



## **Preclinical Appraisal of Hematinic Potential of Mandura Bhasma for Treating Anemia**

**Deepak S. Khobragade<sup>1</sup>, Mrunali S. Potbhare<sup>2\*</sup>, Awdhut Pimpale<sup>1</sup>,  
Sagar B. Wankhede<sup>1</sup> and Chandrashekhar R. Tempe<sup>3</sup>**

<sup>1</sup>Datta Meghe College of Pharmacy, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi(M), Wardha, Maharashtra, India.

<sup>2</sup>GITAM Institute of Pharmacy, Vizag, Andhra Pradesh, India.

<sup>3</sup>Government College of Pharmacy, Amravati, Maharashtra, India.

### **Authors' contributions**

All authors have contributed in finalizing the research problem, experimental design or procedures, analyzing result and compiling manuscript. All authors have read and approved the manuscript.

### **Article Information**

DOI: 10.9734/JPRI/2021/v33i30B31656

Editor(s):

(1) Dr. Wenbin Zeng, Xiangya School of Pharmaceutical Sciences, Central South University, China.

Reviewers:

(1) B. Puvarajan, VCRI Orathanadu, India.

(2) Diego da Silva, Universitário Anchieta, Brasil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/68930>

**Original Research Article**

**Received 20 March 2021**

**Accepted 24 May 2021**

**Published 07 June 2021**

### **ABSTRACT**

**Aims:** To evaluate hematinic potential of mandura bhasma.

**Study Design:** Experimental study.

**Place and Duration of Study:** Institute of Pharmaceutical Education and Research, Wardha, Maharashtra, India. 6 Months.

**Methodology:** The anti-anaemic potential of Mandura bhasma in Wistar rats was investigated. Anaemia was induced in rats with phenyl hydrazine hydrochloride at a dose of 10 mg kg<sup>-1</sup> body weight by oral administration. Anaemia was treated with mandura bhasma administered in three different doses based on body weight.

**Results:** *In vivo* investigation showed that though the dose of 6mg kg<sup>-1</sup> body weight of mandura bhasm produced only a minimal antianaemic (hematinic) effect, oral daily dose of 11 mg kg<sup>-1</sup> body weight and 22mg kg<sup>-1</sup> body weight a produced a significant (P < 0.05) antianaemic effect when compared to standard drug ferrous sulphate indicating dose dependent activity.

**Conclusions:** The results indicate that Mandura Bhasm have very potential dose dependant hematinic activity and can be a safe and effective drug for treating anemia.

\*Corresponding author: E-mail: [mrunalipotbhare@gmail.com](mailto:mrunalipotbhare@gmail.com);

**Keywords:** Anaemia; haematinic activity; mandura bhasma; phenyl hydrazine; ferrous sulphate.

## 1. INTRODUCTION

Anemia is a one of the most common and serious blood disorder globally affecting health and social and economic development of one third of the population of developing and under developed countries (WHO 2005) due to inadequate and imbalanced food intake [1-2]. Anemia can affect people of all ages, but the elderly, young women and the infants but pregnant women are most susceptible due to high demand from the developing fetus [3].

There are large number of anemia (over 400 types), most of them rare but all having depleted or damaged circulating RBC. Some of the most common types of anemia with causes are due to defects in RBC production (Aplastic anaemia), RBC maturation (Megaloblastic anaemia), haemoglobin synthesis (Iron deficiency anaemia), genetic (Sickle cell anaemia and Thalassaemia), physical loss of red cells (Haemolytic anaemia) and vit B deficiency (Pernicious anaemia) [4-11].

Though anemia can be treated by many modern drugs, modern medicines and therapies are not affordable by poor population due to financial constrains or unavailability in rural areas. The patients in these areas use plants and herbal products for the treatment of diseases including anemia. Herbal drugs are very effective in treating anemia and are proved by many studies [12-14].

Anemia is likely to increase in future and cost-effective and better treatment strategies for it are need of the hour. Mandura bhasma is traditionally used in ayurveda for treating blood many disorders including anemia. The scientific evaluation of its effectiveness in treating anemia is desired. This study investigate the anti-anaemic potential of mandura bhasma in wistar rats administered in three different doses and compared with standard iron supplement drug. Various blood parameters such as hemoglobin, RBCs, WBCs PCV, MCH, MCV were evaluated as factors contributing improvement of anemia.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials and Methods

#### 2.1.1 Animals

The albino rats (Wistar strain) of either sex weighing 150-200 g, were selected for the study.

The animals were housed in polypropylene cages at a temperature of  $25 \pm 2^\circ\text{C}$  with relative humidity of 40-60 % and 12 hrs light dark cycle, with maximum of 6 animals per cage. Animals were fed with a balanced diet and water *ad libitum* during the complete experimental period.

#### 2.1.2 Chemicals

Chemicals like EDTA sodium salt, phenylhydrazine, standard drug ferrous sulphate of analytical grades required for animal study were procured from institutional store.

Mandura bhasma was prepared in Institute laboratory using standard procedure. In the present study, the three different doses of mandura bhasma were decided to be as 6mg, 11mg and 22mg/kg of body weight. The doses were calculated by extrapolating the human dose to animal dose based on the body surface area ratio.

#### 2.1.3 Study protocol

The albino rats (Wistar strain) of either sex weighing 150-200 g, were randomly divided into 6 groups, each containing 6 animals.

#### 2.1.4 Induction of anaemia

In the present study phenyl hydrazine was used to induce anemia in rats. Solution of phenyl hydrazine (50mg / ml) was prepared by dissolving phenyl hydrazine in absolute alcohol and diluting with distilled water [15,16] to induce anemia. Solution of phenylhydrazine 10mg/kg was administered by oral route for period 8 days [17].

Drug suspension (mandura bhasma in 3 doses 6mg/kg, 11mg/kg, 22mg/kg ) was prepared in 3% gum acacia solution in distilled water [15,18]. All administrations of drug were done by oral intubation. The treatment schedule was kept for 21 days .Every 7 days the blood parameters were checked.

The treatment schedule was as follow.

#### 2.1.5 Analysis of haematological parameters

Red blood cell count (RBC), haemoglobin (HB) concentration, packed cell volume (pcv) , mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration

(MCHC), and white blood cells (WBC) count were analysed for evaluating hematinic potential of mandure bhasm Blood was collected (1-2 ml) from the retro-orbital plexus, under slight chloroform anaesthesia, in stoppered vials with EDTA solution. The blood was sampled on day 0 before phenyl hydrazine administration, and on days 1, 7, 14 and 21, days after phenyl hydrazine administration and drug treatment [19,20]. All the aforementioned parameters were determined by using Auto cell counter 920<sup>E</sup>, swelab ltd.

### 2.1.6 Statistical analysis

Statistic Results are expressed as the mean ± SEM, n=6. The data were statistically analyzed using a one-way ANOVA followed by Dunnett test. Statistical significance was determined at a level of p < 0.05.

## 3. RESULTS AND DISCUSSION

Lauha bhasma and mandura bhasma are the two most commonly used ayurvedic preparations. Apart from haematinic and astringent, they are used in disorders of liver, spleen and kidney disease. They are also useful in treating intestinal worms, nervous disease, and neuralgia. Being a powerful haematinic and general tonic they are very valuable in the

treatment of haemolytic anaemia and jaundice [21,22].

The reported acute toxicity studies as per OECD guidelines and Globally Harmonized System (GHS) for the classification of acute toxic substances, the mandura bhasma was classified in the Category 5 with a LD<sub>50</sub> higher than 2000 mg/kg [23,24] Based on the earlier reports, a lower dose of 11 mg/kg and higher dose of 22 mg/kg was selected in the present study [25]. Haematinic activity of mandura bhasma was evaluated in vivo in phenyl hydrazine induced anaemic albino rats.

Changes in body weight, RBC, HGB, HCT, MCV, MCH, MCHC and WBC were evaluated to assess and compare the haematinic activity.

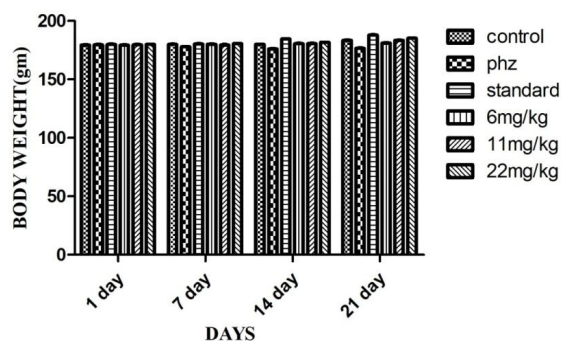
### 3.1 Evaluation of Changes in Body Weight

Anemic patients, generally suffer from the loss of the body weight which does not increase easily. Slight increment or at least maintenance of the body weight in can be a sign of better anemic control. The study shows the Mandura bhasm show significant improvement in body weight of anemic animals comparable to standard drug. The results are depicted in Fig. 1.

**Table 1. Animal groups and respective treatment schedules**

Group	Treatment	Dose
Group 1	Treated control animals	50 ml distilled water with 1ml absolute alcohol
Group2	Phenyl hydrazine(phz)	10mg/kg,per oral for 8 days
Group 3*	Ferrous sulphate(std)	50 mg/kg per oral for 21 days
Group 4*	Mandura bhasma test 1	6mg/kg per oral for 21 days
Group 5*	Mandura bhasma test2	11mg/kg per oral for 21 days
Group6*	Mandura bhasma test3	22mg/kg per oral for 21 days

\* Treatment after induction of anaemia by administering Phenyl hydrazine(phz) 10mg/kg,per oral for 8 days



**Fig. 1. Changes in body weight in experimental animals**

### 3.2 Evaluation of Changes in RBC Count

The changes in RBC count of experimental animals during course of study are given in Fig. 2. Increased RBC count is considered as one of the most important goals of the anemia treatment. When RBC count of experimental animals was evaluated, as expected, the standard showed significant improvement ( $p < 0.05$ ) in RBCs count. Mandura bhasma also produced significant increase ( $p < 0.01$ ) in RBCs count when compared with anemic control groups. The results indicate strong anti anemic potential of Mandura bhasma.

### 3.3 Evaluation of Changes in HB Count

Anemia causes depletion in hemoglobin count and this is reason which makes anemia a very challenging health problem. Depletion in hemoglobin count is primary reason for the complications of anemia. Mandura bhasma increases HB content significantly ( $p < 0.05$  respectively) when compared with anemic

control. The results are comparable with Standard. The results are graphically represented in Fig. 3.

### 3.4 Evaluation of Changes MCH

Standard drug and mandura bhasma produced a significant decrease ( $p < 0.01$ ) in MCH as compared to anemic control (Fig. 4). The results indicate that MB has good reducing effect on MCH indicating improved anemic condition.

### 3.5 Evaluation of Changes in MCV

Significant decrease in the mean cell volume after treatment indicates that the drug treatment is good enough for anemia. Mandura bhasma show significant decrease ( $p < 0.05$ ) in the mean cell volume when compared with anemic control. The decrease is significant compared to standard (Fig. 5). The results indicate that mandura bhasm is a as good as standard in treating anemia.

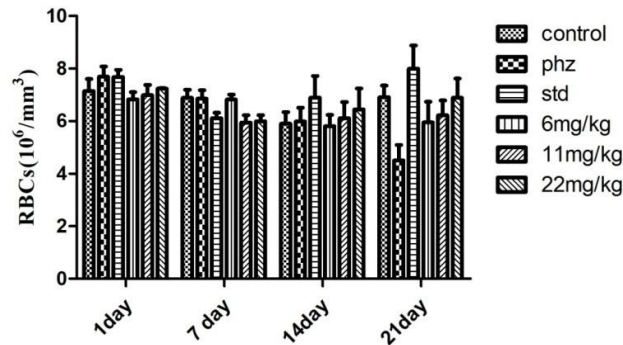


Fig. 2. Changes in RBC count in experimental animals

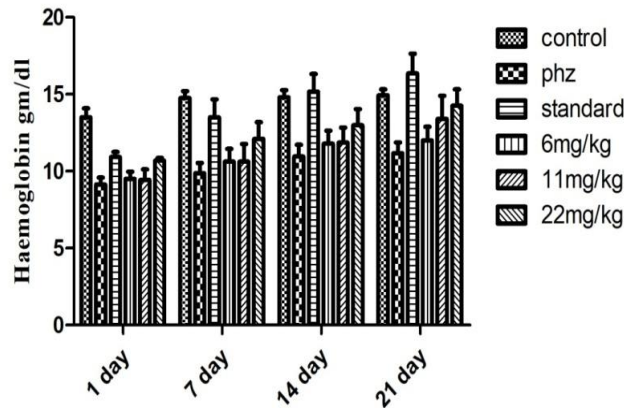


Fig. 3. Changes in HB count in experimental animals

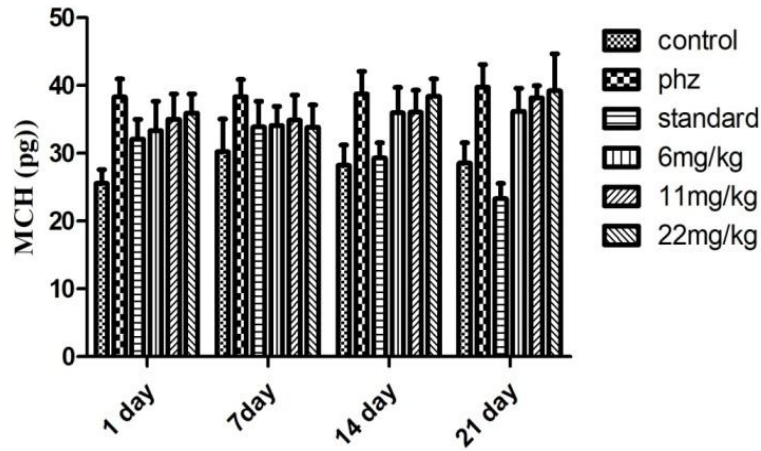


Fig. 4. Changes in MCH in experimental animals

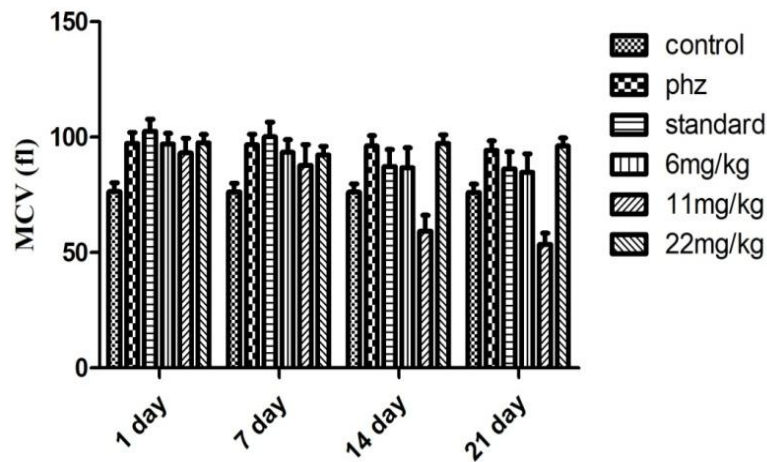


Fig. 5. Changes in MCV in experimental animals

### 3.6 Evaluation of Changes in MCHC

Decreased Mean Cell Hemoglobin Concentration is a indication of the recovery of anemic condition. From Fig. 6, It can be observed that MB shows a significant decrease ( $p < 0.05$ ) in MCHC in experimental anemic animals. The results are comparable with standard indicating as good activity as that of standard.

### 3.7 Evaluation of changes in PCV

Packed cell volume or Hematocrit is decreased in anemia. Any viable treatment for anemia must restore hematocrit in anaemic condition. From the Fig. 7 it is clearly observed that mandura

bhasma produced a significant increase ( $p < 0.01$ ) in PCV/HCT (%), when compared with anaemic control.

### 3.8 Evaluation of WBC

Increase in WBC count is caused by most of health challenges to body including anemic condition. Effective haematinic agent must be able to decrease increased WBC count in blood after anemia or at least it should prevent WBC count from increasing drastically. The mandura bhasma produced a significant decrease ( $p < 0.05$ ) in WBC count in anemic animals as compared to anaemic control. The effect on WBC was comparable to standard drug.

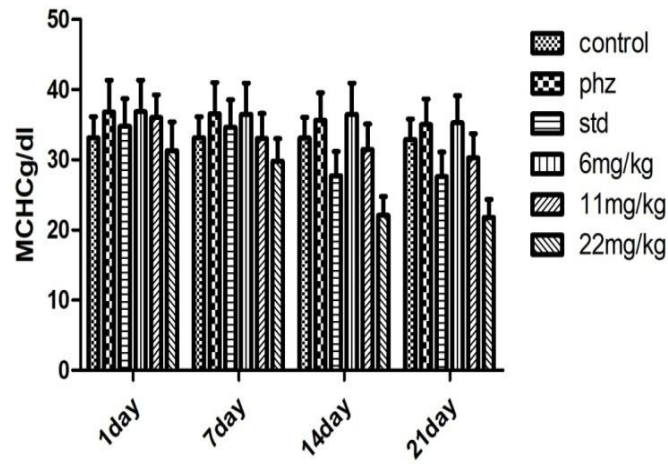


Fig. 6. Changes in MCHC in experimental animals

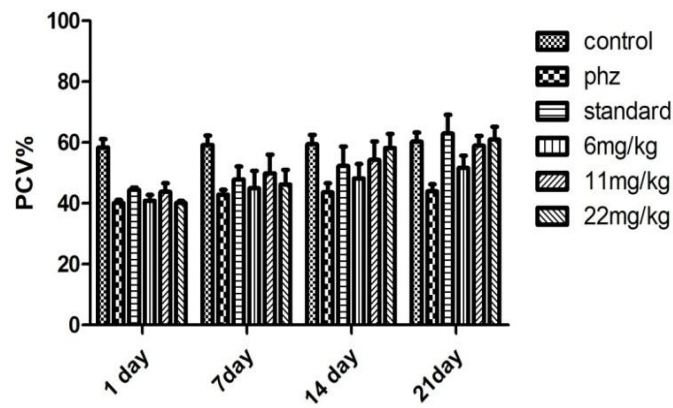


Fig. 7. Changes in PCV in experimental animals

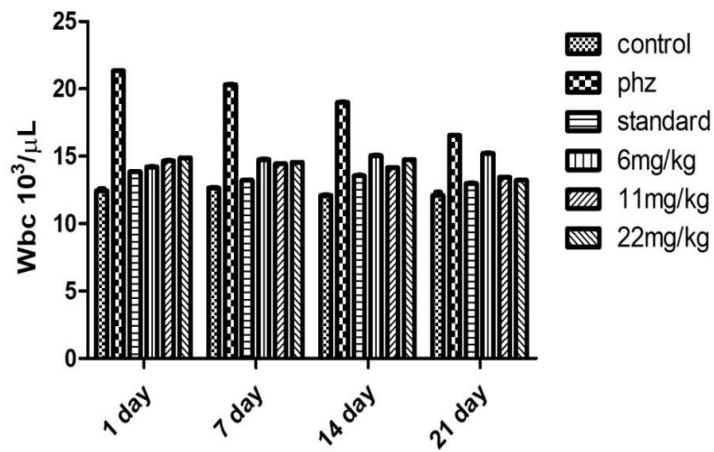


Fig. 8. Changes in WBC count in experimental animals

#### 4. CONCLUSIONS

The study indicates that not only the standard drug but Mandura Bhasma show significant haematinic activity with the significant increase in blood RBC, HB and the % HCT/PCV count. The results indicate and confirm the potential effect of mandura bhasma in treating anemia.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

All the authors hereby declare that All animal experiments were approved by the Institutional Animal Ethical Committee (Registration No. 535/02/a/CPCSEA/Jan2002) of Institute of Pharmaceutical Education and Research, Wardha.

#### ACKNOWLEDGEMENT

The authors would like to acknowledge that the HOI and all technical and supporting staff of animal house for supporting wholeheartedly during this study.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Ezzati M, Lopez AD, Dogers A, Vander HS, Murray C. Selected major risk factors and global and regional burden of disease. *Lancet*. 2002;360:1347-60.
2. Pasricha SR, Flecknoe-Brown SC, Allen KJ, et al. Diagnosis and management of iron deficiency anaemia: a clinical update. *The Medical Journal of Australia*. 2010;193(9):525-32.
3. Baliga S, Chaudhary M, Bhat S, Bhansali P, Agrawal A, Gundawar S. Estimation of Malondialdehyde Levels in Serum and Saliva of Children Affected with Sickle Cell Anemia. *Journal of Indian Society of Pedodontics and Preventive Dentistry*. 2018;36(1):43.
4. Bhatt A, Vinayak N, Acharya N. Role of Intravenous Iron Sucrose in Comparison to Oral Ferrous Sulfate for Prophylaxis of Anemia in Pregnant Women. *Journal of Datta Meghe Institute of Medical Sciences University*. 2020;15(1):12–15.
5. Singh A, Agrawal A, Kale YS. Effects of Iron Deficiency Anemia on HBA1C Levels in Non-Diabetic and Diabetic Patients. *International Journal of Pharmaceutical Research*. 2019;11(1) :1187–92.
6. Sarkar PK, Prajapati PK, Choudhary AK, Sukla VJ, Ravishankar B. Haematinic evaluation of lauha bhasma and mandura bhasma on Hg-Cl<sub>2</sub> induced anemia in rats. *Indian Journal of Pharmaceutical Sciences*. 2008;791-795.
7. Mukherjee KL, Ghosh S. Medical laboratory Technology. Procedure Manual for Routine Diagnostic Tests. Noida McGraw Hill Education. 2012;2(11):263-266.
8. Dacie JV. and Lewis M. Blood counts. *Practical hematology 5<sup>th</sup> edition* New York Churchill Livingstone. 1997;5:20-40.
9. Scheinberg P, Young NS. How I treat acquired aplastic anemia *Blood*. 2012; 120(6):1185- 96.
10. Varma A, Vagha J. Agrawal A, Meshram R, Damke S and Thakur S. Sociodemographic Determinants in Prevalence of Anemia in Adolescents of Rural Area of Maharashtra. *Journal of Datta Meghe Institute of Medical Sciences University*. 2020;15( 2):209–14.
11. Inamdar N, Edalat S, Kotwal V, Pawar S. Care with Nature's cure. *Herbal Drugs Pharmacognosy Reviews*. 2007;1(2):361-368.
12. Potbhare MS, Phendarkar N, Khobragade D. In vitro Evaluation of Antioxidant Potential of N –Acetyl-D-Glucosamine. *Asian Journal of Research in Pharmaceutical Sciences*. 2017;7(2).
13. Breazile JE. *Textbook of Veterinary physiology 1st edition*, Philadelphia Lea and Fabiger. 1971; 205-210.
14. Potbhare MS, Khobragade D. In Vitro Evaluation of Antioxidant Potential of Ayurvedic Preparations Lauha Bhasma

- and Mandura Bhasma. Asian Journal of Pharmaceutical Research. 2017;7(2):63-66.
15. Dina OA, Adedapo AA, Oyinloye OP, Saba AB. Effect of *T. occidentalis* extract on experimentally induced anaemia in domestic rabbits. African Journal of Biomedical Research. 2000;3 :181-183.
  16. Akah PA, Okolo CE, African EC. The haematinic activity of the methanol leaf extract of *Brillantasia nitens* Lindau (Acanthaceae) in rats. Journal of Biotechnology. 2009;8(10):2389-2393.
  17. Christian Peter EA, Ezike A. The haematinic activity of the methanol leaf extract of *Brillantasia nitens* Lindau (Acanthaceae) in rats. African Journal of Biotechnology. 2009;8(10):2389-2393.
  18. Dash N, Dwivedi M, Khilnani, GD. Toxicity study of LauBhasma (calcined iron) in albino rats. Ancient Science of Life. 2016;35(3):159–166.
  19. Agbor AG, Odetola AA. Haematological studies of *Parquetina nigrescens* on haemorrhagic anaemic rats. African Journal of Medical Science. 2001;30:105-109.
  20. Sanni FS, Ibrahim S, Esievo K, Sanni S. Effect of oral administration of aqueous extract of *khaya Senegalensis* stem bark on phenylhydrazine-induced anaemia in rats. Pakistani Journal of Biological Sciences. 2005;8(2):255-258.
  21. OgbelRJ, Adoga GI, Abu AH. Antianaemic potentials of some plant extracts on phenyl hydrazine-induced anaemia in rabbits. Journal of Medicinal Plants Research. 2010;4(8):680-684.
  22. Potbhare MS, Tenpe CR, Khobragade DS. Evaluation of Ayurvedic preparation *lauha bhasma* as a potential haematinic agent. International Journal of Pharmacological Research. 2016; 6(12).
  23. Jaiswal S, Banait S, Daigavane S. A Comparative Study on Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Iron-Deficiency Anemia to Normal Population. Journal of Datta Meghe Institute of Medical Sciences University. 2018;13(1):9–11.
  24. Richard H. Chinese Herbal Medicine. Ancient art and modern Science. New York Shocker Brook. 1978;49-52.
  25. Wajpeyi SM, Badwaik P, Patil M. Protocol for Evaluation of Comparative Efficacy of Dietary Supplement Acquired from Kitchen Garden (*Parasbag*) and *Gudharitaki Awaleha* in *Pandu Roga* (Iron Deficiency Anemia). European Journal of Molecular and Clinical Medicine. 2020;7(7):2122–29.

© 2021 Khobragade et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<http://www.sdiarticle4.com/review-history/68930>