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# PHARMACOLOGICAL ASSESSMENT OF ANTI-ANAEMIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *CURCUBITA PEPO* SEEDS AND *MACROTYLOMA UNIFLORUM* GRAINS IN RATS

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**Keywords:**

*Macrotyloma uniflorum, Curcubita pepo*, Seeds, Grains, Iron, RBCs

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**ABSTRACT:** This study aimed to assess the anti- anemic activity of *Macrotyloma uniflorum* grains and *Curcubita pepo* seeds against Phenylhydrazine- induced anemia in albino rats. It was done by two extraction methods. In extract-1, *Macrotyloma uniflorum* was collected and soaked in water for whole night and next morning the grains were tied in a clean cloth and left for 3-4 days (Sprouted). The sprouted grains were dried under shade. Then the sprouted grains are fine powdered in a mill. The powder was macerated with 50% of alcohol for 7 days and filtered. The filtrate was evaporated after the complete evaporation of the crude extract. This extract was given to the animals for 14 days and on 15$^{th}$ day, the blood samples were collected and examined. In extract-2, *Curcubita pepo* was collected, outer shell was removed and dried under shade. The dried material was fine powdered in a mill. The powder was macerated with 50% of alcohol for 7 days and filtered. The filtrate was evaporated. After the complete evaporation of the crude extract. The extract was given to the animals for 14 days and on 15$^{th}$ day the blood samples are collected & examined. Both groups' blood samples are examined for Hb, PCV, MCH, MCHC, RDW, RBC, WBC were considered to determine the anti-anemic activity. Hydroalcoholic extract of *Macrotyloma uniflorum* and *Curcubita pepo* has increased the anemic condition of rats to normal level and showed anti-anemic activity against Phenyl hydrazine- induced anemia by significant increase in the levels of RBC.

**INTRODUCTION:** Anemia is a blood disorder characterized a reduction in the total number of red blood cells, haemoglobin concentration, and packed cell volume [1]. Hemoglobin is a main part of RBC that makes the blood cells red which binds with oxygen. There is an insufficient oxygen supply to the body in case of anemia.

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The reduction in RBC cells results in impaired oxygen delivery to tissues, giving rise to physiologic consequences of tissue hypoxia, fatigue, weakness, dizziness, headache, numbness, coldness in hands and feet, low body temperature, pale skin, rapid or irregular heartbeat, shortness of breath, chest pain and irritability [2].

The normal level of hemoglobin is generally different for men and women. For men, anemia is typically defined as hemoglobin level of less than 13.5g/100ml and in women as haemoglobin of less than 12.5g/100ml [3].

Anaemia is caused by blood loss, decreased red blood cell production, destruction of red blood cell and low haemoglobin level [4].

**Types of Anemia:**

**Iron Deficiency Anemia:** very little healthy RBCs due to very less iron in the body.

**Hemolyticanemia:** This type of anemia occurs when RBCs break down faster than the body can replace them.

**Idiopathic *Aplastic anemia*:** this is a condition that occurs when our body stops producing the enough red blood cells.

***Megaloblastic anemia*:** This is a type of anemia which produces abnormality structure and large in red blood cells.

***Pernicious anemia*:** this is a condition of decreased in red blood cells when the body cannot absorb enough vitamin $B_{12}$.

**Sickle Cell Anemia:** this is an inherited disease in which the red blood cells have an abnormal crescent shape, block small blood vessels, and do not last as normal RBCs.

**Vitamins Deficiency Anemia:** This type of anemia occurs most often in people whose bodies cannot absorb vitamin B12 from food because of an autoimmune disorder. It also can happen because of intestinal problems [5].

**Folate Deficiency Anaemia:** This type of anemia can occur if the enough folate is not consumed or if having problems absorbing vitamins. It also may occur during the third trimester of pregnancy, when the body needs extra folate.

**Causes of Anaemia:**

1. Destruction of RBC
2. Blood loss
3. Ulcers
4. Colon polyps or colon cancer
5. Regular use of aspirin and other drugs for pain
6. Infections
7. Severe injury
8. Surgery
9. Kidney dysfunction
10. Inherited blood disease
11. Cancer treatments

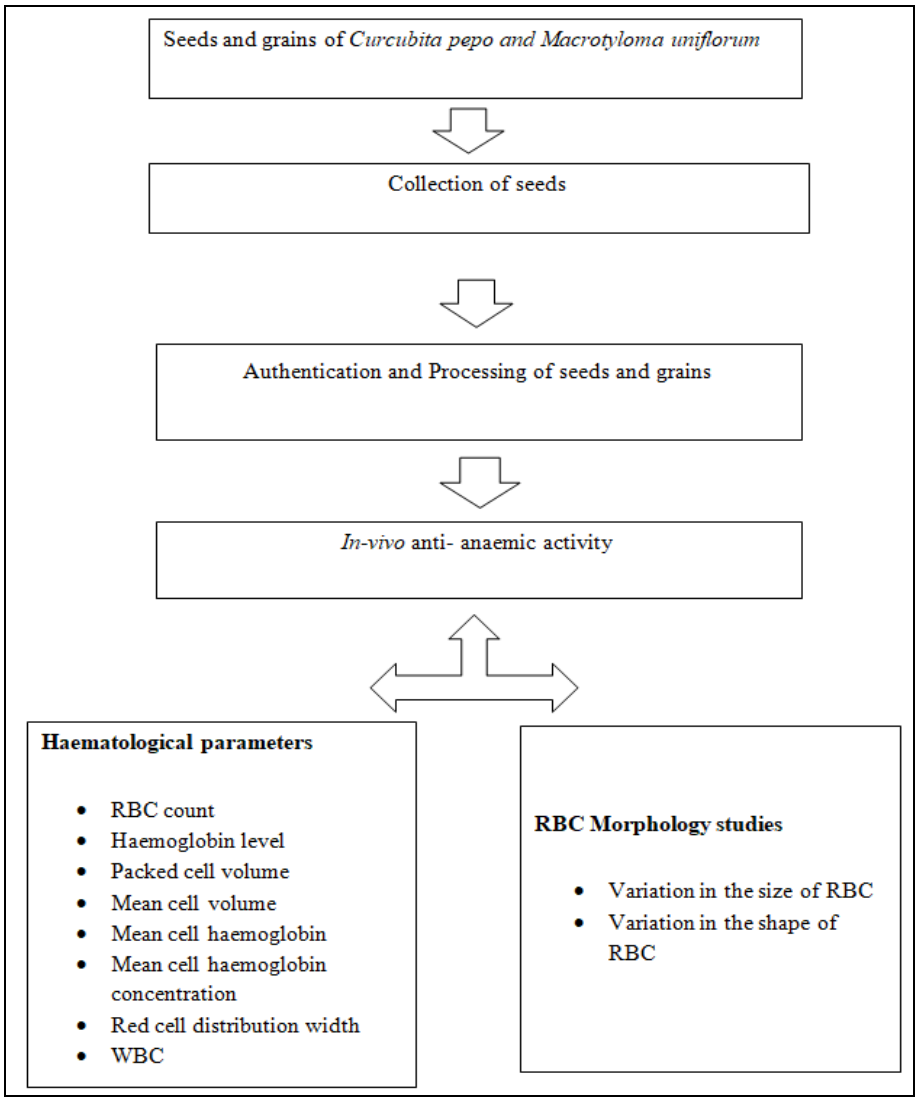
**Symptoms:**

- Dizziness
- Fatigue
- Light-headedness
- Malaise
- Fast heart beat /palpitations
- Brittle nails
- Headache
- Pallor
- Shortness of breath
- Weakness
- Pale skin/ yellowish skin
- Cold hands and feet
- Chest pain
- Inflammation or soreness of your tongue
- Poor appetite
- Unusual cravings for non-nutritive substances

**Treatment:**

- Dietary supplement
- Blood transfusion
- Vitamins
- Iron supplements
- Iv iron therapy
- Erythropoiesis stimulating agents

**Plan of Work:**




## MATERIALS AND METHODOLOGY:

### Materials:

### Collection and Identification of Plant Material:

**Authentication of the Plant:** The seeds and grains of *Curcubita pepo* and *Macrotyloma uniflorum* were collected from the local market, in Bangalore.

The sample was authenticated by Dr. P. E. Rajasekharan, Principal Scientist & Nodal officer GAC, Division of Plant Genetic Resources, Indian Institue of Horticulture Research, Hessaraghatta Lake Post, Bangalore-560089.

### Preparation of Plant Extract:

**TABLE 1: LIST OF CHEMICALS**

| Phenylhydrazine | Inducing agent |
|---|---|
| Carboxymethyl cellulose | Vehicle |
| Normal saline | Vehicle |
| Thiopental sodium | Anesthesia |
| Ethanol+Water | Isolation solvent |

### Maceration:

- The grains of *Macrotyloma uniflorum* were washed with clean water and tied in a clean cloth for 3 days removed from the tied cloth (Sprouted grains) spread under shade dried at room temperature and then subjected to size reduction to a coarse powder by using Willey mill.
- The seeds of *Curcubita pepo* were dried, removed from the hulls of seeds, dried under shade at room temperature, and then subjected to size reduction to a coarse powder using a Willey mill.
- The powdered grains of *Macrotyloma uniflorum* and *Curcubita pepo* were placed in a stoppered container separately with distilled water and ethanol at the ratio of 50:50 at room

temperature for 7 days with frequent agitation until the soluble matter had dissolved. The mixtures were strained, the marc was pressed, and the combined liquids were clarified by filtration or decantation after standing. The hydroalcoholic extract was concentrated in a rotary evaporator. The concentrated extracts were taken for the *in-vivo* studies.


**FIG. 1 & 2: RAW PICTURES OF *MACROTYLOMA UNIFLORUM* AND *CURCUBITA PEPO***



**FIG. 3, 4 & 5: MACERATION PROCESS PICTURES**

**TABLE 2: PERCENTAGE YIELD FROM THE HYDRO-ALCOHOLIC EXTRACT OF *MACROTYLOMA UNIFLORUM AND CURCUBITA PEPO SEEDS***

| Extract | Plant material used | Weight of the plant material (g) | Yield (g) | Percentage yield (%) |
|---|---|---|---|---|
| Ethanol: Water (50:50) | *Macrotyloma uniflorum grains* (Sprouted grains) | 500g | 24.562g | 4.9124% |
| Ethanol: Water (50:50) | *Curcubita pepo seeds* (Dried seeds) | 500g | 29.326g | 5.8652% |

**Screening of Anti-anemic Activity:**

**Experimental Animals:** Healthy Wistar rats (150-200g) were bought from Animal Experimental Laboratory: *in-vivo* Biosciences, Janashakthi Nagar, Kamath Layout, Bangalore- 91. The study was approved by the Institutional Animal Ethics Committee which is certified by the Committee for the Control and Supervision of Experiments on Animals (CPCSEA), India. IAEC Approval Number: MCP085/2019-2020

**Maintainance of Animals:** The animals were kept in clean and dry polypropylene cages with a stainless-steel top grill having facilities for pelleted food and water. The animals were maintained in a well-ventilated animal house in 12 hours and 12 hours dark cycle at a temperature of 28ºC ± 2ºC and were acclimatized to laboratory conditions for 10 days after the commencement of the experiment. The animals were fed with a standard pellet diet and water *ad libitum*. All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC). Paddy husk was used as bedding material and changed twice a week[6].

**Animal Model:** The model used to evaluate anti-anemic activity was PHZ⁻induced anemia in rats [7].

**Procedure:** Anemia was induced in rats by administration of Phenylhydrazine for 7 days at the dose of 40mg/kg, oral route. All the animals were weighed and divided into four groups, each group containing six animals [8].

**TABLE 3: ANTI-ANAEMIC ACTIVITY OF GRAINS & SEEDS EXTRACT OF *MACROTYLOMA UNIFLORUM & CURCUBITA PEPO* IN RATS [9]**

| Sl. no. | Group | Name of the group | Dose | Number of animals | Duration of dosage |
|---|---|---|---|---|---|
| 1 | Group I | control | 10ml/kg of 0.5% CMC | 6 | 14 days |

| | | | | | |
|---|---|---|---|---|---|
| 2 | Group II | Negative control | CMC (10m/kg)+ Phenyl hydrazine 40mg/kg | 6 | 14 days CMC+7 days PHZ |
| 3 | Group III | Standard | Vitamin B12 syrup (1ml/day) + PHZ, 40mg/kg | 6 | 14 days syrup+ 7 days PHZ |
| 4 | Group IV | *Macrotyloma uniflorum* grains extract Low dose | 100mg/kg *Macrotyloma uniflorum*+ 40mg/kg PHZ | 6 | 14 days L.D+ 7 days PHZ |
| 5 | Group V | *Macrotyloma uniflorum* grains extract High dose | 200mg/kg *Macrotyloma uniflorum*+40mg/kg PHZ | 6 | 14 Days H.D+ 7 Days PHZ |
| 6 | Group VI | *Curcubita pepo* seeds extract Low dose | 100mg/kg*Curcubita pepo* + 40mg/kg PHZ | 6 | 14days syrup L.D+ 7days PHZ |
| 7 | Group VII | *Curcubita Pepo* seeds extract High dose | 200mg/kg kg*Curcubitapepo* +40mg/kg PHZ | 6 | 14days H.D +7days PHZ |

The blood samples were collected before and after the induction of anemia by retro-orbital sinus with a glass capillary tube and used for the estimation of hematological parameters [10]. Then the blood was allowed to clot for 30 minutes at room temperature. The clear serum was separated by centrifugation at 2500 rpm for 5 minutes and used for the RBC morphology studies [11].

**Parameters Evaluated:**
**Hematological Analysis:**

1. Red Blood Cell Volume (RBC)
2. Hemoglobin (Hb)
3. Packed Cell Volume (PCV)
4. Mean cell volume (MCV)
5. Mean cell hemoglobin (MCH)
6. Mean cell hemoglobin concentration (MCHC)
7. Red cell distribution width (RDW)

**RBC Morphology:**

- Variation in the size of RBC
- Variation in the shape of RBC

**Statistical Analysis:** The results of the hematological estimations were presented as mean ± SD of six animals in each group. Total variations, present in a set of data were estimated by One Way Analysis Of Variance (ANOVA). A P value of <0.05 was considered statistically significant**.**

**Haematological Analysis:** Hematology analyzers are used widely in patient and research settings to count and characterize blood cells for disease detection and monitoring [12]. Basic analyzers return a complete blood count (CBC) with a three-part differential white blood cell (WBC) count. Sophisticated analyzers measure cell morphology and can detect small cell populations to diagnose rare blood conditions [13].

**RBC Morphology:**
**Normal and Pathologic Red Blood Cells:** The normal red blood cell is a biconcave disk, 6 to 9µm in diameter and 1.5 to 2.5µm thick. The hemoglobin imparts a uniform pink to orange-red color to the cytoplasm that is typically without inclusions [14]. Normally all red blood cells are relatively uniform in size and shape. Numerous disease states affect the size, shape,and hemoglobin content of red cells. Variation in the size is referred to as "anisocytosis," and variation in shape is termed "poikilocytosis." Pathologic red cells may be larger or smaller than normal, may be abnormally shaped, or may contain inclusions [15].

**Procedure:** 1ml of blood will be collected from the rat eye by sinus orbital puncture and centrifuged at 2000rpm for 5 min and the plasma was discarded. The packed cells are reconstituted as 10% v/v suspension with 0.9% normal Saline. A Drop of this suspension will be put on a glass slide under a cover slip and studied under High Power Microscope at 40 X for assessment of morphological changes in the red blood cells.

Morphological changes in RBCs at $0^{th}$ day, after induction of anemia and, after 6 weeks of treatment with *Macrotyloma uniflorum* and *Curucubita pepo of* the hydroalcoholic extract was assessed using the following scoring pattern [16].

***Macrotyloma uniflorum:***
**High dose:** total count- 4800 cells/cumm

RBC count-5.52 mill/cumm

**Low dose:** total count- 7300 cells/cumm

RBC count-6.19 mill/cumm

***Curcubita pepo:***
**High dose:** total count-11900 cells/cumm

RBC count-7.32 mill/cumm

**Low dose:** total count- 11300 cells/cumm, RBC count- 6.65 mill/cumm

**RESULTS:** Anemia is considered as a major health problem throughout the world. Anemia is a condition in which there is a deficiency of red blood cells or of haemoglobin in the blood, resulting in pallor and weariness. More than half of the world's population experience some forms of anemia in their life time. Such prevalence has been attributed to various aggravating factors such as poor nutrition, pregnancy, high prevalence of blood parasites for example, plasmodium, trypanosome and helminthes infection. Prolonged use of non-steroidal anti-inflammatory drugs as well as exposure to toxic chemicals such as phenyl hydrazine have also been implicated to cause anemia. Due to the high prevalence and possibility of even further increase, there is the need to prevent it or seek for more cost effective and better treatment strategies. From ancient time, medicinal plants in Ayurveda are believed to be useful in strengthening the hematopoietic and immune system of an individual. Ayurvedic physicians suggested various herbs for the treatment of haematological disorders as a source of iron and other minerals. Various researchers successfully evaluated the potential of several medicinal plants in the treatment of anemia using various experimental animal models.

*Ageratum conyzoides, Boerhavia diffusa, Centella asiatica, Hemidesmus indicus, Ichnocarpus frutescens, Momordica charantia, Moringa oleifera, Phyllun thusamarus, Punicagranatum, Ocimum tenuiflorum, Solanum americanum* are the some plants used in the treatment of anaemia.

*Macrotylomauni florum* traditionally used in asthma, bronchitis, urinary problems, jaundice, peptic ulcer, haemorrhoids and even menstrual problems. It is also beneficial for extracting phlegm and controlling fever. The astringent properties of horse gram are helpful in treating skin disease leucoderma. It is also used as a facial pack to prevent skin problems and clean the skin. Horse gram (raw, unsprouted) following a meal can reduce the glycemic index, by slowing down carbohydrate digestion and reducing insulin resistance. It has a natural qualities that work as fat burners. It can reduce the LDL cholesterol and increase the HDL cholestrol and favourable in melting body fat and gives a proper shape to the body. These filters and purifies the blood and detoxifying chemicals in the body. The Phytochemical analysis of hydroalcoholic extract of grains of *Macrotylomauniflorum* revealed the presence of carbohydrtae, protein, dietary fiber, fat, calcium, phosphorus, iron, calories, vitamins like thiamine, riboflavin and niacin. Ethnopharmacological studies show that *Curcubitapepo*is used in many countries for treating numerous diseases e.g., as an anti-inflammatory, antiviral, analgesic, urinary disorders, anti-ulcer, antidiabetic and antioxidant. Traditional medicine, particularly Ayurvedic systems, and Chinesehave used different parts of the plant including flesh of the fruits and seeds. Reporting that pumpkin exhibiting important physiological properties as wound healing, tumour growth inhibition, hypoglycaemic effects and immunomodulating.

The seeds are used as a vermifuge, treat problems of the urinary system, hypertension, prevents the formation of kidney stones, alleviate prostate diseases and enhanced the erysipelas skin infection. The Phytochemical analysis of hydroalcoholic extract of seeds of *Curcubitapepo* revealed the presence of total sugars, protein, fibre, fat, ash and iron. To induce anemia in the wistar rats are administered to Phenylhydrazine 40mg/kg by oral route for seven days, on the 8th day the blood was collected and haematological studies are performed then the blood was centrifuged and the serum was separated and used for the RBC morphology studies.

**TABLE 4: HEMATOLOGICAL PARAMETERS AFTER THE TREATMENT WITH THE HYDROALCOHOLIC EXTRACT OF *MACROTYLOMA UNIFLORUM* AT THE END OF 15 DAYS**

| Sl. no. | Parameters | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|---|
| 1 | RBC Count ($X10^{12}$/L) | 10.45±0.56 | 5.98±0.62[a] | 8.45±0.54 | 9.29±0.62 | 10.13±0.69 |
| 2 | Haemoglobin(g/DL) | 17.20±0.35 | 6.20±0.39[a] | 15.31±0.58 | 14.36±0.56 | 15.59±0.69[b] |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | Hematocrit(%) | 48.30±0.41 | 29.35±0.58[a] | 46.45±0.54 | 44.46±0.61 | 46.58±0.78[b] |
| 4 | Mean Cell Volume (fL) | 59.52±0.31 | 31.63±0.63[a] | 55.96±0.71 | 54.74±0.81 | 57.63±0.32[b] |
| 5 | Mean Cell Hemoglobin (pg) | 19.20±0.35 | 7.26±0.38[a] | 18.40±0.43 | 16.63±0.74 | 17.63±0.34[b] |
| 6 | Mean Cell Hemoglobin Concentration(g/DL) | 35.69±0.46 | 20.31±0.58[a] | 33.71±0.82 | 29.63±0.25 | 31.87±0.93[b] |
| 7 | Red Cell Distribution Width (%) | 13.6±0.26 | 7.3±0.17[a] | 11.6±0.32 | 10.3±0.57 | 12.6±0.61[b] |
| 8 | WBC Count | 6600cel/cumm | 3200cel/cumm[a] | 5600cel/cumm | 4800cel/cumm | 7300cel/cumm[b] |

Values are expressed as mean ± SD (n=6). 'b' P<0.001 compared with the negative control group a' P<0.001 compared with negative control group.

There is a decrease in RBC count, Hemoglobin level, Hematocrit, Mean cell volume, Mean cell hemoglobin, Mean cell hemoglobin concentration, Red cell distribution width, and WBC after the administration of PHZ in negative control group II these levels are increased in standard group III and with the administration of hydro alcoholic extract of low dose and high dose of *Macrotyloma unifloruum* treated group and the P values are also significant (P<0.001).

**TABLE 5: HEMATOLOGICAL PARAMETERS AFTER THE TREATMENT WITH THE HYDROALCOHOLIC EXTRACT OF *CURCUBITA PEPO* AT THE END OF 15 DAYS**

| Sl. no. | Parameters | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|---|
| 1 | RBC Count ($X10^{12}$/L) | 10.56±0.13 | 6.32±0.51[a] | 8.26±0.39 | 9.85±0.53 | 10.32±0.62 |
| 2 | Haemoglobin (g/DL) | 17.13±0.29 | 6.10±0.63[a] | 14.28±0.89 | 13.39±0.65 | 15.28±0.56[b] |
| 3 | Hematocrit (%) | 48.15±0.56 | 28.38±0.15[a] | 44.56±0.52 | 43.41±0.71 | 46.05±0.74[b] |
| 4 | Mean Cell Volume (fL) | 58.12±0.24 | 31.53±0.53[a] | 54.26±0.41 | 54.64±0.53 | 56.53±0.12[b] |
| 5 | Mean Cell Hemoglobin(pg) | 18.19±0.15 | 7.56±0.18[a] | 17.44±0.23 | 15.53±0.64 | 16.93±0.61[b] |
| 6 | Mean Cell Hemoglobin Concentration (g/DL) | 36.29±0.56 | 21.11±0.58[a] | 32.60±0.72 | 29.65±0.54 | 31.23±0.53[b] |
| 7 | Red Cell Distribution Width (%) | 13.8±0.21 | 7.6±0.26[a] | 11.2±0.61 | 10.3±0.28 | 12.7±0.55[b] |
| 8 | WBC Count | 7100cel/cu Mm | 3500cel/cu mm[a] | 5800cel/cu Mm | 11300cel/cu Mm | 11900cel/cu mm[b] |

Values are expressed as mean ± SD (n=6). 'b' P<0.001 compared with the negative control group 'a' P<0.001 compared with the negative control group.

There is a decrease in RBC count, Hemoglobin level, Hematocrit, Mean cell volume, Mean cell hemoglobin, Mean cell hemoglobin concentration, Red cell distribution width, and WBC after the administration of PHZ in negative control group II these levels are increased in standard group III and with the administration of hydroalcoholic extract of low dose and high dose of *Curcubitapepo treated* group and the P values are also significant (P<0.001).

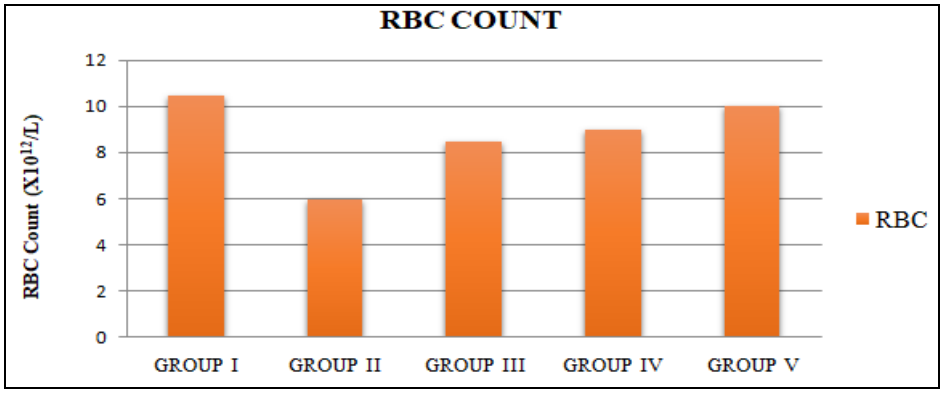



**FIG. 6: RBC COUNT AFTER THE TREATMENT WITH HYDROALCOHOLIC EXTRACT OF THE *MACROTYLOMA UNIFLORUM* GRAINSAND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in RBC count whereas the RBC count of the normal group rats remained the same. Administration of hydroalcoholic extract of grains of *Macrotyloma uniflorum* for 15 days significantly (P<0.001) increased the RBC count and brought back RBC count towards a normal level.

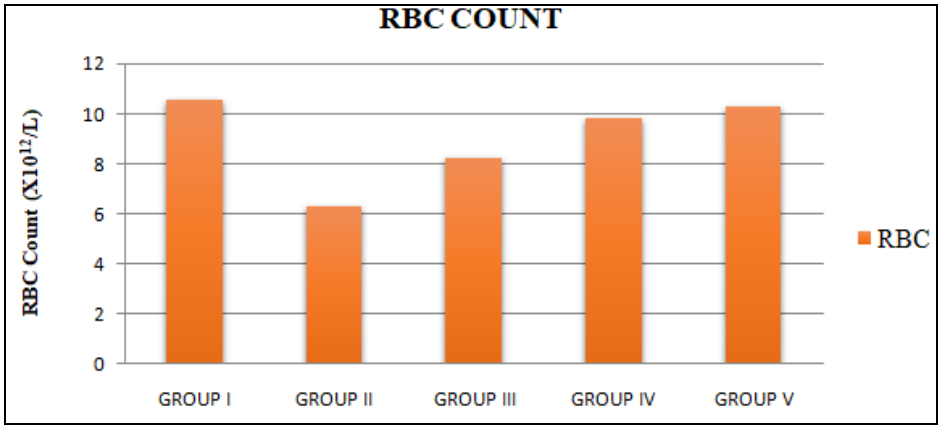



**FIG. 7: RBC COUNT AFTER THE TREATMENT WITH HYDROALCOHOLIC EXTRACT OF THE *CURCUBITA PEPO* SEEDS AND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in RBC count whereas the RBC count of the normal group rats remained the same.

Administration of hydroalcoholic extract of seeds of *Curcubita pepo* for 15 days significantly ($P<0.001$) increase the RBC count and brought back RBC count towards normal level.

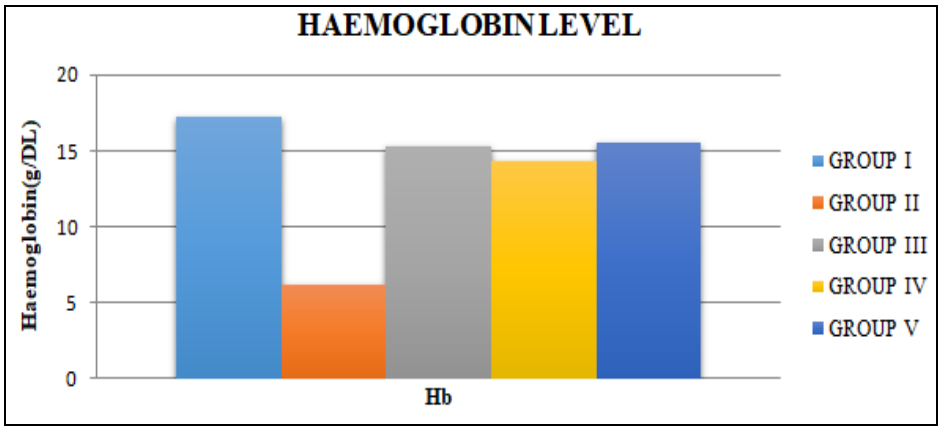



**FIG. 8: HAEMOGLOBIN LEVEL AFTER THE TREATMENT WITH THE HYDROALCHOLIC EXTRACT OF *MACROTYLOMA UNIFLORUM* GRAINS AND AT THE END OF 15DAYS**

The results are tabulated in the above table. Anaemic rats showed decrease in Hb level whereas the Hb level of the normal group rats remained the same. Administration of the hydroalcoholic extract of grains of *Macrotyloma uniflorum* for 15 days significantly ($P<0.001$) increase the Hb level and brought back Hb level towards normal.

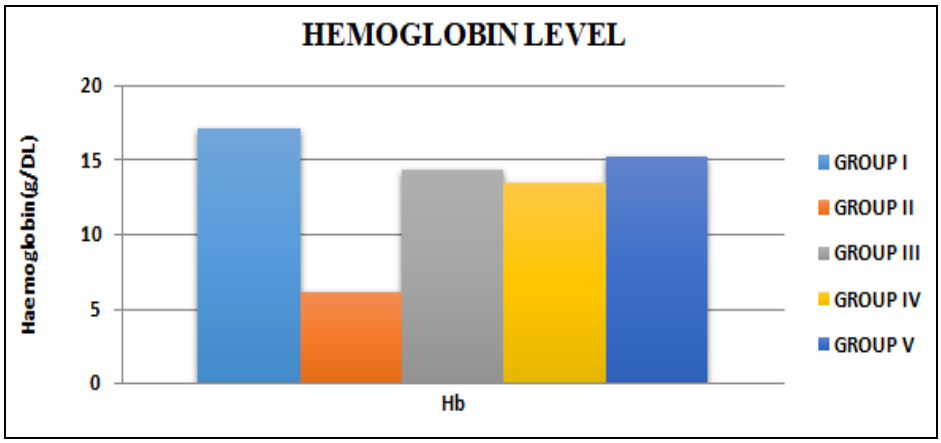



**FIG 9: HAEMOGLOBIN LEVEL AFTER THE TREATMENT WITH THE HYDROALCHOLIC EXTRACT OF *CURCUBITA PEPO* SEEDS AND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in Hb level whereas the Hb level of the normal group rats remained the same. Administration of the hydroalcoholic extract of seeds of *Curcubita pepo* for 15 days significantly (P<0.001) increased the Hb level and brought back Hb level towards normal.

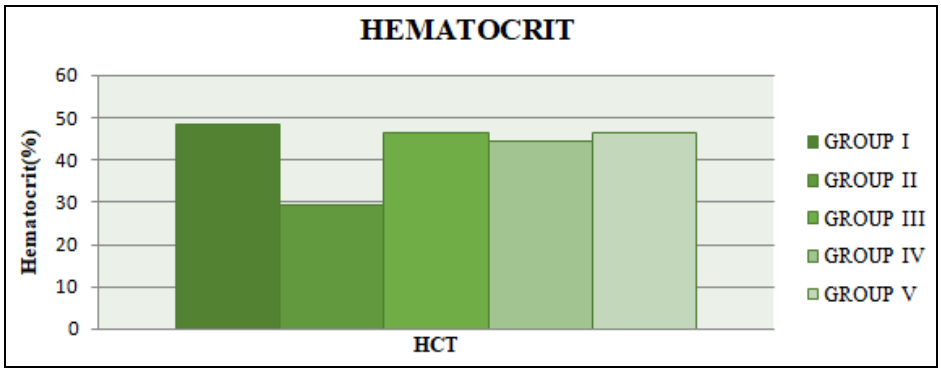



**FIG. 10: HEMATOCRIT AFTER THE TREATMENT WITH HYDROALCOHOLIC EXTRACT OF *MACROTYLOMA UNIFLORUM* GRAINS AND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in Hematocrit whereas the Hematocrit of the normal group rats remained the same. Administration of hydroalcoholic extract of grains of *Macrotyloma uniflorum* for 15 days significantly (P<0.001) increased the Hematocrit and brought back Hematocrit towards normal.

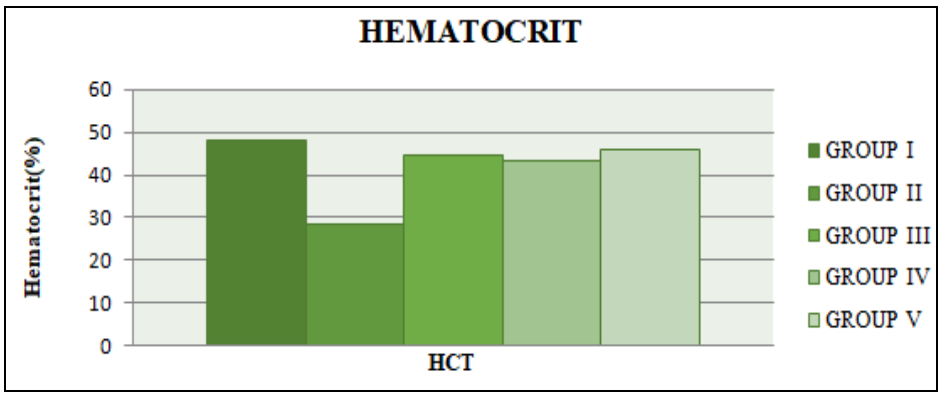



**FIG. 11: HEMATOCRIT AFTER THE TREATMENT WITH HYDROALCOHOLIC EXTRACT OF *CURCUBITA PEPO* SEEDS AND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in Hematocrit whereas the Hematocrit of the normal group rats remained the same. Administration of hydroalcoholic extract of seeds of *Curcubita pepo* for 15 days significantly (P<0.001) increased the Hematocrit and brought back Hematocrit towards normal.

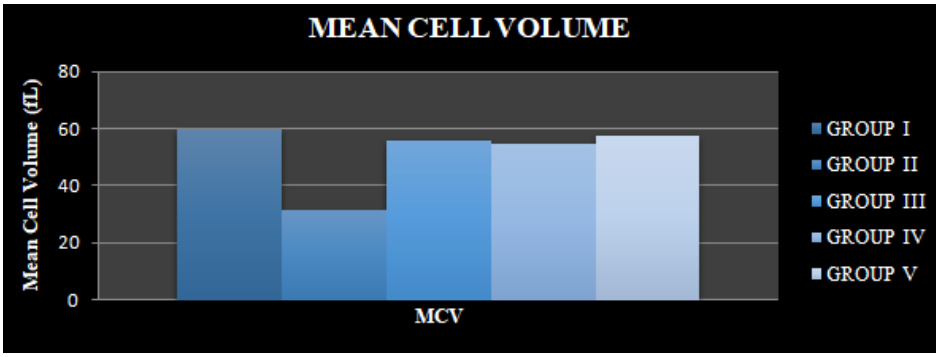



**FIG. 12: MEAN CELL VOLUME AFTER THE TREATMENT WITH HYDROALCOHOLIC EXTRACT OF *MACROTYLOMA UNIFLORUM* GRAINS AND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in Mean Cell Volume whereas the Mean Cell Volume of the normal group rats remained the same. Administration of hydroalcoholic extract of grains of *Macrotyloma uniflorum* for 15 days significantly (P<0.001) increased the Mean Cell Volume and brought back Mean Cell Volume towards normal.

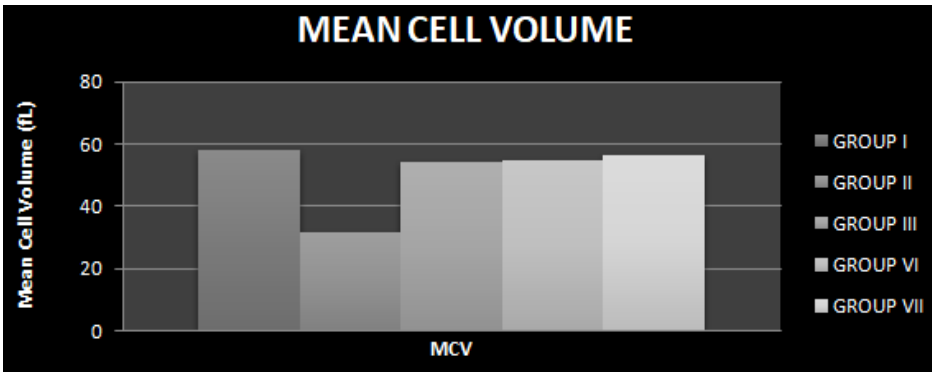



**FIG. 13: MEAN CELL VOLUME AFTER THE TREATMENT WITH HYDROALCOHOLIC EXTRACT OF *CURCUBITA PEPO* SEEDS AND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in Mean Cell Volume whereas the Mean Cell Volume of the normal group rats remained the same. Administration of hydroalcoholic extract of seeds of *Curcubita pepo* for 15 days significantly (P<0.001) increased the Mean Cell Volume and brought back Mean Cell Volume towards normal.

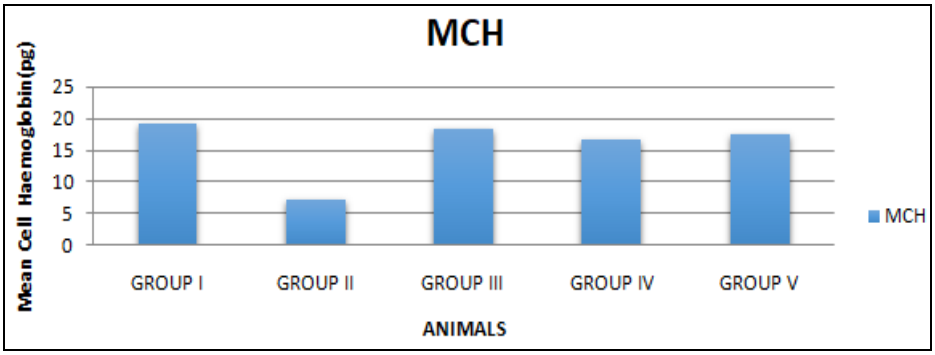



**FIG. 14: MEAN CELL HAEMOGLOBIN AFTER THE TREATMENT WITH THE HYDROALCOHOLIC EXTRACT OF *MACROTYLOMA UNIFLORUM* GARINS AND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in mean Cell haemoglobin level whereas the Mean Cell haemoglobin level of the normal group rats remained the same. Administration of hydroalcoholic extract of grains of *Macrotyloma uniflorum* for 15 days significantly (P<0.001) increased the mean Cell haemoglobin level and brought back mean Cell haemoglobin level towards normal.

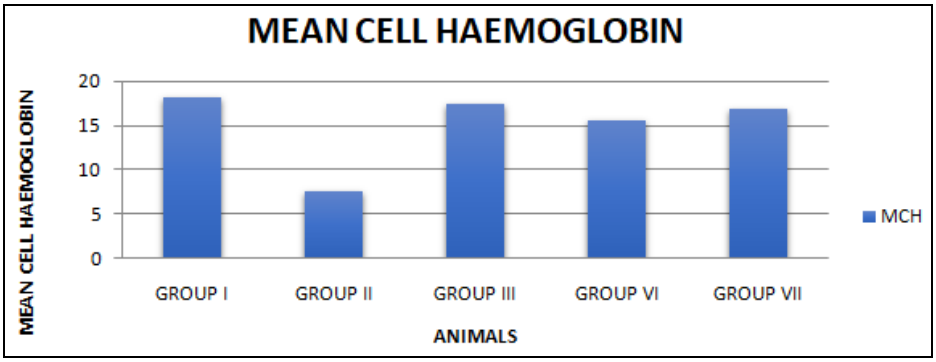



**FIG. 15: MEAN CELL HAEMOGLOBIN AFTER THE TREATMENT WITH THE HYDROALCOHOLIC EXTRACT OF *CURCUBITA PEPO* SEEDS AND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in Mean Cellhaemoglobin level whereas the Mean Cell haemoglobin level of the normal group rats remained the same. Administration of hydroalcoholic extract of seeds of *Curcubita pepo* for 15 days significantly (P<0.001) increased the Mean Cell haemoglobin level and bring back the Mean Cell haemoglobin level to normal.

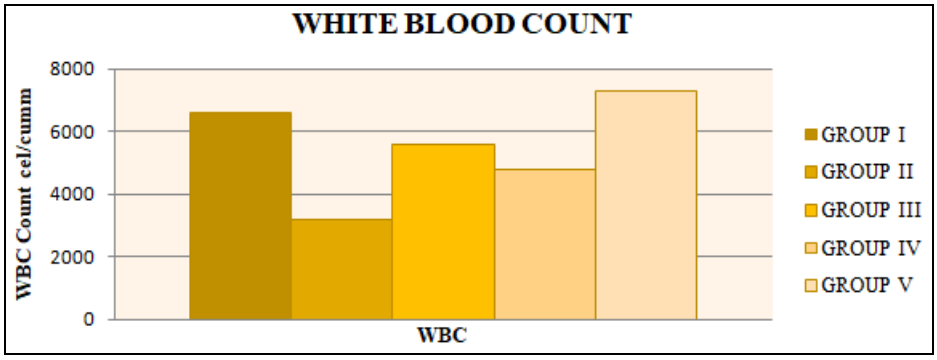



**FIG. 16: WHITE BLOOD CELL COUNT AFTER THE TREATMENT WITH THE HYDROALCOHOLIC EXTRACT OF *MACROTYLOMA UNIFLORUM* GRAINS AND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in WBC whereas the WBC count of the normal group rats remained the same. Administration of hydroalcoholic extract of grains of *Macrotyloma uniflorum* for 15 days significantly (P<0.001) increased the WBC and brought back WBC count towards normal.

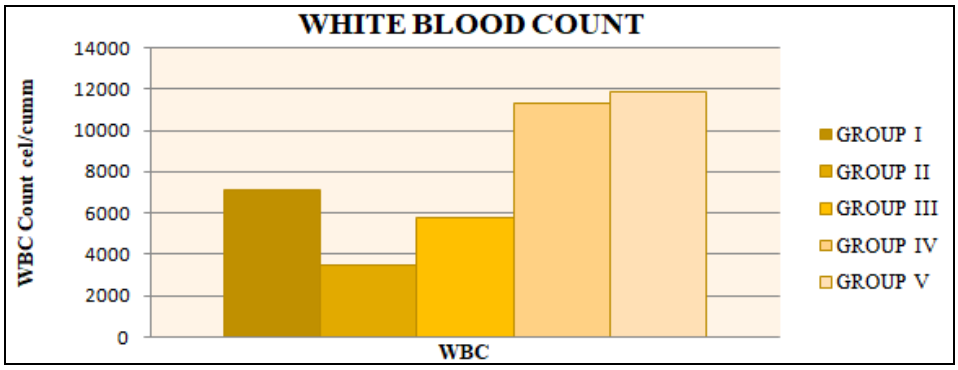
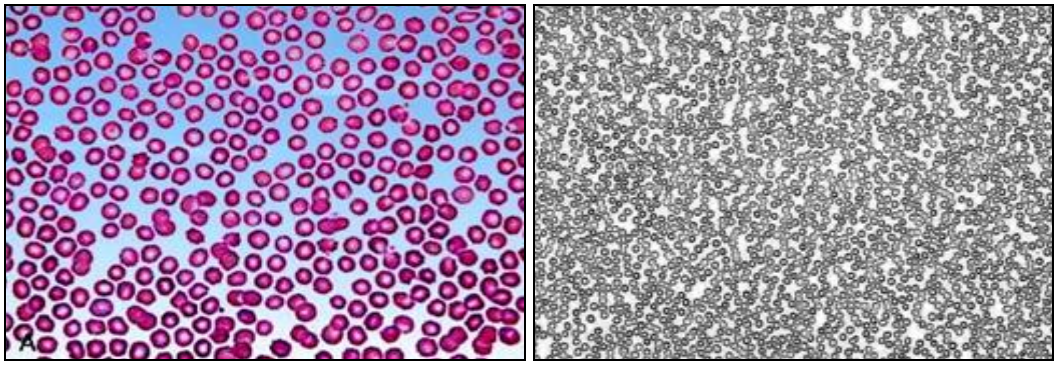



**FIG. 17: WHITE BLOOD CELL COUNT AFTER THE TREATMENT WITH THE HYDROALCOHOLIC EXTRACT OF *CURCUBITA PEPO* SEEDS AND AT THE END OF 15 DAYS**

The results are tabulated in the above table. Anemic rats showed a decrease in WBC whereas the WBC count of the normal group rats remained the same. Administration of hydroalcoholic extract of seeds of *curcubita pepo* for 15 days significantly (P<0.001) increased the WBC and brought back the WBC count towards normal.

**RBC Morphology:**



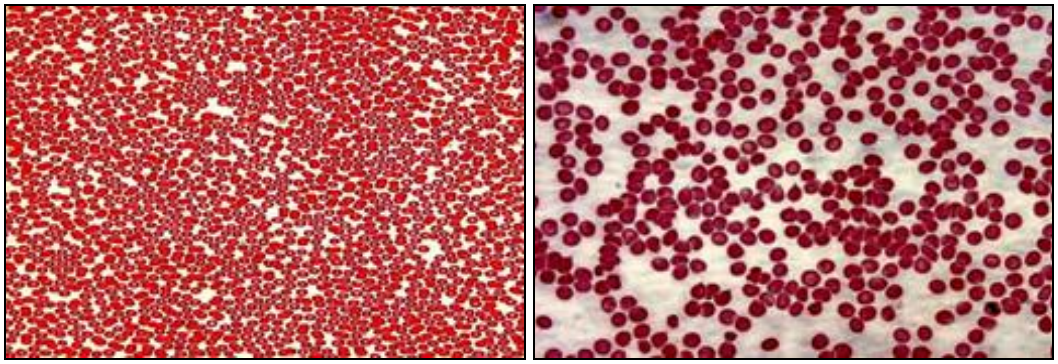


**FIG. 18: MORPHOLOGY OF RBC AT 0 DAYS**

The morphology of RBC was observed on 0 day. All the cells are in the same size and shape when focused under the microscope.

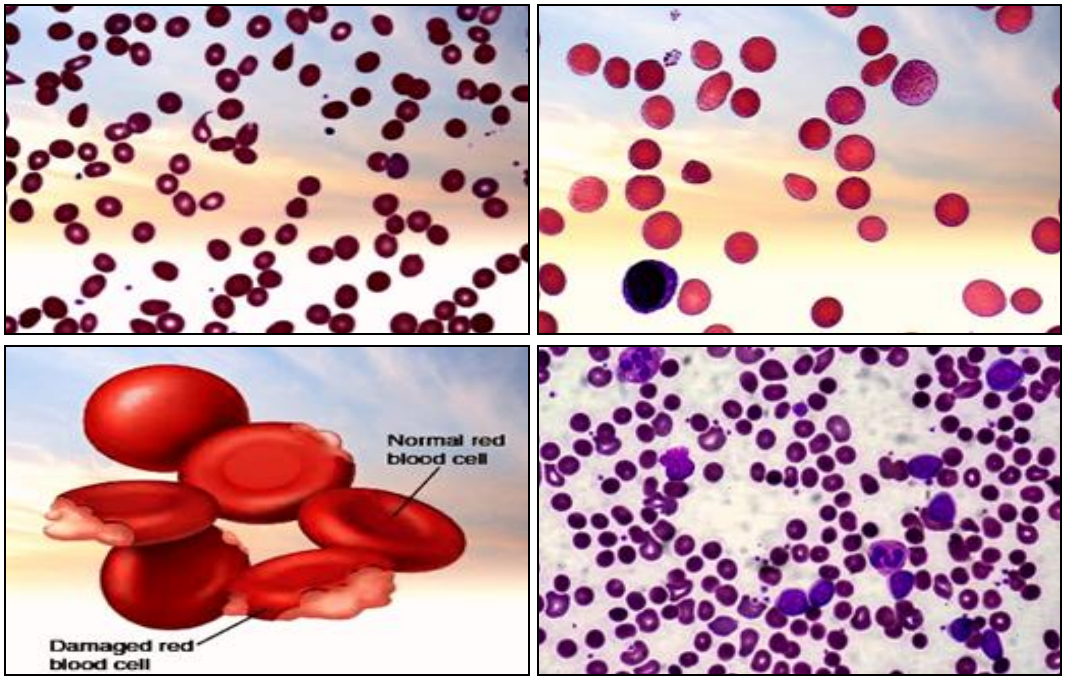



**FIG. 19: MORPHOLOGY OF RBC AFTER THE ADMINISTRATION OF PHENYLHYDRAZINE**

The morphology of RBC was observed on 7$^{th}$day of phenylhydrazine administration. Heinz bodies and RBC damage were observed with crenated edges and changes in the shape of red blood cells were observed.

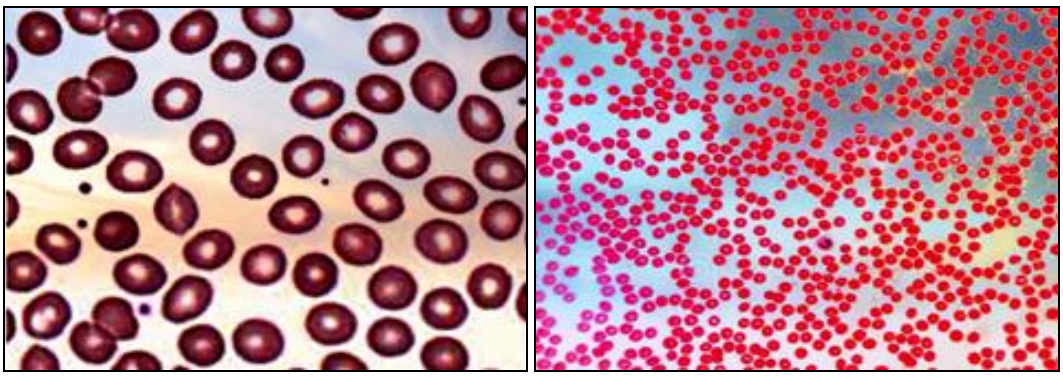


**FIG. 20: MORPHOLOGY OF RBC AFTER THE ADMINISTRATION OF HYDROALCOHOLIC EXTRACT OF *MACROTYLOMA UNIFLORUM* FOR 15 DAYS**

There is a reduction in the number of Heinz bodies and recovery of RBC cells after the administration of hydroalcoholic extract of *Macrotyloma uniflorum* grains for 15 days.

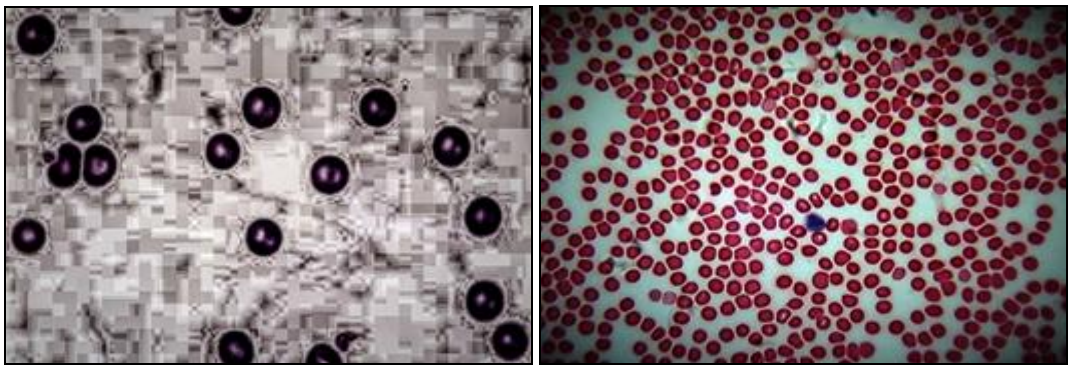


**FIG. 21: MORPHOLOGY OF RBC AFTER THE ADMINISTRATION OF HYDROALCOHOLIC EXTRACT OF *CURCUBITA PEPO* SEEDS FOR 15 DAYS**

There is a reduction in the number of Heinz bodies, and also recovery of RBC cells and also increase in the WBC count after the administration of hydroalcoholic extract of *Curcubita pepo* seeds for 15 days.

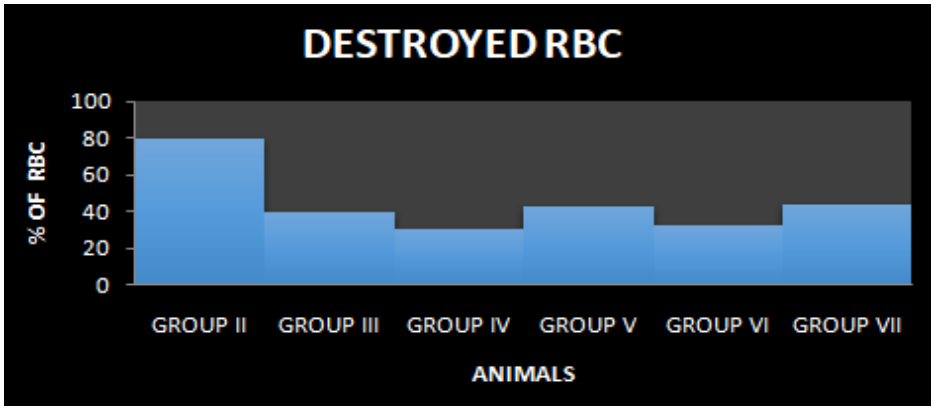



**FIG. 22: PERCENTAGE OF DESTROYED RBC AFTER THE ADMINISTRATION OF PHENYLHYDRAZINE**

About 91% of red blood cells were affected by the administration of phenylhydrazine and the presence of Heinz bodies with crenated edges was observed.

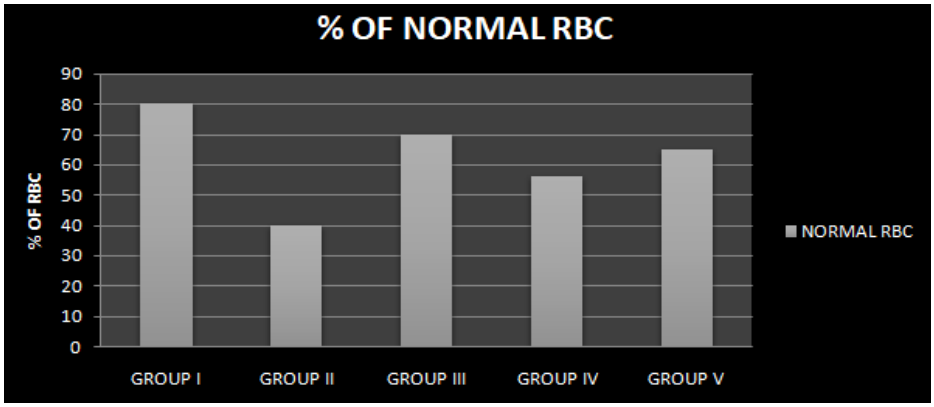



**FIG. 23: PERCENTAGE OF THE NORMAL RBC AFTER THE ADMINISTRATION OF HYDROALCOHOLIC EXTRACT OF *MACROTYLOMA UNIFLORUM* GRAINS AND *CURCUBITA PEPO* SEEDS AT THE END OF 15 DAYS**

About 70% of Heinz bodies and breakage of RBCs were treated with the administration of *Macrotyloma uniflorum* and *Curcubita pepo* for 15 days and came to their normal shape and the results

showed that *Macrotyloma uniflorum* and *Curcubita pepo* prevents the destruction of RBC.

**CONCULSION:** In this present study it has been shown that the hydroalcoholic extract of *Macrotyloma uniflorum* grains and *Curcubita pepo* seedsincreases the RBC count, Haemoglobin level, Haematocrit, Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration, Red Cell Distribution Width and also WBC. On the basis of the above results the RBC morphological studies, reduction in the percentage of Heinz Bodies after the administration of hydroalcoholic extract of *Macrotyloma uniflorum* grains and *Curcubita pepo*seedswhen compared with negative control group (Group II) of animals, Hence it was suggested that the grains of hydroalcoholic extract of *Macrotyloma uniflorum* and *Curcubita pepo* possessed significant anti-anaemic effect

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