

Evaluation of haematinic activity of *Kumara Veeriya Kaantha Chendhuram* in phenyl hydrazine induced Anemia in experimental rats

Sasi Priya. T ^{1,*} and Visweswaran. S ²

¹ Lecturer, Department of Gunapadam Marunthakkaviyal, Sri Sai Ram Siddha Medical College and Research Center, Chennai-44, India.

² Associate Professor, Department of Gunapadam, National Institute of Siddha, Chennai – 600 047, India.

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Abstract

The science of medicine is one of the fundamental requirements to well being of humans and their survival. Siddha system is one of the traditional systems of medicine. According to WHO, anaemia is one of the leading causes of death in worldwide. Anaemia is the most common blood disorder, affecting about one third of the global population. In classical Siddha literature, *Kumara Veeriya Kaantha Chendhuram* is one of the formulation mentioned in *Anupoga Vaithiya Navaneetham* – part I helps in overcoming iron deficiency anaemia. The ingredients of the formulation include *Kaantham* (magnetic oxide of iron) and *Thara* (*Mollugo oppositifolia*). The present study aims to evaluate haematinic activity by Phenyl hydrazine induced anaemia in animal models. The evaluation was carried out in Wistar albino rats. The effect of standard drug was comparable to those of the test drug. The blood parameters in animals treated with the test drug exhibited a highly statistical significant elevation when compared to control group.

Keywords: Siddha science; Anemia; Haematinic activity; Phenyl hydrazine method

1. Introduction

Generally Siddha system of medicine not only illustrates curative procedure of disease and also gives preventive aspects to diseases stating the proverb, 'Prevention is better than cure'. Siddhars, after achieving their goal, had contributed their means and acknowledgements through their writings. This collection of writings formed the basis of Siddha system of medicine - a glorious ancient medicine. It restores the normal functioning of organs and maintains the ratio of the *mukkutram-vadham*, *pitham* and *kapham*, there providing a healthy state of equilibrium to the body. The drugs used in Siddha medicine were classified on the basis of five properties.

- *Suvai* (Taste)
- *Gunam* (Character)
- *Veeryam* (Potency)
- *Pirivu* (Class)
- *Mahimai* (Action).

Two main principles under lie all descriptive animal toxicity testing. The first is that the effect of drug in laboratory animals, when properly quantified is applicable to humans. Anemia is a decrease in the total amount of red blood cells or hemoglobin in the blood, or a lowered ability of the blood to carry oxygen ⁽¹⁾. Iron-deficiency anemia affects nearly 1 billion people. In 2013, anemia due to iron deficiency resulted in about 183,000 deaths down from 213,000 deaths in 1990⁽²⁾. It is more common in women during pregnancy, in children and the elderly. Anemia increases costs of medical

*Corresponding author: Sasi Priya. T

care and lowers a person's productivity through a decreased ability to work. The word anemia is derived from Ancient Greek meaning "Lack of blood".

Causes of Anemia includes dietary deficiency, mal absorption, inherited disorders, autoimmune disorders, chronic diseases, hormone disorders, bone marrow disorders, blood loss, drugs and medications, mechanical destruction, infection, periods of rapid growth or high energy requirements. Depending on the severity, the symptoms of anemia may include pale skin, fatigue, weakness, tiring easily, breathlessness, drop in blood pressure when standing from a sitting or lying position (ortho static hypotension) – this may happen after acute blood loss, like a heavy period frequent headaches, racing heart or palpitations becoming irritated easily, concentration difficulties, cracked or reddened tongue, loss of appetite and strange food cravings^(3,4).

Iron deficiency or sideropaenia or hypoferremia, is the state in which a body lacks enough iron to supply its needs. Iron is present in all cells in the human body and has several vital functions, such as carrying oxygen to the tissues from the lungs as a key component of the hemoglobin protein, acting as a transport medium for electrons within the cells in the form of cytochromes, and facilitating oxygen enzyme reactions in various tissues. Too little iron can interfere with these vital functions and may lead to morbidity and death.

Total body iron averages approximately 3.8grams in men and 2.3grams in women. In blood plasma, iron is carried tightly bound to the protein transferrin. There are several mechanisms that control human iron metabolism and safeguard against iron deficiency. The main regulatory mechanism is situated in the gastro intestinal tract^(5,6). When loss of iron is not sufficiently compensated by intake of iron from the diet, a state of iron-deficiency anemia occurs. Before anemia occurs, the medical condition of iron deficiency without anemia is called latent iron deficiency (LID) or iron-deficient erythropoiesis (IDE).

According to Siddha literature, *Kumara Veeriya Kaantha Chendhuram*⁽⁷⁾ is mentioned for the treatment of anaemia and its related complications.

Nowadays the usage of herbal medicines is tremendously increased because of its therapeutic potency without or less side effects. In classical Siddha literature so many preparations are available which are more valuable and clinically very effective. Amongst the drug *Kumara Veeriya Kaantha Chendhuram* is one of the medicine mentioned in *Anupoga Vaithiya Navaneetham* - part I helps in rejuvenation and also increases the longevity. This is very well indicated in the name of the drug.

- Kumara – Young
- Veeriya - Power

This medicine is not evaluated so far in the aspect of Hematinic activity. The ingredients of *Kumara Veeriya Kaantha Chendhuram* are easily available and the preparation of the medicine is also cost effective to explicit the safety and pharmacological effect of trial medicine to the scientific world.

2. Material and methods

2.1. Ingredients: [7]

- Purified *Kaantham* (Magnetic oxide of Iron) - 8 *palam* (280gms)
- *Thara* leaf juice (*Mollugo oppositifolia* Linn.,) – as required



Figure 1 *Kantham* (Magnetic oxide of Iron)



Figure 2 *Thara* (*Mollugo oppositifolia*)

2.2. Method of Preparation

Purified Magnetic oxide of Iron was taken and placed in the mortar and grounded with adding sufficient quantity of *Thara* (*Mollugo oppositifolia*) leaf juice for 4 *samam* (12 hours) then made into a small sized pellets and dried in sunlight. Pellets are placed in the mud plate and closed it with similar mud plate. The margins of those plates were covered with 7 layers of mud coated cloth piece, dried and kept in the pit under earth. *Pudam* is made with 1000 cow dung cakes (*Kaja pudam*). The mud plate is removed after heat reduced and ground in mortar. Then processed *Kumara Veeriya Kantha Chendhuram* was collected and stored in an air tight container.



Figure 3 *Kumara Veeriya Kantha Chendhuram*

2.3. Determination of Hematinic Activity

The drug material was prepared by the following method. The suspension of *Kumara Veeriya Kantha Chendhuram* was distilled with 2% CMC to get 200mg/dL stock solution and used in this study.

2.4. Procurement and rearing of experimental animals

Both male and female albino rats (250 ± 25 g bodyweight) were used for this study. The animals were obtained from animal house, TANUVAS, Madhavaram, Chennai and the study was conducted in National Institute of Siddha, Chennai. The animals were housed in groups of three in polypropylene cages at ambient temperature ($25 \pm 2^\circ\text{C}$), relative humidity ($55 \pm 5\%$) and 12hrs/12hrs light-dark cycle. Animals had free access to commercial brand pellet diet and water given *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethical Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, certificate no. NIS/IAEC-VII/ 28.08.18/10. The studies were conducted according to the guidelines of CPCSEA. [8]

2.5. Animal treatment and experimental design: [9,10]

A total of 30 albino rats were used for this experiment. Six rats were kept as normal control group (Group 1), while 24 rats were made anemic by oral administration of Phenylhydrazine (10mg/kg of body weight) daily for 8 days. Rats that developed anemia with haemoglobin concentration <14gm/dl were included for the study. Anemic rats randomly divided into 5 groups (2 to 6) and treated as follows,

- Group I : Vehicle control - received only honey orally once in a day during the entire study period.
- Group II: Received distilled water daily (AnemiaControl).
- Group III: Test Group I-Received *Kumara Veeriya Kaantha Chendhuram* orally 35mg/kg/day with honey.
- Group IV: Test Group II-Received *Kumara Veeriya Kaantha Chendhuram* orally 70mg/kg/day with honey.
- Group V: Test Drug Control - Received oral single dose of Hematinic syrup 2ml/kg/day.

The treatment was continued for 15 days.

Blood collected from the retro orbital vein of experimental animals after an overnight fast and after one and two weeks of treatment with *Kumara Veeriya Kaantha Chendhuram* was also collected and used for the determination of red blood cell count (RBC), Haemoglobin (Hb) concentration, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated^[11].

2.6. Statistical analysis

Experimental data were analyzed using analysis of Variance (ANOVA) and Dunnett's 't' test to determine significant difference between mean values. The statistical analysis system (INSTAT-V3) package was used for this analysis.

3. Results

Table 1 Hematological findings of Hb concentration (g/dl), RBC count (million cells/cu.mm), PCV, MCV, MCH and MCHC after 8 days treatment with Phenylhydrazine

Blood parameter	Group-I (Vehicle Control)	Group-II (Anemia control)	Group-III (KVKC-35mg/kg with honey)	Group-IV (KVKC-70mg/kg with honey)	Group-V (Standard 2ml/kg)
Hb (g/dl)	17.31±0.45	11.8±0.25**	12.48±0.65**	12.75±0.35**	12.74±0.31**
PCV	51.10±1.45	37.76±2.32**	40.11±1.82*	42.10±2.21**	42.17±2.72**
RBC (×10 ⁶ /ml)	5.85±0.15	4.52±0.22**	4.24±0.24**	4.51±0.20**	4.73±0.32**
MCV (fl)	69.11±2.65	81.34±3.3	84.14±0.11*	85.45±3.15**	88.40±3.60**
MCH (pg)	21.74±1.42	25.28±1.30	28.18±1.64*	30.21±1.11**	31.23±2.42**
MCHC (g/dl)	35.62±0.34	32.10±0.4	34.01±0.24	33.61±1.45	30.08±2.20*

Values are mean ± S.E.M. (Dunnett's test). * P < 0.05; ** P < 0.01 Vs Control N=6

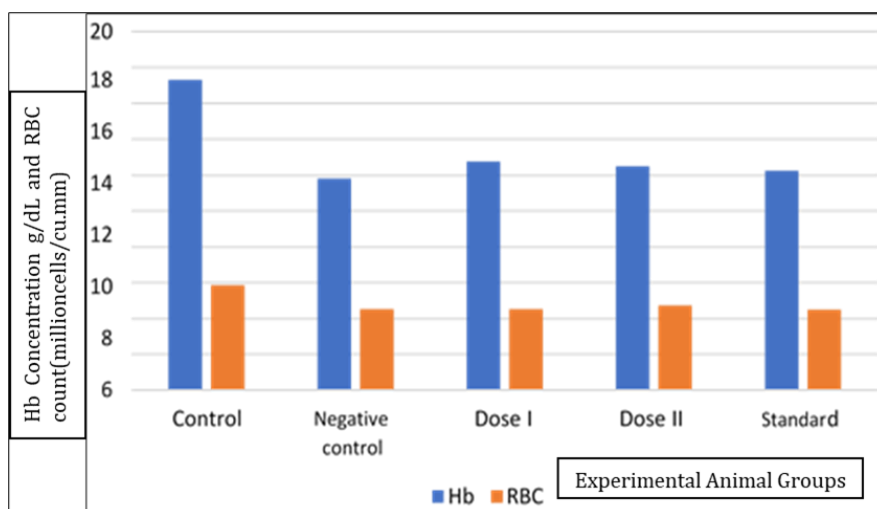


Figure 4 Effect of Hb concentration(g/dl)and RBC count(million cells/cu.mm)after8 days treatment with Phenyl hydrazine

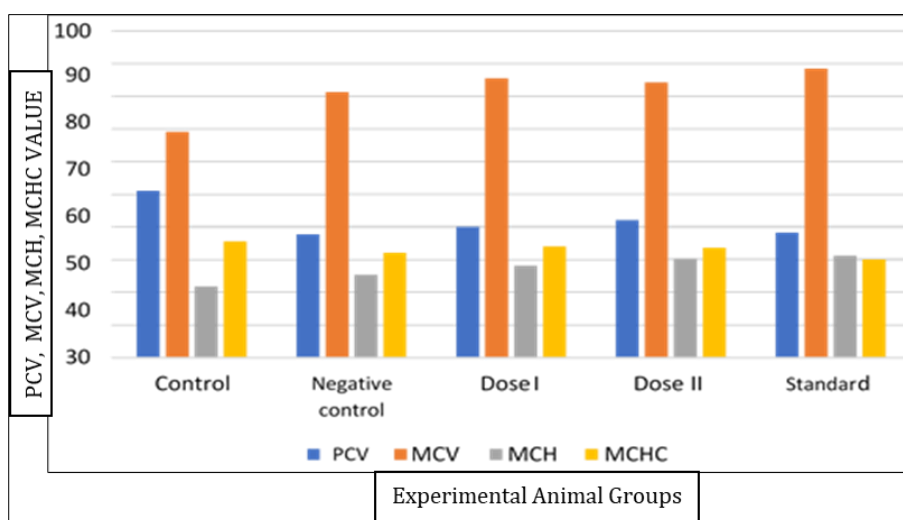


Figure 5 Effect of PCV, MCV, MCH and MCHC after 8 days treatment with Phenyl hydrazine

Table 2 Hematological findings of Hb concentration (g/dl), RBC count (million cells/cu.mm), PCV, MCV, MCH and MCHC after 7 days treatment with *Kumara Veeriy Kaantha Chendhuram*.

Blood parameter	Group-I(Control)	Group-II (Anemia control)	Group-III (KVKC-35mg/kg with honey)	Group-IV (KVKC-70mg/kg with honey)	Group-V (Standard 2ml/kg)
Hb (g/dl)	17.10±1.25	10.8±1.52**	11.64±0.47*	14.47±1.23	19.65±1.40**
PCV	51.10±1.33	40.42±2.5	42.24±2.21	44.34±2.1	54.27±2.32**
RBC(×10 ⁶ /ml)	6.16±0.25	4.23±0.51**	6.85±0.24	7.35±0.12**	8.45±1.01**
MCV(fl)	75.16±2.35	74.43±2.42	76.15±1.5	78.42±2.50	75.36±2.51
MCH(pg)	25.23±1.66	28.12±1.45	21.24±1.4	24.01±1.3	26.55±1.32
MCHC(g/dl)	34.28±2.87	32.14±1.14	28.64±1.7*	27.66±1.4*	32.15±1.3

Values are mean±S.E.M. (Dunnett's test). * P < 0.05; **P < 0.01 Vs Control N=6

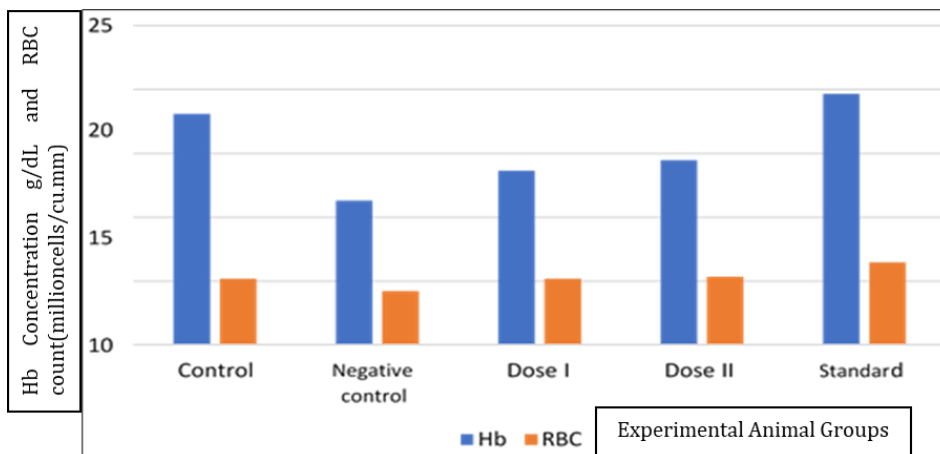


Figure 6 Effect of Hb concentration (g/dl) and RBC count (million cells/cu.mm) after 7 days treatment with *Kumara Veeriya Kaantha Chendhuram*

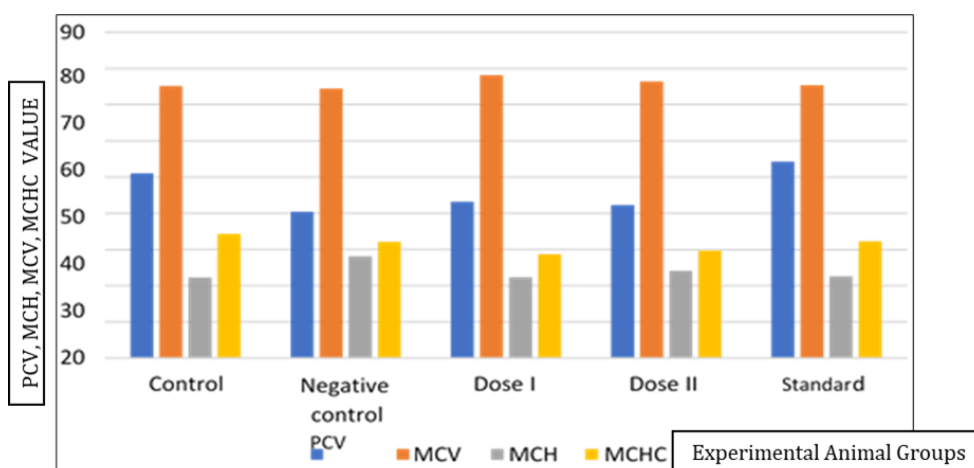


Figure 7 Effect of PCV, MCV, MCH and MCHC after 7 days treatment with *Kumara Veeriya Kaantha Chendhuram*

Table 3 Hematological findings of Hb concentration (g/dl), RBC count (million cells/cu.mm), PCV, MCV and MCHC after 15 days treatment with *Kumara Veeriya Kaantha Chendhuram*

Blood parameter	Group-I (Control)	Group-II (Anemia control)	Group-III (KVKC- 35mg/kg with honey)	Group-IV (KVKC- 70mg/kg with honey)	Group-V (Standard 2ml/kg)
Hb (g/dl)	16.1±1.57	9.78±1.15**	12.11±0.65*	14.24±0.56**	20.55±1.35**
PCV	43.35±1.30	41.01±1.86	40.14±3.03	42.55±2.1	55.17±1.82**
RBC (×10 ⁶ /ml)	5.25±0.25	3.26±0.52**	4.01±0.36	4.82±0.35	5.23±0.54**
MCV (fl)	72.51±2.07	87.65±2.84**	80.16±2.41**	5.12±1.60	71.21±2.45
MCH (pg)	29.33±2.84	32.28±2.25	30.11±1.47	29.44±1.73	27.64±2.26
MCHC (g/dl)	31.25±2.87	35.14±1.35	33.01±1.33	32.89±0.65	31.98±3.04

Values are mean ± S.E.M. (Dunnett's test). *P<0.05; **P <0.01 Vs Control N=6

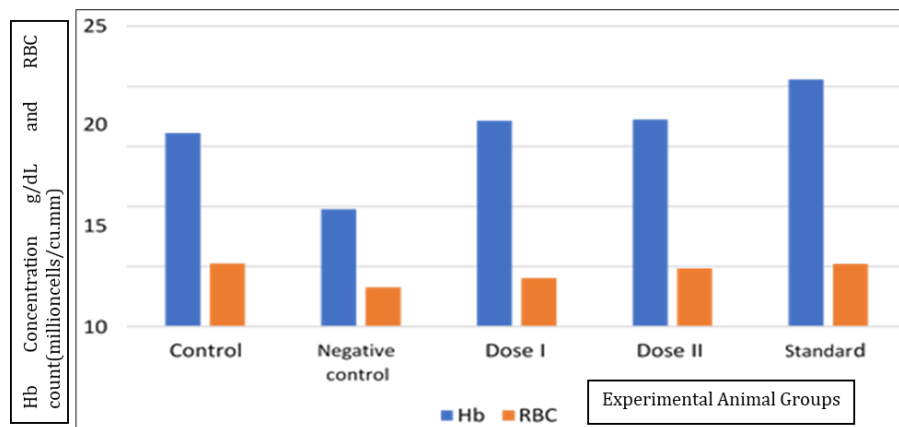


Figure 8 Effect of Hb concentration (g/dl) and RBC count (million cells/cu.mm) after 15 days treatment with *Kumara Veeriya Kaantha Chendhuram*

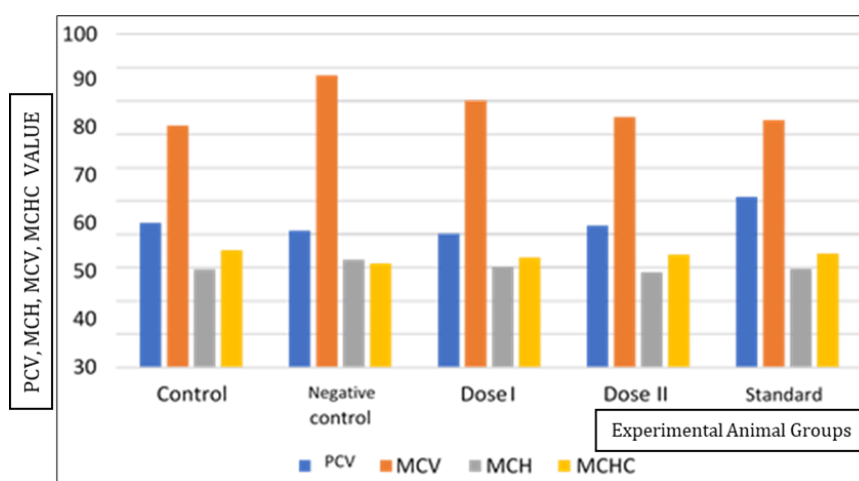


Figure 9 Effect of PCV, MCV, MCH and MCHC after 15 days treatment with *Kumara Veeriya Kaantha Chendhuram*

4. Discussion

This study aimed to evaluate the effect of *Kumara Veeriya Kaantha Chendhuram* on anemia induced by phenyl hydrazine in albino rats. In the untreated control rats (Phenylhydrazine induced) shown significant ($p < 0.05$) decrease in Hemoglobin, Total red blood cell count indicates the development of anemia. The administration of *Kumara Veeriya Kaantha Chendhuram* produced a significant ($p < 0.05$) increase in hematological parameters. The phenyl hydrazine induced anemia was very significantly ($p < 0.01$) reversed after 15 days treatment with *Kumara Veeriya Kaantha Chendhuram* at the dose level of 70mg/kg towards almost normal. In the anemia control the Hemoglobin level was 10.8 ± 1.52 gm/dl at day seven and this was improved to 11.64 ± 0.47 gm/dl and 14.47 ± 1.23 gm/dl at dose levels of 35mg/kg and 70mg/kg respectively.

Similarly after 15 days treatment with *Kumara Veeriya Kaantha Chendhuram* at 70mg/kg the hemoglobin level was increased from 9.78 ± 1.15 gm/dl to 12.11 ± 0.65 gm/dl and 14.24 ± 0.56 gm/dl at doses of 35mg/kg and 70mg/kg respectively. Same kind of beneficial and significant ($p < 0.05$) changes were recorded in the other hematological parameters at the higher dose of *Kumara Veeriya Kaantha Chendhuram*. The effect of standard drug (Hematinic syrup) was comparable to those of the test drug. The red blood cell count ($p < 0.01$) in animals treated with 70mg/kg of *Kumara Veeriya Kaantha Chendhuram* exhibited a highly statistical significant elevation when compared to control group.

5. Conclusion

The hematinic activity was evaluated using phenyl hydrazine induced method in Wistar albino rats model. A total of 30 albino rats were used for this experiment. Six rats were kept as normal control group (Group 1), while 24 rats were

made anemic by oral administration of Phenyl hydrazine(10mg/kg of body weight)daily for 8days. Anemic rats randomly were further divided into groups and treated with test drug and standard drug. The treatment period was scheduled for 7 days and15 days. Then blood sample was collected and used for the determination of red blood cell count (RBC), Haemoglobin (Hb) concentration, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC). The results indicate that *Kumara Veeriya Kaantha Chendhuram* at the dose level of both 35mg/kg and 70mg/kg exhibited very significant ($p<0.01$) hematinic activity in Wistar albino rats when compared with control group.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There are no conflicts of interest.

Statement of ethical approval

The research work was approved by the IAEC. The approval number was NIS/IAEC-VII/28.08.18/10.

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