

# In vivo assessment of bioactivity of *Trichosanthes dioica* Roxb for the management of haemolytic anaemia.

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**Abstract:** Anemia is severe blood disorder in developing and underdeveloped countries, which can have very diverse origin. The objective of the present work is to assess the effect of aqueous extracts of fruits of *Trichosanthes dioica* Roxb (Family-Cucurbitaceae) on blood parameters in drug induced hemolytic anemia.

Dried and powdered fruits of *Trichosanthes dioica* were extracted by maceration with distil water. Aqueous extract are tested for the presence of alkaloids, glycosides, phenolic compounds, protein & amino acids, tannins, iron, in fruit.

This aqueous extract of *Trichosanthes dioica* fruits were tested for their effects on hemoglobin concentration (Hb), red blood cell count (RBC), reticulocyte count, packed cell volume (PCV), cell shape, morphology and osmotic resistance in phenyl hydrazine induced hemolytic anemia model in albino rats.

Recovery study revealed that after 16 days, the groups of anemic rats treated with aqueous extract exhibited significant ( $P < 0.01$ ) improvement in hemoglobin concentration, red blood cell count, reticulocyte count, packed cell volume (mean values being 14.15gm/dl,  $7.66 \times 10^6/\mu\text{l}$ , 0.75%, 48.4% respectively) when compared to group of anemic rats left untreated (mean values 8.54gm/dl,  $4.16 \times 10^6/\mu\text{l}$ , 1.316 % and 39.16 % respectively). Treatment with aqueous extract during recovery period resulted in increased osmotic resistance in larger proportion of cells showing regular normal shape and cell integrity than that observed with only phenyl hydrazine treated group. The aqueous extract of *Trichosanthes dioica* significantly altered values of most of the parameters associated with hemolytic anemia.

**Keywords:** *Trichosanthes dioica*, Anaemia, osmotic resistances, hemolytic anemia, Phenyl hydrazine.

## 1. INTRODUCTION:

Anemia is the most common blood disorder in developing countries especially in India that affects people of all ages, although the people at greater risk are the elderly, young women of child-bearing age and the infants. According a study, more than half of the world population experience some or the other type of anemia in their life times<sup>1</sup>. This condition is not a disease but could develop as a result of various diseases. Presently, more than half of the world's

population will experience some forms of anaemia in their life time<sup>2</sup>. Anemia is a medical condition in which the red blood cell count or hemoglobin is less than normal leading to reduced oxygen carrying capacity. The normal level of hemoglobin is generally different in males and females. For men, anemia is typically defined as hemoglobin level of less than 13.5 gram/dL and in women as hemoglobin of less than 12.0 gram/dL<sup>3</sup>. Infant has higher concentration 15g/dL at the time of birth and 9.5 g/dl at the time of 3 month. Normal life span of a red blood cell is typically around

120 days. Any process that can disrupt the normal life span of a red blood cell may cause anemia<sup>4-8</sup>. Anemia is caused essentially through two basic pathways: by a decrease in production of red blood cell or hemoglobin, or by a loss or destruction of blood.

Hemolytic Anemia caused by the premature destruction of red blood cells is known as hemolytic anemia. In this type of anemia, antibodies produced by the immune system damage red blood cells. This condition is sometimes associated with disorders such as systemic lupus, or lymphoma. Toxic materials such as lead, copper, and benzene can also cause the destruction of red blood cells. Hemolytic anemia can be acquired or inherited. Sick cell disease and thalassemia are both inherited types of hemolytic anemia.

Medicinal plants play an important role in health care as well as in personal care of mankind alongside the therapeutically active substances. They have occupied an important position in the socio-cultural, spiritual and medicinal area of rural and tribal lives of India<sup>9</sup>. A good number of medicinal plants are traditionally employed to alleviate anaemia. Some of these plants include *Brillantasia nitens*, *telferia occidentalis*, *Jatropha curcas*, *combretum dolichopetalum*, *Psorospermum ferbrifugum*, and *Flacourtia flavescens*<sup>1,10-15</sup>. The leaves of *B. nitens* are commonly used as haematinic and are claimed to be very effective in the treatment of malaria-induced and other types of anaemias.

Anaemia constitutes a serious health problem in many tropical countries because of the prevalence of malaria and other parasitic infection<sup>16</sup>. The prevalence of anaemia is higher in the third world than in developed countries due to the presence of many aggravating factors such as poor nutrition, high prevalence of blood parasites examples, plasmodium, trypanosomas and helminthes infection. From ancient time, herbal drugs are useful in the treatment of various disorders and supports traditional and medicinal value in the society. The selected plant *Trichosanthes dioica* is commonly found in India. The plant *Trichosanthes doica* Roxb is belonging to family Cucurbitaceae. Colloquially, in India it is often called green potato. *Trichosanthes dioica* fruit, which is a common vegetable in South Asia, has been used traditionally in treatment of fever, skin infection, wounds and hypoglycemia<sup>17-21</sup>.

## **2. EXPERIMENTAL:**

### **2.1. Plant:**

The fresh fruit of *Trichosanthes dioica* Roxb were collected in the month of June from the local market of Bhopal. The *Trichosanthes dioica* fruit was authenticated by Botanist Dr. Zeaul Hasan, Head of

Department of Botany Saifia College of Science, Peer Gate, Bhopal (M.P.).

### **2.2. Animals:**

Adult male albino rats (150–200 g each) were housed in animal house of pharmacology division, VNS Institute of Pharmacy, Bhopal (M.P.), India. They were housed in isolated cages under standard conditions (dark/light, 12/12) at 30°C and 50–55% humidity. They were provided food (Purina chow) and water *ad libitum*. All experimental procedures were conducted in accordance to the ethical guidelines of International Association for the study of anaemia. This procedure were reviewed and approved by the institutional animal ethics committee (Registration no. 778/03/c/CPCSEA).

### **2.3. Extraction:**

Fresh raw deseeded fruits of TD (1Kg) were peeled, washed, cut into small pieces, and homogenized in a warring blender, with 2 litres of distilled water. The extraction was carried at a temperature of 200 ± 10C, with constant stirring overnight. The homogenate was then squeezed through a cheese cloth, and was centrifuged at 2000 rpm for 10min at 0-40C. The supernatant being the TD fruit extract, it was decanted and used for experiments. The each liquid extracts were collected in a tarred conical flask. The solvent removed by heating on water bath and last traces of solvent being removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w basis was calculated, and stored in tight closed container.

### **2.4. Phytochemical screening of extracts:**

The aqueous extract was subjected to phytochemical investigation using the methods described by Harbone (1988) to assess the presence of alkaloids, glycosides, proteins, amino acids, flavonoids, sterols, carbohydrates, phenolic compounds, tannins, acidic compounds and resins<sup>2,3,10,11,15,19-23,24-36</sup>.

### **2.5. Acute toxicity study:**

Toxicological studies were performed on albino rats of uniform weight (150-200 g) with no prior history of drug treatment. Rats were kept with standard diet for 7 days before start experiment. Test animals were divided into six groups of three males and three females in each group. Group I was treated with aqueous extract of *Trichosanthes dioica* (100 mg/kg body weight orally), group II was treated with aqueous extract of TD (200 mg/kg body weight orally), group III was treated with aqueous extract of TD (500 mg/kg

body weight orally), group IV was treated with aqueous extract of TD (800 mg/kg body weight orally), group V was treated with aqueous extract of TD (1000 mg/kg body weight orally), group VI was treated with aqueous extract of TD (1250 mg/kg body weight orally) for seven days.

## 2.6. Preparation of dose of plant extracts form:

Extract dose of crude drug were freshly prepared in the distilled water. Calculated amount of each extracts were dissolved in the distilled water by shaking and stored in airtight bottles in a cool place.

## 2.7 Induction of anemia and Treatment of phenyl hydrazine induced anemia with plant extract

Rats were randomly divided into five groups with six animals in each group. Hemolytic anemia was induced in groups II, IV, V, by Phenyl hydrazine at the dose of 10 mg/kg body weight, i.p. for four days. Group II was treated with Phenyl hydrazine alone and groups IV, V, were administered with Phenyl hydrazine along with aqueous extract (orally) at different doses. Group III was treated orally with aqueous extract of TD at the dose of 200 mg/kg body weight. On the fourth days, blood samples were collected from the retro- orbital plexus vein of the rat's eyes in vials containing EDTA as the anticoagulant. These samples were evaluated for hematological parameters using hematological cell counter, with each measurement being repeated five times to the check the reproducibility of the results.

After 4th day administration of Phz was discontinued in all the groups. Excluding group I and group II, rest of the groups were treated with aqueous extract once a day, at the dose of 100 or 200 mg/kg body weight continuously up to next 12 days, as mentioned previously. Animals in group II were administered only vehicle. Blood sample were collected on the 16th day and evaluated for hematological parameters. On 4th and 16th days, slides of blood cells were prepared and stained by cresol blue and the concentration of reticulocyte is determined based on the whole red blood cells<sup>11,37-38</sup>.

## 2.8 Osmotic resistance of the red blood cells:

At day 8 the osmotic resistance of red blood cells was evaluated according to the method described early by Redondo *et al*<sup>39</sup>. The method consists to induce the lysis of red blood cell in hypotonic solution. For this study, the procedure used is as follow: A range of saline solutions from 0% to 0.9% were prepared and blood samples from normal control group (group I), Phz control group (group II), Phz (10mg/kg) + aqueous (100 mg/kg body weight) group (group IV), Phz (10mg/kg) + aqueous (200 mg/kg body weight) group

(group V), were collected from the retro- orbital plexus vein of the rats eyes in vials containing EDTA as the anticoagulant. 50 µl of blood of each group were mixed with different saline solutions respectively. Then each mixture was slightly shaken and incubated for 60 min at room temperature. After the incubation, the mixtures were centrifuged at 1500 rpm for 10 min. The supernatant were collected and the absorbance was determined at 540 nm with spectrophotometer. The percentage of hemolysis was determined by multiplying the ratio of absorbance of supernatant from the test animal to the test to the absorbance observed in samples undergone full hemolysis (induced by distilled water or 0 % saline solution) by 100. haemolysis curve of each group was generated as a function of sodium chloride concentration<sup>13</sup>.

## 2.9 Morphological study of blood cells:

The blood smear was prepared by placing a small drop of blood near an end of a slide and bringing the edge of another slide in contact with the drop and allowing the drop to bank evenly behind the spreader. The smear was fixed for at least 30 second in absolute methanol and methanol was removed by tilting the slide. Staining solution (Wright stain) was applied and slide was horizontally positioned for 2 min. Aliquot of the buffer solution (Sorensen's buffer solution) was gently mixed without any of the stain running off the slide and without disturbing the surface of the blood film on the slide. Slide was left for 3 minutes and rinsed with the distilled water for 30 seconds. Slide was dried in a tilted position, covered with a glass cover slip and examined under light microscope<sup>40</sup>.

## 2.10 Statistical analysis:

Data obtained from animal experiments were expressed as mean  $\pm$  S.E.M. (standard error mean). Statistical difference between the treated and the control group were evaluated by ANOVA, followed by the Dennett's test.  $P < 0.05$  was considered statistically significant.

## 3. RESULTS:

Aqueous extracts of *Trichosanthes dioica* fruit did not show any sign and symptom of toxicity and morbidity up to 1250 mg/kg body weight when administered orally after the seven day of treatment.

Intraperitoneal administration of phenyl hydrazine, in the control group exhibited significant ( $P < 0.05$ ) reduction in hemoglobin concentration, red blood cell count and packed cell volume and increase the reticulocyte count after four day treatment with phenyl hydrazine, comparison to the normal, untreated control group. This reduction is reversed naturally and

progressively to 8.54 g/dl, 4.16  $10^6/\mu\text{l}$ , 39.16 %, and reticulocyte was 1.316 % respectively at 16 day after phenyl hydrazine administration.

When the rats were treated with the aqueous extract of *T. dioica* fruits at 100 mg/kg body weight, with phenyl hydrazine (10mg/kg) the rate of haemoglobin count, red blood cell count, packed cell volume and the reticulocyte count after four day treatment is 8.28 g/dl, 4.17  $10^6/\mu\text{l}$ , 38.67 %, and 1.36 %. In presence of the same dose of extract at day 16, the concentrations of haemoglobin count, red blood cell count, packed cell volume and the reticulocyte count are 13.20 g/dl, 7.33  $10^6/\mu\text{l}$ , 46.83 %, and 0.75 % respectively. In the presence of the extract at 200 mg/kg body weight, with phenyl hydrazine (10mg/kg) the rate of haemoglobin count, red blood cell count, packed cell volume and the reticulocyte count after four day treatment is 9.36 g/dl, 4.5  $10^6/\mu\text{l}$ , 39.83 %, 1.42 % and 14.15 g/dl, 7.66  $10^6/\mu\text{l}$ , 48.4 %, 0.75 % at day 16. The values of haemoglobin concentration, red blood cells count, packed cell volume and reticulocyte count at both the

doses (100 mg/kg body weight and 200 mg/kg body weight) of aqueous extracts (Table 3.2) were significantly ( $P < 0.01$  in all) improved toward normal.

Morphology of red blood cells on the 4<sup>th</sup> day in phenyl hydrazine treated group alone was significantly reduced as compared to the normal ( $P < 0.01$ ) as a result of treatment with phenyl hydrazine. Most of the red blood cells in phenyl hydrazine treated group was irregularly shaped (like sickle) and ruptured (Fig 3.10).

The effect of aqueous extracts at dose of 200 mg/kg body weight on the morphology of red blood cells was studied in order to evaluate the potential of these extracts in promoting the cell recovery. Following the treatment with aqueous extracts for 12 days after discontinuation of phenyl hydrazine administration, red blood cells count increased significantly and the proportion of cells showing regular normal shape and integrity also increased (Fig 3.11).

**Table:-3.1: Effect of Aqueous extract of *Trichosanthes dioica* in phenyl hydrazine treated rats on 4<sup>th</sup> day:**

Group/ Parameters	Control ( I )	Phz Treated (10mg/kg) ( II )	Aqueous extract (200mg/kg) ( III )	Phz (10mg/kg) + Aqueous extract (100mg/kg) ( IV )	Phz (10mg/kg) + Aqueous extract (200mg/kg) ( V )
Red blood cells ( $10^6/\mu\text{l}$ )	7.83 $\pm$ 1.077	3.33 $\pm$ 0.210	7.33 $\pm$ 0.391	4.17 $\pm$ 0.267	4.5 $\pm$ 0.244
Packed cell volume (%)	49.5 $\pm$ 3.224	33.67 $\pm$ 2.333	49.17 $\pm$ 2.607	38.67 $\pm$ 2.333	39.83 $\pm$ 2.201
Haemoglobin conc. (gm/dl)	14.24 $\pm$ 2.171	6.52 $\pm$ 1.181	13.73 $\pm$ 0.714	8.28 $\pm$ 0.490	9.36 $\pm$ 0.879
Reticulocyte count (%)	0.73 $\pm$ 0.093	1.16 $\pm$ 0.143	0.733 $\pm$ 0.043	1.36 $\pm$ 0.072	1.42 $\pm$ 0.074

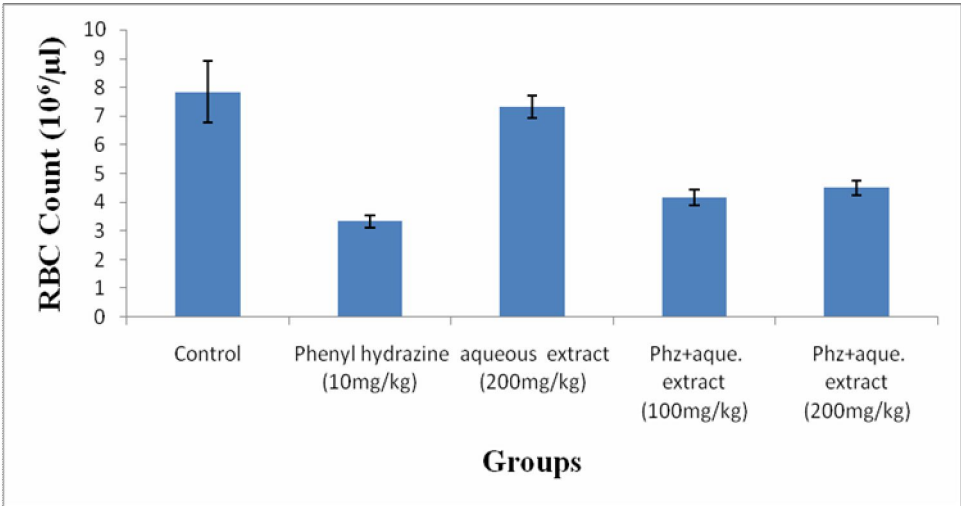


Fig:-3.1: Effect of aqueous extract of *Trichosanthes dioica* on RBC in Phenyl hydrazine treated rats on 4<sup>th</sup> day.

Table:-3.2: Recovery period observation of aqueous extract after withdrawal of phenyl hydrazine from all groups (on 16<sup>th</sup> day):

Group/ Parameters	Control ( I )	Phz Treated (10mg/kg) ( II )	Aqueous extract (200mg/kg) ( III )	Phz (10mg/kg) + Aqueous extract (100mg/kg) ( IV )	Phz (10mg/kg) + Aqueous extract (200mg/kg) ( V )
Red blood cells (10 <sup>6</sup> /μl)	7.83±1.307	4.16±0.307	7.83±0.397	7.33±0.411 <sup>a</sup>	7.66±0.039 <sup>a</sup>
Packed cell volume (%)	49.5±2.224	39.16±2.477	49.17±2.507	46.83±2.907 <sup>a</sup>	48.4±2.562 <sup>a</sup>
Haemoglobin conc. (gm/dl)	14.24±1.171	8.54±0.455	14.23±0.730	13.20±0.779 <sup>a</sup>	14.15±0.879 <sup>a</sup>
Reticulocyte count (%)	0.73±0.133	1.316±0.081	0.733±0.039	0.766±0.041 <sup>a</sup>	0.75±0.042 <sup>a</sup>

All values represent Mean ± SEM; (n= 6), ns= not significant; a=P<0.01, b=P<0.05, P values are according to one way ANOVA followed by Dunnett test.

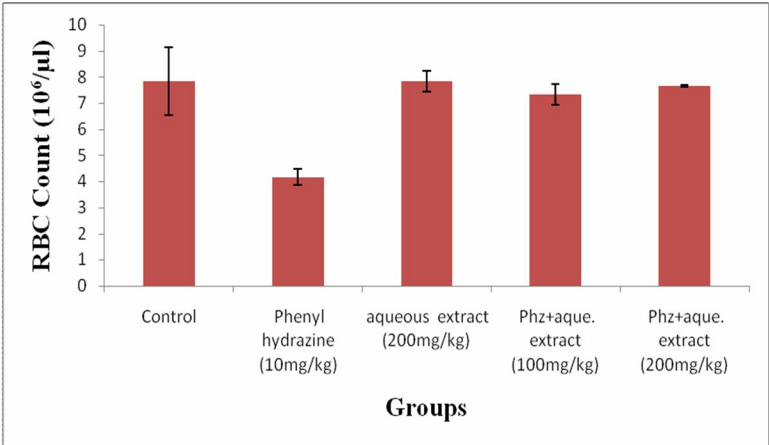
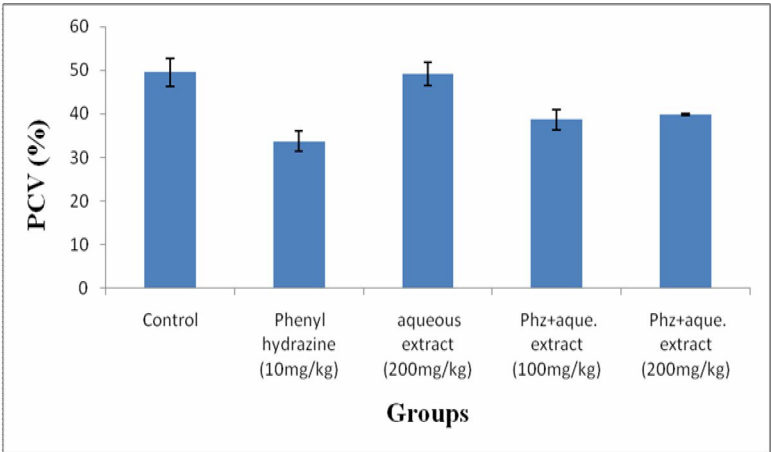
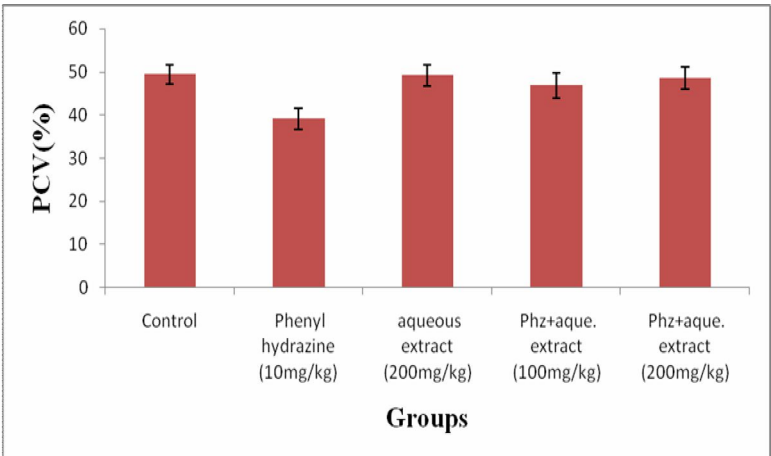


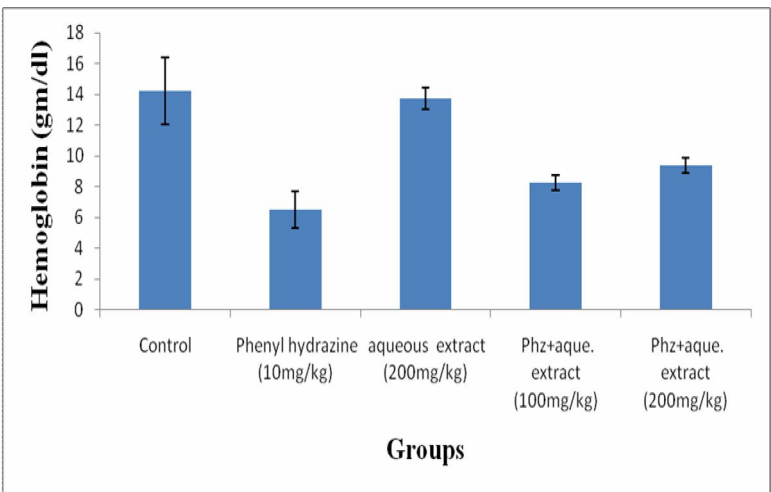
Fig:-3.2: Recovery period observation of aqueous extract on RBC after withdrawal of phenyl hydrazine from all groups on 16<sup>th</sup> day.



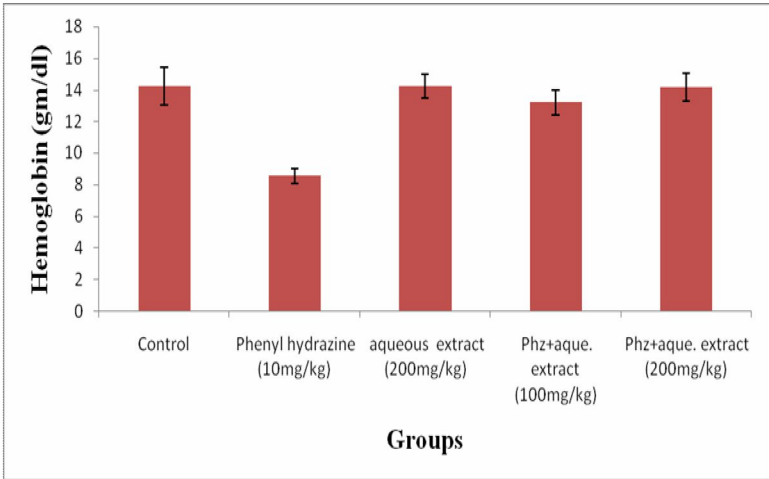
**Fig:-3.3: Effect of aqueous extract of *Trichosanthes dioica* on PCV in Phenyl hydrazine treated rats on 4<sup>th</sup> day.**



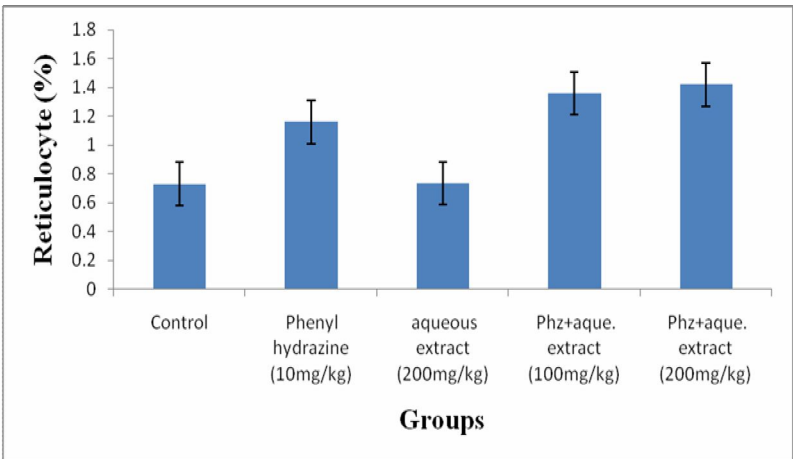
**Fig:-3.4: Recovery period observation of aqueous extract on PCV after withdrawal of phenyl hydrazine from all groups on 16<sup>th</sup> day.**



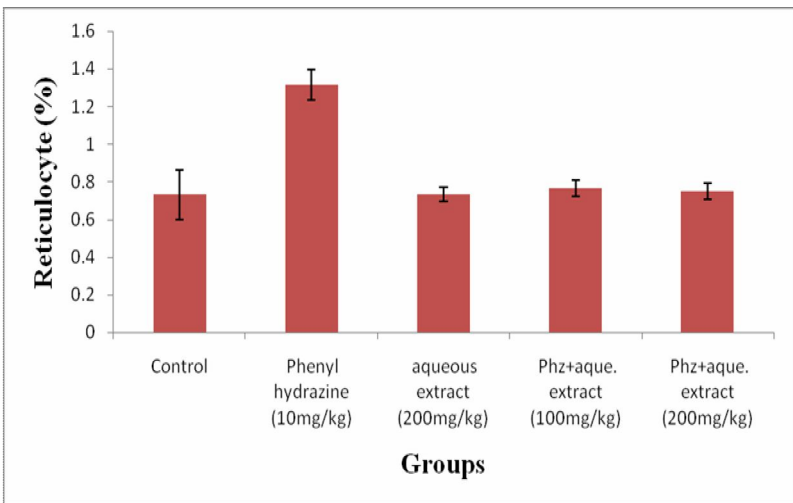
**Fig:-3.5: Effect of aqueous extract of *Trichosanthes dioica* on Hemoglobin in phenyl hydrazine treated rats on 4<sup>th</sup> day.**



**Fig:-3.6: Recovery period observation of aqueous extract on Hemoglobin after withdrawal of phenyl hydrazine from all groups on 16<sup>th</sup> day.**

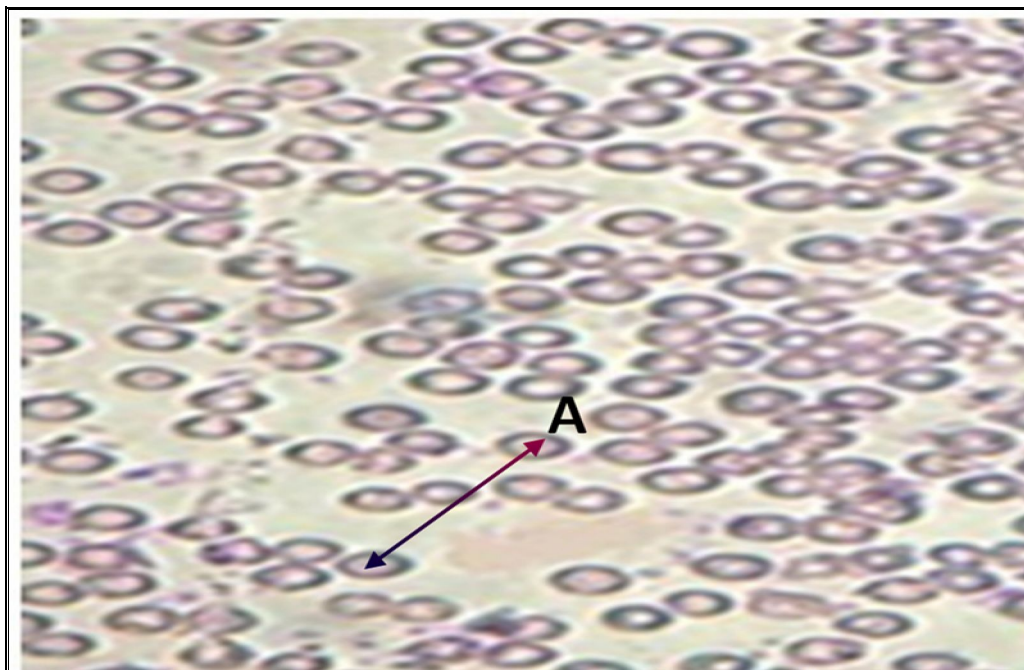


**Fig:-3.7: Effect of aqueous extract of *Trichosanthes dioica* on Reticulocytes in phenyl hydrazine treated rats on 4<sup>th</sup> day.**

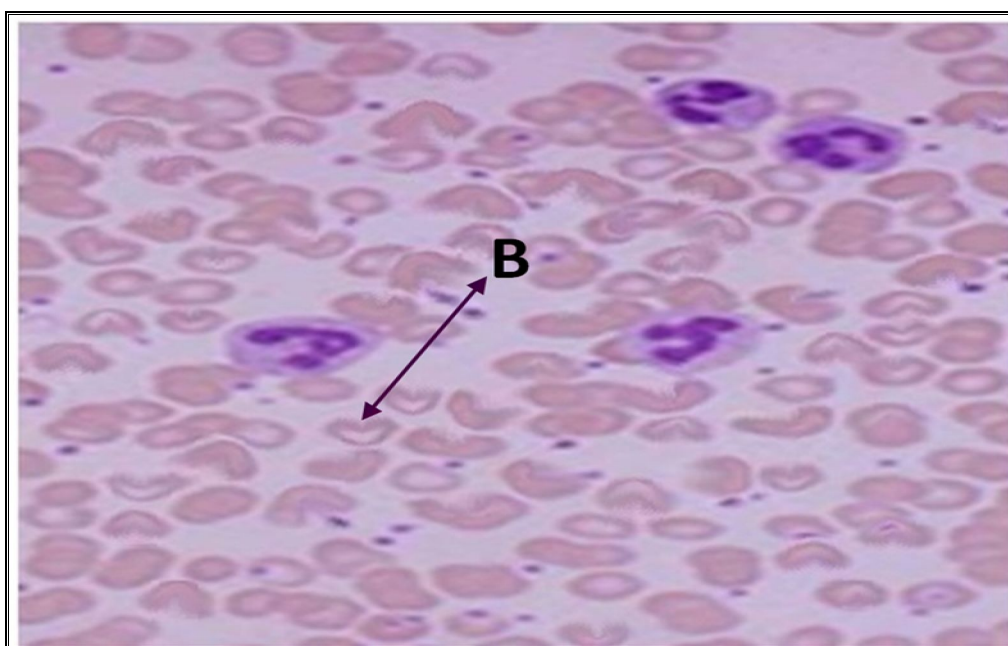


**Fig:-3.8: Recovery period observation of aqueous extract on Reticulocytes after withdrawal of phenyl hydrazine from all groups on 16<sup>th</sup> day.**



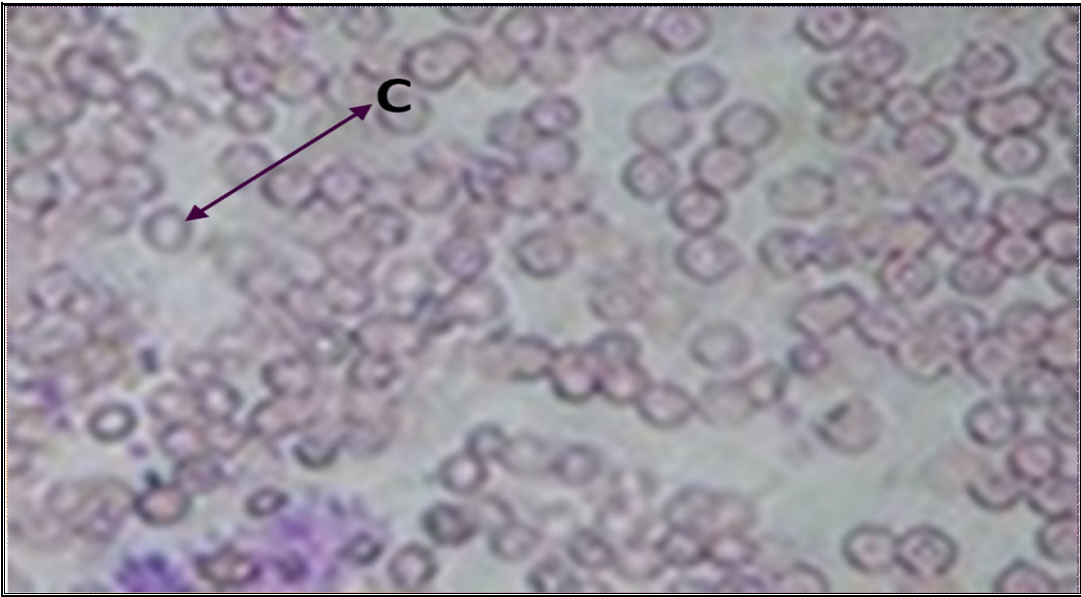


**Fig.3.9:** Photomicrograph on 4<sup>th</sup> day after vehicle administered shows red blood cells with normal shape and count (A=red blood cells with normal shape).



**Fig.3.10:** Photomicrograph on 4<sup>th</sup> day after Phenyl hydrazine treatment at the dose of 10mg/kg body weight shows less number and ruptured red blood cells. Leishman X 40 (B= ruptured red blood cells or sickle cell shaped).



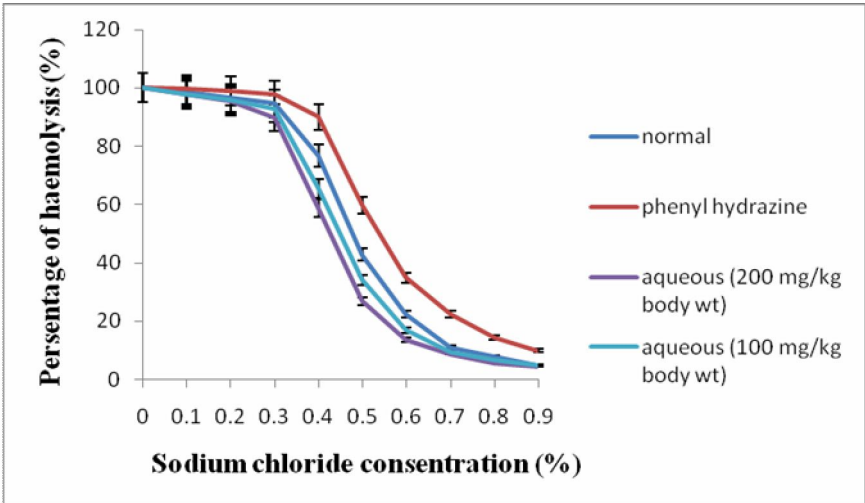


**Fig.3.11:** Photomicrograph on 16<sup>th</sup> day after aqueous extract treatment at the dose of 200 mg/kg body weight shows red blood cells with normal shape and count (C= red blood cells with normal shape).

**Table:-3.3: Effect of extracts of *Trichosanthes dioica* on osmotic resistance of red blood cells:**

Concentration	Positive control group	Phenyl hydrazine group (10mg/kg)	Aqueous group (200mg/kg)	Aqueous group (100mg/kg)
0%	100±5.44	100±6.48	100±7.6	100±5.34
0.1%	98.627±6.76	99.480±6.32	97.647±5.82	97.840±6.27
0.2%	96.515±6.34	98.857±5.76	95.508±6.19	95.897±4.31
0.3%	94.509±5.29	97.713±6.25	89.839±4.43	92.764±4.28
0.4%	76.874±4.30	89.917±5.19	58.823±3.37	65.443±4.19
0.5%	42.872±2.22	59.771±3.19	26.952±1.431	34.125±2.73
0.6%	22.492±1.44	34.927±1.96	13.583±0.734	16.847±0.93
0.7%	10.982±0.610	22.453±1.25	8.877±0.437	9.287±0.531
0.8%	7.919±0.433	14.345±0.727	5.775±0.323	6.587±0.433
0.9%	4.857±0.272	9.875±0.519	4.492±0.277	4.644±0.319

All values represent Mean ± SEM; (n= 6), result expressed in percentage of hemolysis of red blood cells in different concentration of saline solution (range 0.0 to 0.9%).



**Fig:-3.12:** Effect of *Trichosanthes dioica* extracts on the osmotic resistance of red blood cells. The result are mean ±S.E.M. with N=6: \*P<0.05, \*\*P<0.01.

At the 8 day aqueous extract of *Trichosanthes dioica* fruit enhances the osmotic resistance of red blood cells of albino rats in treated groups. Hemolysis curves obtained from groups of animals having hemolytic anemia induced by phenyl hydrazine treated with aqueous (200mg/kg and 100 mg/kg body weight) extracts of *Trichosanthes dioica* were shifted to the left of the hemolysis curve of the normal rats (positive control) (Fig 3.12). In other words, the percentage of hemolysis of red blood from groups of animals having hemolytic anemia induced by phenyl hydrazine treated with aqueous extract of *Trichosanthes dioica* fruit was less than the percentage of hemolysis observed in phenyl hydrazine treated animals (Table 3.3).

#### 4. DISCUSSION:

This study aimed to evaluate the effect of fruits of *Trichosanthes dioica* aqueous extract on the haemolytic anaemia induced by phenyl hydrazine in albino rats. It has been demonstrated previously that intraperitoneal administration of phenyl hydrazine decreases haemoglobin concentration, red blood cells number and packed cell volume in rat<sup>38,40</sup>. This anaemia which resulted from the early lysis of the red blood cells was naturally reversed 4 days later by the regeneration of these blood cells due to the increase of the reticulocytes after discontinues of the phenyl hydrazine. Our results indicate that the total extract of *Trichosanthes dioica* fruit increases significantly the concentration of haemoglobin, osmotic resistance of

red blood cells and the number of reticulocytes, after discontinues of phenyl hydrazine administration. Moreover, the extract of *Trichosanthes dioica* potentiates the increase of the number of reticulocytes; Increase of the number of young red blood cells (reticulocytes) explains the strong osmotic resistance of the red blood cells in rats treated with the extract.

At day 8 the osmotic resistance of red blood cells was evaluated. Percentage hemolysis of red blood cells from the animals treated with aqueous extract animal groups was less than the percentage of hemolysis of positive control. These results suggest that the aqueous extract of *Trichosanthes dioica* fruits enhance the osmotic resistance of red blood cells. It has been reported that phenyl hydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species (Clemens et al., 1984; Hill and Thornalley, 1982). presence of antioxidants in the plant extracts reverses the damaging effect of phenyl hydrazine. A study on *Trichosanthes dioica* Roxb (fruits) (Pawar R.S et al., February 10, 2010) showed that *Trichosanthes dioica*, has antioxidant property.

Finally, it is concluded from the data obtained that treatment with aqueous extract of *Trichosanthes dioica* increase significantly the concentration of hemoglobin, packed cell volume, as well as reform and increase concentration of red blood cells after extract administration. Aqueous extracts of the fruit successfully countered the drug induced hemolytic anemia after daily administration for 16 days.

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