeneous drug formulation improves oxygen supply to the body tissues via blood flow through the circulatory system. The renal parenchyma of testgroup and standard group did not present any sign of particular abnormalities. The histopathological section of the spleen obtained from the PHz induced group, there is decreased cellularity of white pulp

seen in few slides and there is no histological changes like decrease or increase in cellularity of white pulp and red pulp seen in both standard and test group.

CONCLUSION

The physiological changes seen in animals are the concluding factors to understand the intrinsic changes that take place. This study showed that the administration of *indigeneous drug formulation* effectively increased the hemoglobin levels of pregnant rats with anemia and also increased concentration of serum iron. The average mean hemoglobin levels of pregnant rats after being given indigeneous drug formulation, increased from 9.41gm/dl to 13.8gm/dl and no toxic effects have been observed throughout the duration of the study.

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