IJPSR (2013), Vol. 4, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 April, 2013; received in revised form, 10 June, 2013; accepted, 14 August, 2013; published 01 October, 2013

EVALUATION OF HAEMATINIC ACTIVITY OF POLYHERBAL FORMULATION IN $HgCl_2$ INDUCED ANAEMIA IN LABORATORY ANIMALS

Pradnya N. Jagtap*, Vaishali R. Undale and A.V. Bhosale

PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad, Tal- Purandar, Dist- Pune, Maharashtra, India

Keywords:

anaemia, Haema Tablet, Haemoglobin, Red blood cell

Correspondence to Author:

Pradnya N. Jagtap

PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad, Tal- Purandar, Dist- Pune, Maharashtra, India

E-mail: pradnyajagtap67@yahoo.co.in

ABSTRACT: Anaemia constitutes serious health problem in many countries. It is widespread public health problem, and the fourth leading cause of hospital admissions and the second factor contributing to death. Anaemia is characterized by low count of haemoglobin. The empirical use of herbal preparations in the treatment of anaemia dates from ancient times. Despite the obvious effectiveness and efficacy of iron supplementation, there are certain limitations which include gastrointestinal side effects like nausea, constipation, vomiting, and stained teeth. $HgCl_2$ was administered for induction of anaemia in rats by oral route at 9mg/kg for 30 days simulaneousaly with test & standard drug. After 1 week blood was withdrawn to check all haematological parameters. The animals were treated with standard and test drug. After 2, 3, 4 weeks blood was withdrawn to check haematological & biochaemical parameters. Preliminary phytochemical screening by various tests was done. Acute oral toxicity was also done at 2000mg/kg and 5000mg/kg. $HgCl_2$ induced significant decrease in the blood parameters indicating anaemia which was significantly increase in test and standard treated animals. Acute toxicity studies in mice established the oral LD50 greater than 5000 mg/kg. Haema tablet assessed for the haematinic activity by using: $HgCl_2$ induced anaemia in rats. In the present study, the pharmacological evaluation of Haema tablet has done for the haematinic effect. In the present study, it can be concluded that animals treated with standard & Haema tablet in dose 500 mg/kg of body weight was effective.

INTRODUCTION: Anaemia is characterized by low haemoglobin count. WHO defines anaemia as Haemoglobin levels less than 13 g/dl in males & less than 12 g/dl in females. In adults, the lower extreme of the normal haemoglobin is taken as 14.0 -16 g/dl for males and 12 -14 g/dl for females. Newborn infants have higher haemoglobin level and, therefore 15 g/dl is taken as the lower limit at birth, whereas at 3 months the lower level is 9.5 g/dl. Although haemoglobin value is employed as the major parameter for determination anaemia.

The low Hb levels results in a corresponding decrease in the oxygen carrying capacity of blood[1] and other parameters such as total no. of RBCs, PCV, MCV, and MCHC. Anaemia is a widespread public health problem; it is the fourth leading cause of hospital admissions and the second factor contributing to death [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(10).3938-52

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(10).3938-52

The haemoglobin, hematocrit and Red cell count have gender differences. The rest of the values (red cell indices) do not change with gender. Red cell indices give information about the average red cell volume (MCV) and red cell hemoglobin content (MCH) or concentration (MCHC). MCV is the most useful because it permits separation of microcytic anemia from normocytic and Anaemia is possibly one of the most common condition in the world and results in the significant morbidity & mortality, particularly in the developing world.

Despite the obvious effectiveness and efficacy of iron supplementation, there are certain limitations. The main limitation is the lack of compliance, especially when long-term daily administration is required [3, 1]. General factors affecting compliance with iron supplementation are as follows;

- General constraints on supplementation success
- Lack of knowledge and concern about anaemia
- Forgetfulness or lack of motivation to take a supplement frequently (daily)
- Dose-related gastrointestinal side effects (nausea, diarrhea, and constipation)
- Unacceptable color, taste, or other characteristic of the supplement
- Fear that the supplement is a contraceptive
- Lack of supportive education and counseling
- Lack of compliance by functionaries to their work protocol
- Poor distribution and/or supply of supplements to delivery outlets [4]

Gastrointestinal side effects associated with oral iron therapy included nausea, constipation, anorexia, heartburn, vomiting, and diarrhea. These effects are generally dose-related. In addition, stools may appear darker in color in patients taking products containing iron.

Other side effects associated with oral iron products included stained teeth and iron overload (hemosiderosis). Secondary hemochromatosis due to prolonged iron ingestion has been reported rarely. Stained teeth have primarily occurred following ingestion of iron liquid preparation. Iron overload (i.e., hemosiderosis) has been reported in patients genetically predisposed, or have underlying disorders, that augment the absorption of iron [2, 5].

Metabolic side effects associated with iron have included decreased absorption of thyroxin (T4).

During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries a large proportion relies on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complimentary therapies, including medicinal herbs [6].

There are some polyherbal haematinic formulations which are used to treat the anaemia. These formulations consist of more than one ingredient. In these formulations other plants were used to treat the anaemia, as supportive or to give synergistic action. Some plants when used alone it shows haematinic activity but when it combines with another plant either it will shows synergistic or opposite action.

MATERIAL & METHODS:

Animals: For pharmacological experiments, Sprague Dawley rats of either sex weighing 200-250 gm used for the study. The animals were collected from the animal house of Seth Govind Raghunath Sable College of Pharmacy, SASWAD. All the protocol of the experiments and animal usage were discussed in the Institutional Ethical Committee meeting and permission has been obtained to carry out the parameters selected for the study in accordance to CPCSEA guidelines. The research proposal number is SGRS/IAEC/03/2009-10.

Housing of Animal: Animals were housed in propylene cages under standard laboratory conditions, maintained on a natural light and dark cycle (12 h – 12 h) and were fed with standard laboratory diet and water *ad labium* in the same place.

The bedding material of the cages was changed every day. Animals handling was performed according to Good Laboratory Practice (GLP).

Procurement of Tablets: Haema tablet were collected from Indu Pharma at jejuri.

Composition of Haema Tablet: Amalaki 60 mg, Haritaki 60 mg, Mandur bhasma 40 mg, Mustak 60 mg, Shunthi 60 mg, Vidang 60 mg, Bibhitaki 60 mg, Indryava 60 mg,Suvarna makshik bhasma 40 mg

Acute toxicity study: In order to decide the dose, toxicity study was carried out as per OECD guidelines No. 425. Toxicity study was carried out using a starting dose of 2000 mg/kg body weight. Animals were observed individually after dosing at least once during the first 30 min. periodically during the first 24 h, with special attention given during first 4 h. OECD Guidelines, No. 425 (para-36).

Five animals are treated with 2000 mg/kg of solution of Haema tablet. The LD_{50} is greater than the test dose (2000 mg/kg) when three or more animals survive.

One animal is treated with 5000 mg/kg of solution. If the animal dies, conduct the main test to determine the LD_{50}. If the animal survives, dose two additional animals. If both animals survive, the LD_{50} is greater than the limit dose and the test is terminated .The LD_{50} is less than the test dose (5000 mg/kg) when three or more animals die. So reduce extract dose to determine exact LD50 [6, 7].

Characterization of test solution for various primary & secondary metabolites (chemical constituents) [8, 9, 10]:

Experimental design:

$HgCl_2$ induced anaemia in rats:

Purpose & Rationale: $HgCl_2$ is a severe corrosive and a lead to irritability, stomatitis and colitis. It is toxic to liver and kidney leading to uremia. Reduction in the serum Erythropoietin due to liver and kidney damage and uremia cause haemolysis and bone marrow depression leads to decrease in RBC count, and haemoglobin percentage. Here haemoglobin content of blood and total RBC count decreased in $HgCl_2$ treated group and these were increased in test drug treated group. $HgCl_2$ was administered for induction of haemolytic anaemia [11, 12].

Procedure: The animals were divided into 6 groups each contains 6 animals. Group 1 was normal control, group 2 was anaemic control, group 3 was Standard, and group 4, 5, 6 was test treated groups for different doses.

Group 1 was normal control which received vehicle, group 2 was anaemic control which received $Hgcl_2$ 9mg/kg by oral route, group 3 was Standard which received Multivitamin Syrup with $Hgcl_2$, group 4, 5, 6 was received test solution of different doses like 250, 500 & 1000 mg / kg by oral route along with $HgCl_2$ for 30 days.

Blood was collected by retro orbital puncture of experimental animals after an overnight fast (T=0) & after 4 weeks (30 days). Blood was collected in EDTA bulb for haematological determination & in plain bulb for Biochemical determination [11, 12].

Analysis of parameters: Blood was collected by retro orbital puncture of experimental animals after an overnight fast (T=0).Blood was collected in EDTA bulb for haematological determination & in plain bulb for Biochemical determination.

Haematological Determination: The red blood cell count (RBC), haemoglobin count was determined on 0, 15& 30 day. The mean cell volume (MCV), mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) were determination. Body weight, color index, oxygen carrying capacity also determined.

Histopathology of Kideny, Liver, Spleen:

Biochemical Determination: Hepatic enzymes of clinical significance and serum were determined in samples. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured. Histopathology of Histopathology of Kidney, Liver, and Spleen, Body weight, color index, oxygen carrying capacity also determined.

Experimental data was analyzed using analysis of variance (ANOVA) & Dunnet's test to determine significant difference between means.

RESULTS: Preliminary Phytochemical screening of Polyherbal formulation revealed the presence of secondary metabolites like Alkaloids, Glycosides, Flavonoids, Tannins and absence of Carbohydrates. The oral administration of polyherbal formulation up to 5,000 mg/kg did not produce any toxic effects and no mortality was observed in mice. Results indicated that doses up to 5,000 (mg/kg) are safe for administration. The one tenth of the LD50 i.e. 500 mg/kg was chosen as the effective dose. Similarly, a subtherapeutic and a supertherapeutic dose i.e. 250 mg/kg & 1000 mg/kg was also chosen to check the precise therapeutic effect.

There was no any significant difference found in Haemoglobin concentration in all experimental animals on 0 day $HgCl_2$ (9mg/kg by oral route) was administered for induction of anaemia & s given along with Standard drug & test drug for 30 days. After 15 days treatment in all groups has not showing any significant difference in the concentration of Haemoglobin. After 30 days treatment there was decrease in the haemoglobin concentration in the group 2 experimental animals (anaemic control). The $HgCl_2$ reduces the concentration of haemoglobin which was significantly ($P<0.001$) increased in the animals of group 3 (Standard). The animals treated with various doses of Haema tablet has significantly ($P<0.001$) increased the concentration of Haemoglobin in group 4 (250 mg/kg), group 5 (500 mg/kg), group 6 (1000 mg/kg) as compared to group 2 (Anaemic control), (**See Table 1 & Figure 1**).

TABLE 1: **EFFECT OF HAEMA TABLET ON HAEMOGLOBIN COUNT IN $HgCl_2$ INDUCED ANAEMIA IN RTAS**

Parameter Hb (g/dl)	Group1 Normal Control	Group2 Anaemic Control	Group 3 Standard	Group 4 250 mg/kg	Group 5 500 mg/kg	Group 6 1000mg/kg
0 DAY	17.47± 0.71	17.67±0.76	17.17± 0.60	17.00± 0.57	17.83± 0.70	17.33± 0.66
15 DAY	17.60±0.57	12.97±0.52 x	14.07±0.51	12.77±0.50	13.20±0.61	12.83±0.60
30 DAY	17.27±0.37	11.48±0.58 x	16.03±0.69***	13.27±0.42*	14.85±0.68***	13.73±0.52*

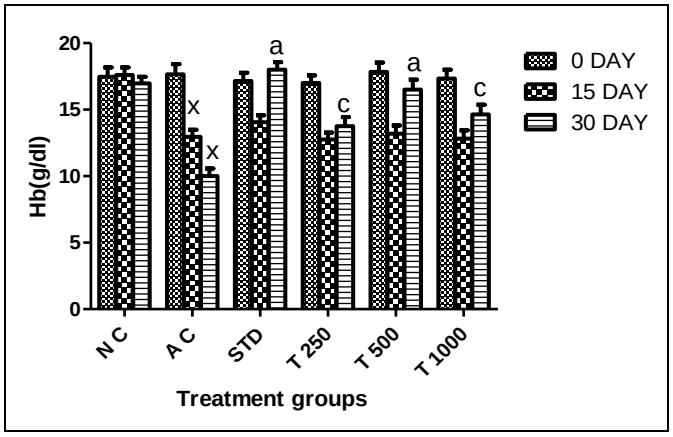

FIGURE 1: EFFECT OF HAEMA TABLET ON HAEMOGLOBIN COUNT IN $HgCl_2$ INDUCED ANAEMIA IN RTAS

The RBC concentration has not showed any significant difference in all experimental animals on 0 day.

$HgCl_2$ (9mg/kg by oral route) was administered for induction of anaemia & given along with standard drug & test drug. After 15 days treatment not any significant difference was found in the concentration of RBC in all animals. After 30 days treatment there was decrease in the RBC concentration in the animals of group 2 (Anaemic control). The reduction in the concentration of RBC due to $HgCl_2$ was significantly ($P<0.001$) increase in the animals of group 3 (Standard).

The animals treated with various doses of Haema tablet has significantly ($P<0.001$) increases the concentration of RBC in group 4 (250 mg/kg), group 5 (500 mg/kg), group 6 (1000 mg/kg) as compared to group 2 (Anaemic control), (**See Table 2 & Figure 2**).

TABLE 2: EFFECT OF HAEMA TABLET ON RBC COUNT IN $HgCl_2$ INDUCED ANAEMIA IN RATS

Parameter RBC ($x10^6$/ul)	Group1 Normal Control	Group2 Anaemic Control	Group 3 Standard	Group 4 250 mg/kg	Group 5 500 mg/kg	Group 6 1000mg/kg
0 DAY	7.20± 0.29	6.86±0.52	7.56± 0.29	7.40± 0.36	7.200± 0.42	7.21± 0.36
15 DAY	7.48±0.27	5.33±0.70 y	6.20±0.37	5.21±0.34	5.78±0.40	5.50±0.48
30 DAY	7.767±0.17	4.383±0.38 x	7.26±0.35***	5.98±0.27**	6.58±0.35***	6.03±0.40**

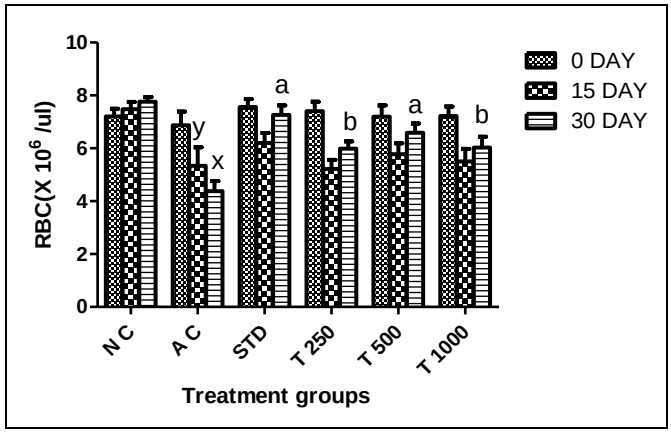

FIGURE 2: EFFECT OF HAEMA TABLET ON RBC COUNT IN $HgCl_2$ INDUCED ANAEMIA IN RATS

The MCV concentration has not shown any significant difference in the experimental animals on 0 day. $HgCl_2$ (9mg/kg by oral route) was administered for induction of anaemia &given along with Standard drug & test drug for 30 days. After 15 days treatment significant difference was found in the concentration of MCV. After 30 days the animals treated with Phenytoin shows increase in the MCV concentration in group 2 (Anaemic control). An increased in the concentration of MCV due to $HgCl_2$ was significantly (P<0.001) reduced in the animals of group 3 (Standard).

The animals treated with various doses of Haema tablet has significantly (P<0.001) decreased the concentration of MCV in group 4 (250 mg/kg), group 5 (500 mg/kg), group 6 (1000 mg/kg) as compared to group 2 (Anaemic control), (**See Table 3 & Figure 3**).

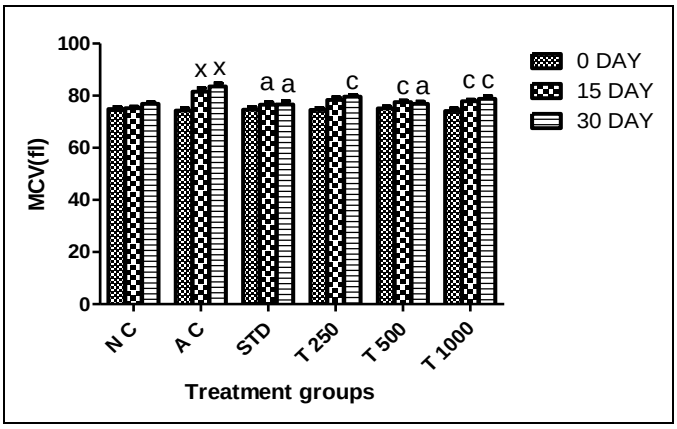

FIGURE 3: EFFECT OF HAEMA TABLET ON MCV CONCENTRATION IN $HgCl_2$ INDUCED ANAEMIA IN RATS

There was no any significant change found in the concentration of MCH after oral administration of $HgCl_2$, Standard drug & test drug in all groups as compared to group 1 (Normal Control), (**See Table 4 & Figure 4**).

TABLE 3: EFFECT OF HAEMA TABLET ON MCV CONCENTRATION IN $HgCl_2$ INDUCED ANAEMIA IN RATS

Parameter MCV(fl)	Group1 Normal Control	Group 2 Anaemic Control	Group 3 Standard	Group 4 250 mg/kg	Group 5 500 mg/kg	Group 6 1000mg/kg
0 DAY	74.83± 0.79	74.25±0.98	74.67± 0.91	74.50± 0.76	75.08± 0.75	74.17± 0.94
15 DAY	75.20±0.62	81.50±1.36 x	76.50±0.99***	78.33±1.05	77.50±0.61*	77.83±0.65*
30 DAY	76.83±0.60	83.50±1.17 x	76.67±1.25***	79.67±0.66*	76.87±0.83***	78.75±1.12*

Statistical analysis done by using ANOVA followed by Dunnett's test (group 1, Group 3, Group 4, Group 5, group 6 compared with Group 2) , Group 2 compared with Group1 (n = 6).

TABLE 4: EFFECT OF HAEMA TABLET ON MCH CONCENTRATION IN $HgCl_2$ INDUCED ANAEMIA IN RATS

Parameter MCH (pg)	Group 1 Normal Control	Group 2 Anaemic Control	Group 3 Standard	Group 4 250 mg/kg	Group 5 500 mg/kg	Group 6 1000mg/kg
0 DAY	24.80± 0.45	24.50±0.67	25.04± 0.81	24.56± 0.68	24.97± 0.66	25.22± 0.52
15 DAY	25.05±0.46	26.00±0.68	25.00±0.57	25.50±0.67	25.83±0.65	25.67±0.55
30 DAY	25.62±0.44	27.83±0.70	25.83±0.79	26.50±0.67	26.08±0.58	26.67±0.55

Statistical analysis done by using ANOVA followed by Dunnett's test (group 1, Group 3, Group 4, Group 5, group 6 compared with Group 2) , Group 2 compared with Group1 (n = 6).

TABLE 5: EFFECT OF HAEMA TABLET ON MCHC CONCENTRATION IN $HgCl_2$ INDUCED ANAEMIA IN RATS

Parameter MCHC (g/dl)	Group 1 Normal Control	Group 2 Anaemic Control	Group 3 Standard	Group 4 250 mg/kg	Group 5 500 mg/kg	Group 6 1000mg/kg
0 DAY	32.00± 0.77	31.67±0.66	32.67± 0.71	32.53± 0.76	32.45± 0.71	32.78± 0.63
15 DAY	33.08±0.77	32.83±0.65	33.50±0.56	33.17±0.70	32.67±0.61	32.83±0.60
30 DAY	33.33±0.84	34.00±0.77	33.08±0.86	33.42±0.98	33.33±0.74	33.83±0.87

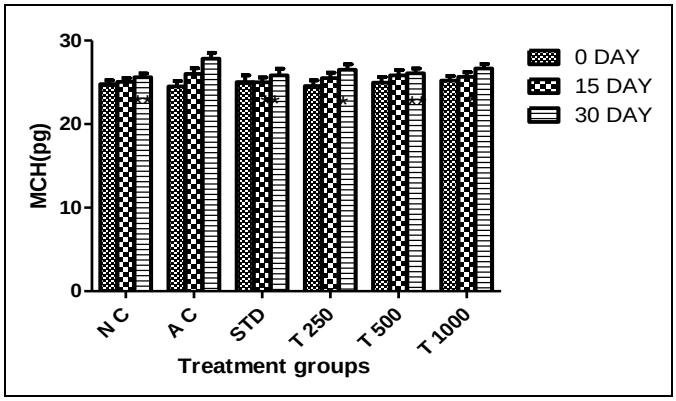

FIGURE 4: EFFECT OF HAEMA TABLET ON MCH CONCENTRATION IN $HgCl_2$ INDUCED ANAEMIA IN RATS

There was no any significant change found in the concentration of MCHC after oral administration of $HgCl_2$, Standard drug & test drug in group 2, group 3, group 4, group 5 & group 6 as compared to group 1 (Normal Control), (**See Table 5 & Figure 5**).

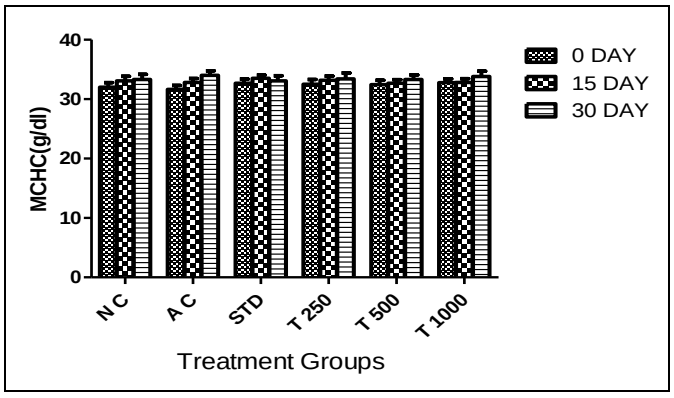

FIGURE 5: EFFECT OF HAEMA TABLET ON MCHC CONCENTRATION IN $HgCl_2$ INDUCED ANAEMIA IN RATS

There was no any significant difference found in Oxygen carrying capacity in all experimental animals on 0 day. $HgCl_2$ (9mg/kg by oral route) was administered for induction of anaemia & given along with standard drug & test drug. After 15 days treatment no any significant difference was found in the oxygen carrying capacity in all treated groups.

The animals treated with $HgCl_2$ for 30 days produced decrease oxygen carrying capacity in the group 2 experimental animals. The reduced oxygen carrying capacity was significantly ($P<0.001$) increased in the animals of group 3 (Standard). The animals treated with various doses of Haema tablet has significantly ($P<0.001$) increased the oxygen carrying capacity in group 4 (250 mg/kg), group 5 (500 mg/kg), group 6 (1000 mg/kg) as compared to group 2 (Anaemic control), (**See Table 6 & Figure 6**).

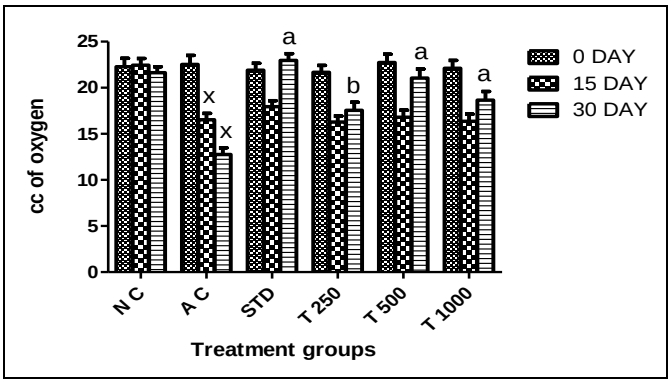

FIGURE 6: EFFECT OF HAEMA TABLET ON OXYGEN CARRYING CAPACITY OF BLOOD IN $HgCl_2$ INDUCED ANAEMIA IN RATS

There was no any significant difference found in color index in all experimental animals on 0 day. $HgCl_2$ (9mg/kg by oral route) was administered for induction of anaemia & given along with standard drug & test drug. After 15 days treatment no any significant difference was found in the color index in all treated groups. After 30 days treatment decrease in the color index in the group 2 experimental animals (anaemic control). The reduced color index was significantly ($P<0.001$) increased in the animals of group 3 (Standard). The animals treated with various doses of Haema tablet has significantly ($P<0.001$) increases the color index in group 4 (250 mg/kg), group 5 (500 mg/kg), group 6 (1000 mg/kg) as compared to group 2 (Anaemic control), (**See Table 7 & Figure 7**).

TABLE 6: EFFECT OF HAEMA TABLET ON OXYGEN CARRYING CAPACITY OF BLOOD IN $HgCl_2$ INDUCED ANAEMIA IN RATS

Parameter Oxygen carrying capacity (cc)	Group 1 Normal Control	Group 2 Anaemic Control	Group 3 Standard	Group 4 250 mg/kg	Group 5 500 mg/kg	Group 6 1000mg/kg
0 DAY	22.28± 0.91	22.54±0.97	21.90± 0.76	21.68± 0.73	22.75± 0.89	22.11± 0.85
15 DAY	22.45±0.73	16.54±0.67 x	17.94±0.65	16.29±0.64	16.81±0.77	16.37±0.76
30 DAY	21.64±0.65	12.75±0.73 x	22.96±0.73***	17.56±0.85**	21.05±0.97***	18.67±0.93***

TABLE 7: EFFECT OF HAEMA TABLET ON COLOUR INDEX IN HgCl2 INDUCED ANAEMIA IN RATS

Parameter Colour Index	Group 1 Normal Control	Group 2 Anaemic Control	Group 3 Standard	Group 4 250 mg/kg	Group 5 500 mg/kg	Group 6 1000mg/kg
0 DAY	1.33± 0.05	1.34±0.05	1.30± 0.04	1.29± 0.04	1.36± 0.05	1.32± 0.05
15 DAY	1.34±0.04	0.98±0.04 x	1.07±0.03	0.970±0.03	1.00±0.04	0.97±0.04
30 DAY	1.293±0.03	0.76±0.04 x	1.37±0.04***	1.04±0.05**	1.25±0.05***	1.11±0.05***

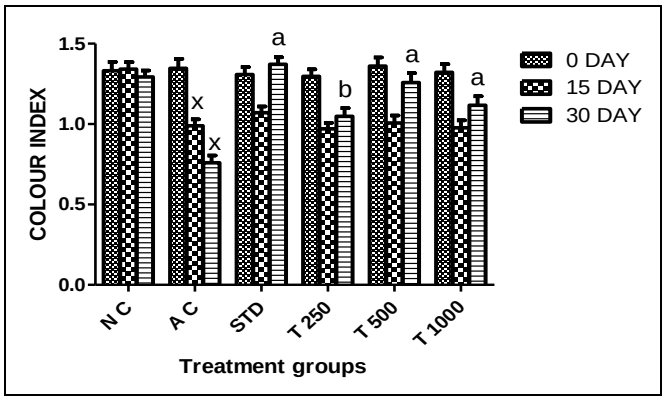

FIGURE 7: EFFECT OF HAEMA TABLET ON COLOUR INDEX IN $HgCl_2$ INDUCED ANAEMIA IN RATS

There was not any significant difference found in the levels of AST in all experimental animals on 0 day. $HgCl_2$ (9mg/kg by oral route) was administered for induction of anaemia & given along with standard drug & test drug for 30 days. After 15 days treatment no any significant difference was found in the levels of AST in all treated groups. After 30 days treatment increase in the levels of AST in group 2 experimental animals (anaemic control).

An increase in the levels of AST due to $HgCl_2$ was significantly (P<0.001) decreased in the animals of group 3 (Standard). The treatment with various doses of Haema tablet has significantly (P<0.001) decreases the AST in group 4 (250 mg/kg), group 5 (500 mg/kg), group 6 (1000 mg/kg) as compared to group 2 (Anaemic control), (**See Table 8 & Figure 8**).

TABLE 8: EFFECT OF HAEMA TABLET ON BIOCHEMICAL PARAMETERS (AST) IN $HgCl_2$ INDUCED ANAEMIA IN RATS

Parameter AST (IU/L)	Group 1 Normal Control	Group 2 Anaemic Control	Group 3 Standard	Group 4 250 mg/kg	Group 5 500 mg/kg	Group 6 1000mg/kg
0 DAY	37.50± 0.76	37.00±1.06	37.67± 0.98	38.00± 0.89	37.17± 0.47	37.33± 0.91
30 DAY	38.17±0.70	103.8±2.24 ###	38.83±1.51***	41.83±2.08***	39.83±1.47***	43.33±1.76***

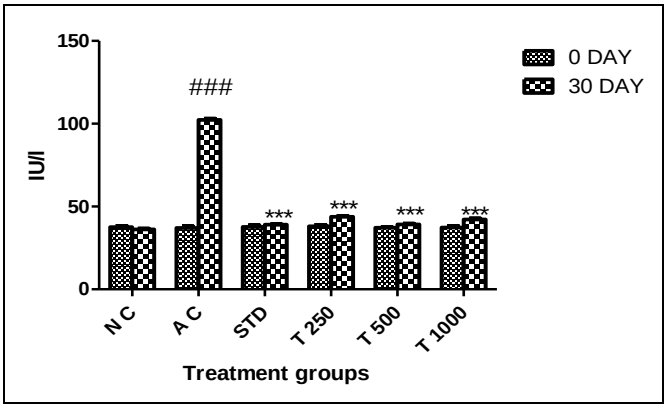

FIGURE 8: EFFECT OF HAEMA TABLET ON BIOCHEMICAL PARAMETERS (AST) IN $HgCl_2$ INDUCED ANAEMIA IN RATS

There was no any significant difference found in the levels of ALT in all experimental animals on 0 day. $HgCl_2$ (9mg/kg by oral route) was administered for induction of anaemia & given along with standard drug & test drug. After 15 days treatment no any significant difference was found in the levels of ALT. After 30 days treatment with $HgCl_2$ increased in the levels of ALT in the group 2 experimental animals (anaemic control). An increase in the levels of ALT due to $HgCl_2$ was significantly (P<0.001) decreased in the animals of group 3 (Standard). The animals treated with various doses of Haema tablet has significantly (P<0.001) decreases the levels of ALT in group 4 (250 mg/kg), group 5 (500 mg/kg), group 6 (1000 mg/kg) as compared to group 2 (Anaemic control), (**See Table 9 & Figure 9**).

TABLE 9: EFFECT OF HAEMA TABLET ON BIOCHEMICAL PARAMETERS (ALT) IN $HgCl_2$ INDUCED ANAEMIA IN RATS

Parameter ALT (IU/L)	Group 1 Normal Control	Group 2 Anaemic Control	Group 3 Standard	Group 4 250 mg/kg	Group 5 500 mg/kg	Group 6 1000mg/kg
0 DAY	66.67± 2.07	68.67±1.90	68.00± 1.94	68.50± 1.64	67.83± 1.81	68.17± 1.64
30 DAY	67.08±2.34	136.2±2.52 ###	69.33±2.67***	85.00±3.21***	74.67±3.13***	87.33±2.41***

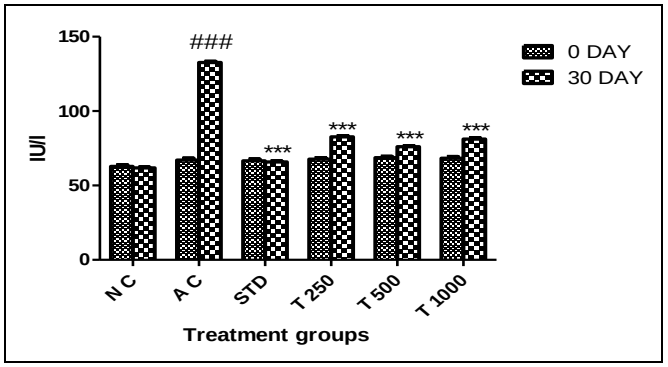

FIGURE 9: EFFECT OF HAEMA TABLET ON BIOCHEMICAL PARAMETERS (ALT) IN $HgCl_2$ INDUCED ANAEMIA IN RATS

The body weight was significantly reduced in the in all animals of treated groups. The $HgCl_2$ reduces the body weight which was significantly ($P<0.001$) increased in animals of group 3 (Standard).

The animals treated with various doses of Haema tablet has significantly ($P<0.001$) increases the body weight in group 4 (250 mg/kg), group 5 (500 mg/kg), group 6 (1000 mg/kg) as compared to group 2 (Anaemic control), (**See Table 10 & Figure 10-14**).

TABLE 10: EFFECT OF HAEMA TABLET ON BODY WEIGHT IN $HgCl_2$ INDUCED ANAEMIA IN RATS

Parameters Body weight gm	Group 1 Normal Control	Group 2 Anaemic control	Group 3 Std	Group 4 250 mg/kg	Group 5 500mg/kg	Group 6 1000mg/kg
0 DAY	223.3±5.72	228.3±5.27	229.5.±5.05	230.0±3.65	231.3±3.61	232.5±3.19
7 DAY	228.5±5.34	219.2±6.11	224.7±5.14	216.7±3.00	226.8±4.65	223.5±3.87
14 DAY	232.2±1.97	219.0±5.13	223.0±5.02	212.0±2.30	221.2±5.41	219.8±3.81
21 DAY	233.0±2.01	209.3±2.98###	228.2±4.67**	217.5±3.09	227.0±4.35**	221.7±4.19
30 DAY	236.8±2.18	201.0±2.38###	235.5±2.81***	223.3±3.47***	233.5±3.22***	225.3±3.84***

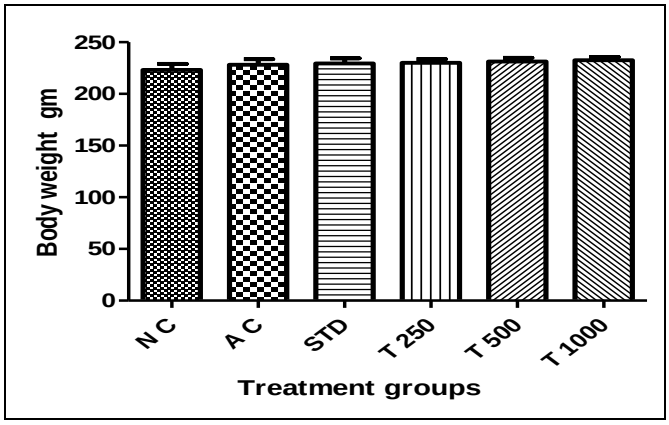

FIGURE 10: BODY WEIGHT ON 0 DAY OF THE STUDY

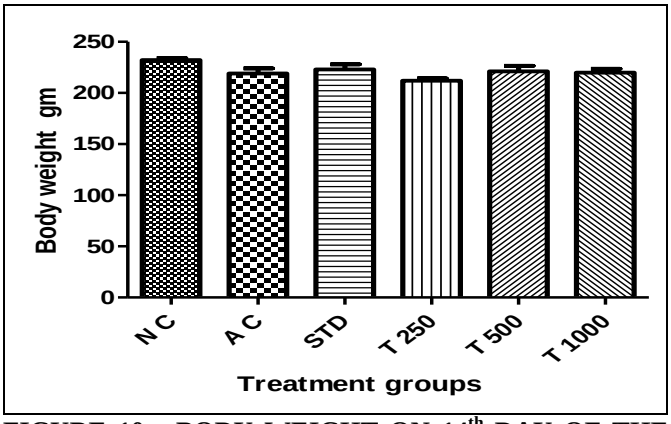

FIGURE 10: BODY WEIGHT ON 14th DAY OF THE STUDY

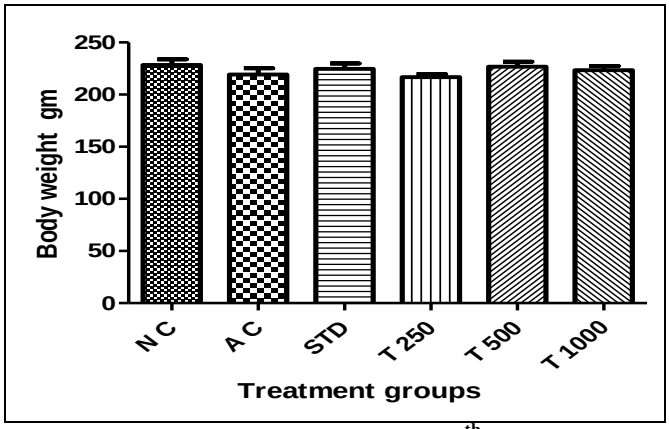

FIGURE 10: BODY WEIGHT ON 7th DAY OF THE STUDY

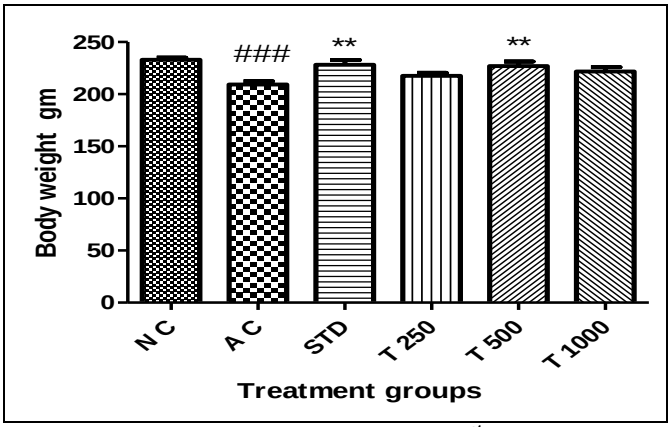

FIGURE 10: BODY WEIGHT ON 21st DAY OF THE STUDY

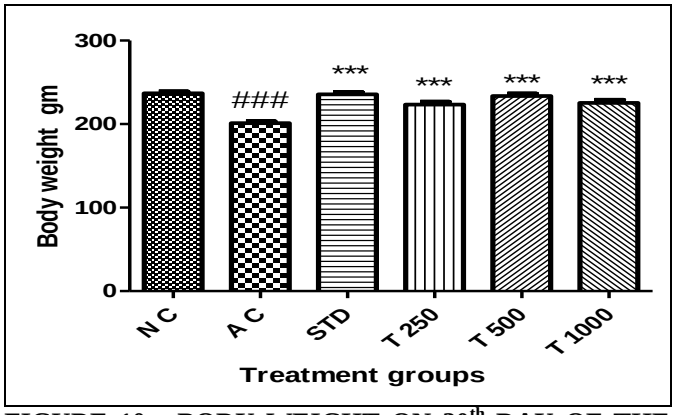

FIGURE 10: BODY WEIGHT ON 30th DAY OF THE STUDY

Statistical analysis done by using ANOVA followed by Dunnett's test (group 1, Group 3, Group 4, Group 5, group 6 compared with Group 2) *** P<0.001= a, ** p< 0.01 = b & Group 2 compared with Group1 *** P<0.001 = x (n = 6)

Histopathology:

Effect of Haema tablet on histopathology of isolated kidneys of Sprague-Dawley rats in $HgCl_2$ induced anaemia (**See Table 11 & Figure 15**).

TABLE 11: EFFECT OF HAEMA TABLET ON HISTOPATHOLOGY OF ISOLATED KIDNEYS OF SPRAGUE-DAWLEY RATS IN $HgCl_2$ INDUCED ANAEMIA

Groups	Treatment	MNC infiltration	Degeneration	Tubular swelling
1	Normal Control	--	--	--
2	Anaemic Control	++++	++++	++++
3	Standard	+	+	+
4	250 mg/kg	+++	+++	+++
5	500 mg/kg	+	+	+
6	1000 mg/kg	++	++	++

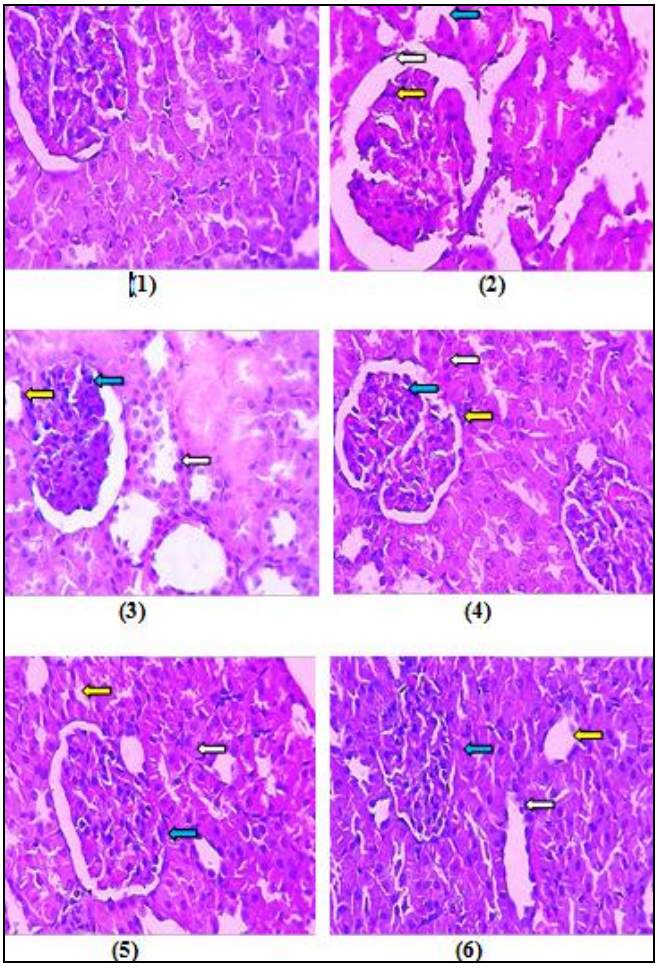

FIGURE 15: EFFECT OF HAEMA TABLET ON HISTOPATHOLOGY OF ISOLATED KIDNEYS OF SPRAGUE-DAWLEY RATS IN $HgCl_2$ INDUCED ANAEMIA

1. **Group 1 (Normal Control):** Histological profile of the kidneys of control animals showed normal glomeruli and tubules. There was no sign of cellular infiltration, glomerular or tubular necrosis in these animals.
2. **Group 2 (Anaemic control):** After four weeks, histopathological examination of the kidneys in $Hgcl_2$ treated animals showed severe to moderate abnormal changes like tubular swelling & necrosis, as well as cellular infiltration and degeneration.
3. **Group 3 (Standard):** After four weeks, on histopathological examination of the kidneys in the animals treated with standard drug Multivitamin syrup. The severity of abnormal changes like tubular swelling, necrosis, as well as cellular infiltration and degeneration were less as compared to group 2 anaemic control.
4. **Group 4 (250 mg/kg):** After four weeks, on histopathological examination of the kidneys in the animals treated with Haema tablet 250 mg/kg of the body weight. The severity of abnormal changes like tubular swelling, necrosis, as well as cellular infiltration and degeneration were mild as compared to group 2 anaemic control. But

these changes are severe as compared to standard treated group.

5. **Group 5 (500 mg/kg):** After four weeks, on histopathological examination of the kidneys in the animals treated with Haema tablet 500 mg/kg of the body weight. The severity of abnormal changes like tubular swelling, necrosis, as well as cellular infiltration and degeneration were less as compared to group 2 anaemic control.

6. **Group 6 (1000 mg/kg):** After four weeks, on histopathological examination of the kidneys in the animals treated with Haema tablet 1000 mg/kg of the body weight. The severity of abnormal changes like tubular swelling, necrosis, as well as cellular infiltration and degeneration were mild as compared to group 2 anaemic control. But these changes are severe as compared to standard treated group.

Effect of Haema tablet on histopathology of isolated liver of Sprague-Dawley rats in $HgCl_2$ induced anaemia. (**See Table 12 & Figure 16**).

TABLE 12: EFFECT OF HAEMA TABLET ON HISTOPATHOLOGY OF ISOLATED LIVER OF SPRAGUE-DAWLEY RATS IN $HgCl_2$ INDUCED ANAEMIA

Groups	Treatment	Cellular infiltration	Degeneration	Swelling of cords
1	Normal Control	--	--	--
2	Anaemic Control	++++	++++	++++
3	Standard	+	+	+
4	250 mg/kg	+++	+++	+++
5	500 mg/kg	+	+	+
6	1000 mg/kg	++	++	++

1. **Group 1 (Normal Control):** Histological profile of the normal control animals showed normal hepatocytes with well-preserved cytoplasm, prominent nucleus, nucleolus and central vein. There was no sign of inflammation, fatty change or necrosis in these animals.

2. **Group 2 (Anaemic control):** After four weeks, histopathological examination of the liver in Phenytoin treated anaemic control group showed severe to moderate abnormal changes like cellular infiltration, degeneration and derangement & swelling of cords.

3. **Group 3 (standard):** After four weeks, on histopathological examination of the kidneys in the animals treated with Standard drug Multivitamin syrup. The severity of abnormal changes like cellular infiltration, degeneration and derangement & swelling of cords were mild as compared to group 2 anaemic control animals.

4. **Group 4 (250 mg/kg):** After four weeks, on histopathological examination of the kidneys in the animals treated with Haema tablet 250 mg/kg of the body weight. The severity of abnormal changes like cellular infiltration, degeneration and derangement & swelling of cords were mild as compared to group 2 anaemic control animals. But these changes are severe as compared to standard treated group.

5. **Group 5 (500 mg/kg):** After four weeks, on histopathological examination of the kidneys in the animals treated with Haema tablet 500 mg/kg of the body weight. The severity of abnormal changes like cellular infiltration, degeneration and derangement & swelling of cords were mild as compared to group 2 anaemic control animals.

6. **Group 6 (1000 mg/kg):** After four weeks, on histopathological examination of the kidneys in the animals treated with Haema tablet 1000 mg/kg of the body weight. The severity of abnormal changes like cellular infiltration, degeneration and derangement & swelling of cords were mild as compared to group 2 anaemic control animals. But these changes are severe as compared to standard treated group.

Effect of Haema tablet on histopathology of isolated spleen of Sprague-Dawley rats in $HgCl_2$ induced anaemia (**See Table 13 & Figure 17**).

TABLE 13: EFFECT OF HAEMA TABLET ON HISTOPATHOLOGY OF ISOLATED SPLEEN OF SPRAGUE-DAWLEY RATS IN $HgCl_2$ INDUCED ANAEMIA

Groups	Treatment	Focal depopulation	Degeneration	Necrosis
1	Normal Control	--	--	--
2	Anaemic Control	++++	++++	++++
3	Standard	+	+	+
4	250 mg/kg	++++	++++	++++
5	500 mg/kg	+	+	+
6	1000 mg/kg	++	++	++

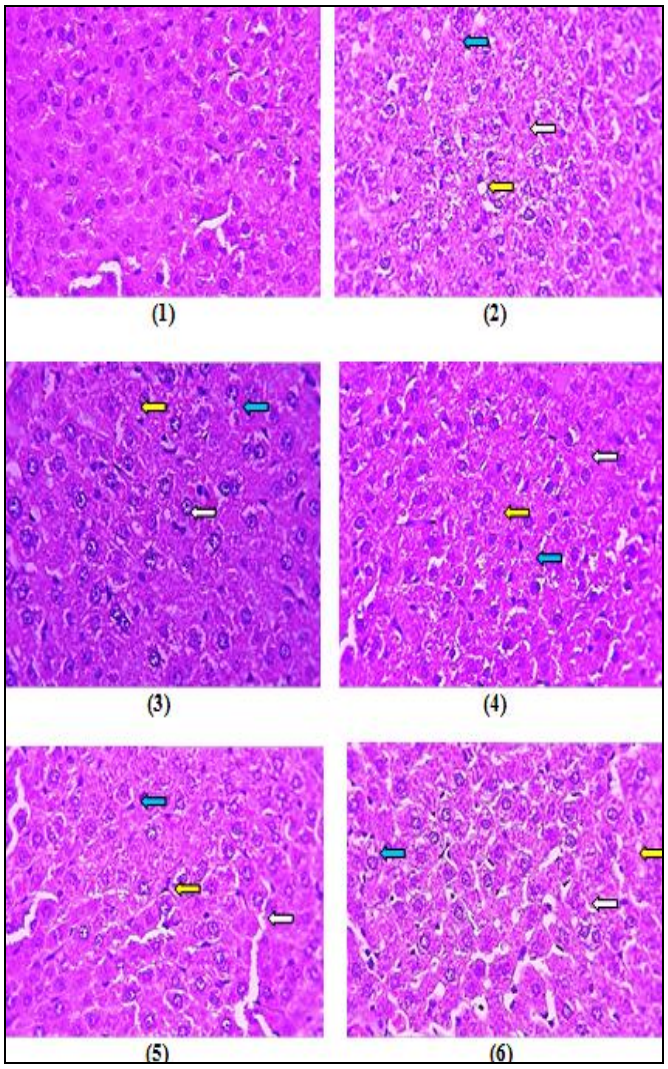

FIGURE 16: EFFECT OF HAEMA TABLET ON HISTOPATHOLOGY OF ISOLATED LIVER OF SPRAGUE-DAWLEY RATS IN $HgCl_2$ INDUCED ANAEMIA

1. **Group 1 (Normal Control):** Histological profile of the normal control animals showed normal spleen with well-preserved fibroblastic capsule. There was no sign of inflammation, Tissue degeneration or necrosis in these animals.

2. **Group 2 (Anaemic control):** After four weeks, histopathological examination of the liver in Phenytoin treated anaemic control group showed severe to moderate abnormal changes like focal depopulation & necrosis.

1. **Group 3 (standard):** After four weeks, on histopathological examination of the Spleen in the animals treated with Standard drug Multivitamin syrup. The severity of abnormal changes like focal depopulation & necrosis were mild as compared to group 2 anaemic control animals.

2. **Group 4 (250 mg/kg):** After four weeks, on histopathological examination of the spleen in the animals treated with Haema tablet 250 mg/kg of the body weight. The severity of abnormal changes like focal depopulation & necrosis were mild as compared to group 2 anaemic control animals. But these changes are severe as compared to standard treated group.

3. **Group 5 (500 mg/kg):** After four weeks, on histopathological examination of the Spleen in the animals treated with Haema tablet 500 mg/kg of the body weight. The severity of abnormal changes like focal depopulation & necrosis were mild as compared to group 2 anaemic control animals.

4. **Group 6 (1000 mg/kg):** After four weeks, on histopathological examination of the spleen in the animals treated with Haema tablet 1000 mg/kg of the body weight. The severity of abnormal changes like focal depopulation & necrosis were mild as compared to group 2 anaemic control animals. But these changes are severe as compared to standard treated group.

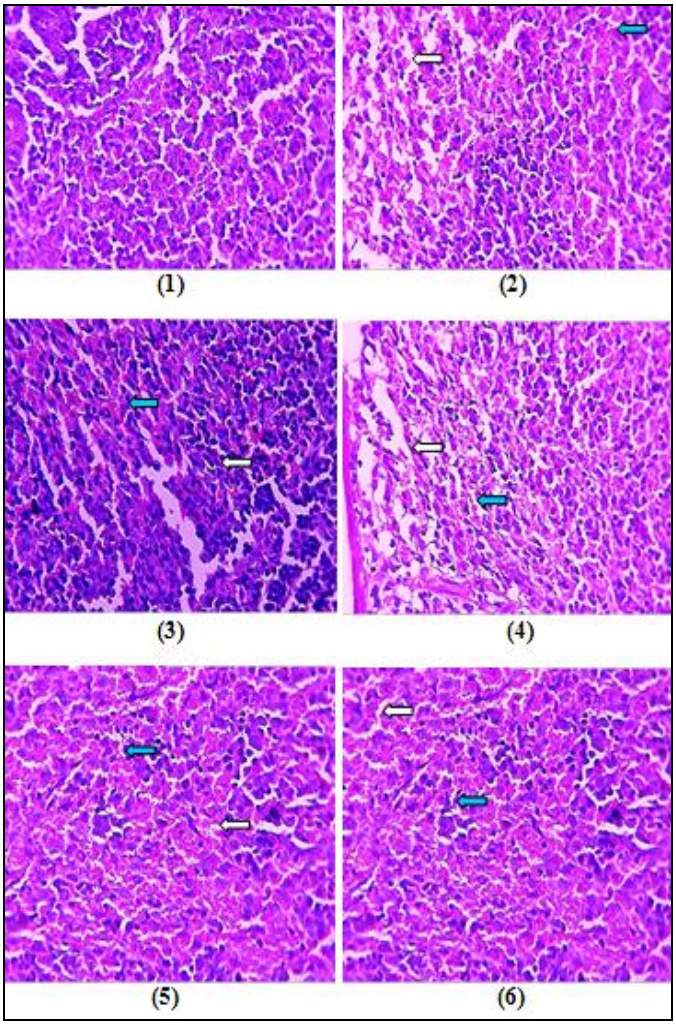

FIGURE 17: EFFECT OF HAEMA TABLET ON HISTOPATHOLOGY OF ISOLATED SPLEEN OF SPRAGUE-DAWLEY RATS IN $HgCl_2$ INDUCED ANAEMIA

Note:

- -- : no abnormality detected
- +: pathological changes up to less than 25 %
- ++: pathological changes up to less than 50 %
- +++: pathological changes up to less 75 %
- ++++: pathological changes up to more than 75 %

DISCUSSION: Anaemia is defined as low levels of haemoglobin concentration in blood. The WHO defines anaemia in adults as Hb levels less than 13 g/dl for males & less than 12 g/dl for females. In adults, the lower extreme of the normal haemoglobin is taken as 14.0 -16 g/dl for males and 12 -14 g/dl for females. Newborn infants have higher haemoglobin level and, therefore 15 g/dl is taken as the lower limit at birth, whereas at 3 months the lower level is 9.5 g/dl.

Haemoglobin value is employed as the major parameter for determination of anaemia. The low Hb levels results in a corresponding decrease in the oxygen carrying capacity of blood & related symptoms [1].

Occupational and Environmental background have a major role in causing certain type of anaemia: Benzene exposure can cause aplastic anemia and acute leukemia. Lead exposure (battery factory work) will cause lead poisoning. Pure vegetarian might cause B12 and iron deficiency. Increase alcohol intake will cause folate deficiency, toxic effect on hematopoies. Unusual cravings (pica) will cause iron deficiency (eats ice) or lead poisoning (eats paint or plaster).

Travel history; parasites (iron deficiency from hookworm, mixed deficiencies from malabsorption due to Giardia), Malaria can cause hemolytic anemia. Exposure to a very cold temperature will cause IgM cold agglutinin, IgM antibodies. The release of histamine from mast cell after warm shower in vulnerable people will cause myeloproliferative disease manifesting itself with itching and autoimune hemolytic anaemia [2].

Most commonly, people with anaemia report non-specific symptoms of a feeling of weakness, or fatigue, general malaise and sometimes poor concentration. They may also report shortness of breath, dyspnea, on exertion. In very severe anaemia, the body may compensate for the lack of oxygen carrying capability of the blood by increasing cardiac output [3].

The most important point in the treatment strategies is that should be directed to the causal factor and towards the underline disease. If the anaemia due to dietary deficiency, Iron, B12, folic acid or Vitamin C is the treatment and according to the deficient element. If there is symptomatic acute or chronic blood loss, packed cell transfusion is the treatment of choice. Bone marrow transfusion is recommended for those with aplastic anaemia. In renal disease Recombinant erythropoietin is the treatment of choice. In sickle cell anaemia the treatment should be directed towards preventing the crises and crisis management, Hydration, analgesics, oxygen, and hydroxyurea.

Other symptomatic and supportive treatment might be recommended [4]. Many synthetic drugs are available for the treatment of anaemia. But there are some limitations of synthetic treatment such as nausea, constipation, anorexia, heartburn, vomiting, and diarrhea. Other side effects associated with oral iron products included stained teeth. Some herbal formulations are also available for the treatment of anaemia with fewer side effects as compared to synthetic drugs. So we have chooses Polyherbal formulation to assessment of activity. Composition of Haema Tablet;

1. Amalaki 60 mg,
2. Haritaki 60 mg,
3. Mandur bhasma 40 mg,
4. Mustak 60 mg ,
5. Shunthi 60 mg,
6. Vidang 60 mg,
7. Bibhitaki 60 mg,
8. Indryava 60 mg,
9. Suvarna makshik bhasma 40 mg.

The tablet was evaluated for its oral toxicity. In the Acute oral toxicity study, no death was observed at 5000 mg/kg. The one tenth of the LD50 i.e. 500 mg/kg was choosen as the effective dose. Similarly, a subtherapeutic and a supertherapeutc dose i.e. 250 mg/kg and 1000 mg/kg was also choosen to check the precise therapeutic effect.

Various animal models can be used for the evaluation of haematinic activity such as;

Toxicogenomics of drug induced haemolytic anaemia by analyzing gene expression profile in the spleen. Trypanosome brucei brucei induced anaema in rats.Erythropoietin restores the anaemia induced reduction in cyclophosphamide cttotoxicity in rat spleen. Phenylhydrazine induced anaemia in rats. Phenytoin induced anaema in rats. $HgCl_2$ induced anaemia in rats.
In-vivo Dopamine metabolism is altered in iron deficient anaemic rats. Lack of protein 4.1a red blood cells of the hereditarily anaemic Belgrade laboratory rat. Change in serum hepcidin levels in acute iron intoxication in a rat model. Anaemia of immobility caused by adipocyte accumulation in bone marrow. Improvement of anaemia in w/v mice by recombinant human erythropoietin mediated through erythropoietin receptors with lowered affinity.

$HgCl_2$ is a severe corrosive and leads to irritability, stomatitis and colitis. It is toxic to liver and kidney leading to uremia [13]. Mercury accumulates in mammalian target organs and damages them. Mercury is not important in modern medicine today, but certain mercury salts are still in the Ayurvedic system of medicine. Metallic mercury is relatively non-toxic.

Mercuric chloride or mercury (II) chloride is a white powder of colorless rhombohedral crystals, somewhat soluble in water. It is also called bichloride of mercury or corrosive sublimate. It is extremely poisonous. Raw egg white may be given as an antidote in case of toxicity, since mercuric chloride reacts with egg albumin to form a nearly insoluble precipitate; & the medical treatment should be sought immediately. Mercuric chloride is sometimes used in dilute solution as an antiseptic for inanimate objects and also as a fungicide. It is also used in preparing other mercury compounds as it reacts with mercury metal to form mercurous chloride.

Mercuric chloride is prepared by reacting mercury with chlorine gas or by subliming a mixture of mercuric sulfate and sodium chloride (common salt) [14]. Only a few substances can reduce its toxicity (Vitamins D & E, Zn and Cu), and costly chelators like BAL and DMSA (dimercapto succinic acid) can mobilize it from the body [1]. So multivitamin syrup was used as a standard in this model 1ml/100 g by oral route. Composition of Syrup was Vitamin B1, B2, B6 Niacinamide, Cynocobalamine, D- panthenol. Reduction in the serum Erythropoietin due to liver and kidney damage and uremia causes hemolysis and bone marrow depression leading to decrease in RBC count, and haemoglobin percentage [15].

The test drug i.e. Haema tablet was administered to test groups in the dose of 250 mg/kg, 500 mg/kg, 1000 mg/kg of the body weight along with $HgCl_2$ in the dose of 9 mg/kg. the multivitamin syrup in the of 1 ml was administered as standard drug along with $HgCl_2$ while in the anaemic control group only $HgCl_2$ in the dose of 9 mg/kg was administered for 30 days.

The rise in the Hb count, RBC count was observed as compared to anaemic group in the groups treated with Haema tablet at the end of the study. Increase in the MCV concentration as observed in the anaemic control group but was decreased in groups treated with Haema tablet. No any significant change in the concentration of MCH & MCHC was observed in all the groups.

The levels of AST were found to increase in the anaemic control group & the levels were decreased in the test treated with Haema tablet. Standard group significantly decreased in the AST levels, and the group treated with Haema tablet at 500 mg/kg observed that is comparable with standard.

The levels of ALT were found to increase in the anaemic control group & the levels were decreased in the test group. The standard group significantly decreased in the ALT levels, and the group treated with Haema tablet at 500 mg/kg observed that is comparable with standard.

Haemoglobin is important to carry oxygen. As each atom of iron can combine with an oxygen molecule, this means that a single haemoglobin molecule can carry up to four molecules of oxygen.In this study the oxygen capacity was increased as increase in the haemoglobin concentration.

The dose of 1000 mg/kg body weight has more oxygen carrying capacity to 46.43% as compared to anaemic control group, while the dose of 500 mg/kg body weight raises it to 65.09%. But the dose of 250 mg/kg body weight produced only 37.71% rise in oxygen carrying capacity. Multivitamin syrup produced the maximum oxygen carrying capacity to 80.07%.

Colour Index is one of the parameter used to differentiate between types of anaemia and in most of anaemia the color index is lower than 1. In the present study the color index of all group was lower than 1. So it was confirmed that there was induction anaemia in all groups. But after the treatment with standard & test drug Color index was greater than 1. The significant increase colour index was observed in a group treated with 500 mg/kg body weight of Haema tablet as compared to standard Multivitamin syrup.

Anaemia is also characterized by weight loss. So the body weight of all the animals was monitored. At the start of study the body weight of all animals was 228-233 gm. After administration of $HgCl_2$ in the anaemic control group it was found to be reduced to 201 gm at the end of the study. The substantial gain in body weight than anaemic control group was observed in the groups treated with Haema tablet in the doses of 250, 500, 1000 mg/kg body weight. Also comparable increase in weight was observed with animals treated with Multivitamin syrup.

$HgCl_2$ was administered for induction of anaemia and it was confirmed by histopathological findings on kidney, liver and spleen. After four weeks treatment with $HgCl_2$, there were severe abnormal changes like necrosis, cellular infiltration, degenration & fatty changes kidney, liver & spleen. This histopathological investigation confirmed the tissue changes as well as inflammatory changes that occurred due to $HgCl_2$ administration. The animals treated with standard (multivitamin syrup) & test drug (Haema tablet) has showed maximum protection against pathological changes in kidney, liver & spleen.

In this present study, animals treated with $HgCl_2$ has showed more than 75% (++++) pathological changes, animals treated with standard & Haema tablet in dose 500 mg/kg of body weight has showed less than 25% (+) pathological changes, animals treated with Haema tablet tin dose of 250 mg/kg & 1000 mg/kg of body weight was showing less than 75% (+++) & less than 50% (++) histopathological changes in kidney, liver & spleen respectively.

From the present study, it can be concluded that animals treated with standard (multivitamin syrup) & Haema tablet in dose 500 mg/kg of body weight both exerted significant protection against necrosis, inflammation, tissue degeneration in kidney, liver & spleen.

ACKNOWLEDGMENT: We are thankful to PDEA's S.G.R.S College of Pharmacy, Saswad for providing research facilities for this work.

REFERENCES:

1. Ross and Wilson: Anatomy and Physiology in health and illnes Elsevier Churchill Livingstone London New York Oxford, 9th edition: 60-75.
2. Gerard J. Tortora: Principles of Anatomy and Physiology Sandra Reynolds Grabowski Purdue University, 10th edition: 634-641.
3. Roger & walker: Clinical Pharmacy & therapeutics. 3rd edition: 725-740
4. Harsh Mohan: Text book of Pathology Jaypee Brothers Medical Publishers New Delhi, 3rd edition :385-444.
5. Joseph T. Dipiro: Pharmacotherapy A pathophysiologic Approach 6th edition :1799-1800 & 1805-1829
6. OECD Guildlines No 424
7. H Panda: Handbook on Herbal Medicines: 241-245.
8. Kirtikar KR and BD Basu. *Indian Medicinal Plants*. Vol. 1-4, 2nd edition: 1935.
9. Khandelwal K.R: Practical Handbook Of Pharmacognosy, Nirali Prakashan: 149-155
10. PK sarka, PK Prajapati,AK Choudhary, VJ Shukla,B Ravishankar: Haematinic evaluation of Lauha Bhasma and Mandur Bhasma on $HgCl_2$ induced anaemia in Rats. Indian Journal of pharmaceutical Sciences2007: 69:6: 791-795.
11. Rathore, H.S. and Varghese, J: Effect of Mercuric Chloride on the Survival, Food-intake, Body Weight, Histological and Haematological Changes in Mice and their Prevention with Liv.52. Indian Journal of Occupational Health Cell Biology Unit, School of Studies in Zoology, Vikram University, Ujjain, India.1997: 40: 1, 1
12. Gabriel A. agbor Julius E: Haematinic activity of Hibiscus cannabinus. African J of Biotechnology: Aug 2005:4(8): 833-837
13. Goldwater LJ: Mercury in the environment .Sci Am 1971: 224:15-21.
14. Friedman, M: In Protein, metal interaction. Advances in Experimental Medicine and Biology. 1971:13:1-15
15. Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL: Haririsons principles of internal medicine The McGraw Hill Companies. 15th edition.

How to cite this article:
Jagtap PN, Undale VR and Bhosale AV: Evaluation of Haematinic activity of polyherbal formulation in $HgCl_2$ induced anaemia in laboratory animals. *Int J Pharm Sci Res* 2013: 4(10); 3938-3952. doi: 10.13040/IJPSR. 0975-8232.4(10).3938-52