Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

ANTI-ANEMIC ACTIVITY OF DHATRYADI GHRITA IN PHENYL HYDRAZINE-TREATED WISTAR RATS

Rashmi Saxena Pal[1]*, Amrita Mishra[2]

1. ***Assistant Professor, Pharmacy Department, PSIT, Kanpur, India,***
2. ***Assistant Professor, SPS, IFTM University, Moradabad, India.***

ARTICLE INFO

Received:
28th Mar 2019
Received in revised form:
08th Aug 2019
Accepted:
12th Aug 2019
Available online:
21th Oct 2019

Keywords: Dhatryadi Ghrita, Anemic, Phenyl hydrazine, Hematological

ABSTRACT

Objective: Herbal formulations based on plants are effective against anemia. The influence of Dhatryadi Ghrita on blood glucose levels and some biochemical parameters were assessed. **Background:** Herbal drugs constitute a major part of all conventional systems of medicine. Researchers have no doubt that nature is still the preeminent synthetic chemist and that in plants, especially; there are almost infinite reserves of chemical constituents with actual and potential impacts on human body. **Methods:** Group I anemic control received distilled water from day 2 to 14. Group II positive control treated with Vitamin B 12 syrup 1 mL/day from day 2 to 14. Group III test group treated with 100 mg/kg/day of Dhatryadi Ghrita from day 2 to 14. Group IV test group treated with 200 mg/kg/day of Dhatryadi Ghrita from day 2 to day 14. Group V test group treated with 300 mg/kg/day of Dhatryadi Ghrita from day 2 to day 14. **Results:** Anemia was induced successfully in Groups I, II, III, IV, and V, which was indicated by a mean reduction of RBC and hemoglobin. Analysis of hematological parameters on days 14 and 28 showed significant effects. **Conclusion:** This study, not only substantiates the traditional uses of Dhatryadi Ghrita but also suggests its inclusion in the treatment of anemia as it exhibited significant anti-anemic activity.

To Cite This Article: Saxena Pal R and Mishra A, (2019). Anti-anemic activity of Dhatryadi Ghrita in phenyl hydrazine-treated Wistar rats. ***Pharmacophore, 10(5),*** *29-36.*

Introduction

Iron deficiency is the most prevalent nutritional disorder, which indicates a depleted and limited supply of iron to different tissues. Anemia emerges when blood lacks enough healthy red blood cells or hemoglobin. [1] Iron deficiency results in depletion of hemoglobin and iron-dependent intracellular enzymes involved in various metabolic pathways. [2] Anemia is characterized by various symptoms such as weight or appetite loss, pallor, fatigue or unknown drowsiness, weakness, energy loss, shortness of breath, etc. [3] Anemia occurs due to iron deficiency, folic acid deficiency, inhibition of absorbance of vitamin B_{12} in GIT, and nutritional deficiency. It is sometimes observed in alcoholics. [4, 5] Based on WHO, anemia influences more than 2 billion people globally, accounting for over 30% of the world's population which is the most occurring public health problem, particularly in developing countries. Anemia is one of the important health problems not only in India but also in most of the south East Asian countries. About 4-16% of maternal death is due to anemia. [6] It also increases the maternal morbidity, as well as fetal and neonatal mortality. It is the most common nutritional problem, which influences women of child-bearing age, especially during pregnancy and lactation in which there is a depleted and a restricted supply of iron to various tissues. [7] This influences many metabolic pathways. [8, 9] Thus, there is a need for proper management of micronutrient deficiencies, most especially iron deficiency. Anemia is a condition commonly seen in developing countries because of lack of nutrition and frequent use of drugs to treat diseases. [10, 11]

Hemolytic anemia is a form of inherited or acquired anemia resulting from either intravascular or extravascular RBC destruction. [12] The exposure to many chemicals causes RBC destruction and hemolytic anemia. [13] The hemolytic activity of aryl hydrazines, such as phenyl hydrazine, dapsone, hydroxylamine causes acute hemolytic anemia in vertebrates. [14] From ancient time, medicinal plants known as Rasayana in Ayurveda are thought to be beneficial in strengthening the hematopoietic and immune system of an individual. Ayurvedic physicians recommended various herbs for the treatment of hematological disorders as a source of iron and other minerals. [15, 16] There is an increasing demand for herbal medicines, health products, pharmaceuticals, food supplements, cosmetics, etc. [17] Herbal drugs are being proved more effective than synthetic drugs with lesser side effects. Nowadays, they are assumed to have greater importance in primary health care

Corresponding Author: Rashmi Saxena Pal; Assistant Professor, Pharmacy Department, PSIT, Kanpur. Email: rashmisaxenapal @ gmail.com

needs. In the present investigation, the objective was to assess the anti-anemic activity of in-house-prepared Dhatryadi ghrita against phenyl hydrazine-induced anemic rats.

Materials and Methods:

Preparation of Ghrita and its extract

Raw drugs were collected from rural farms of Kanpur Dehat and were identified and authenticated in NBRI, Lucknow. Dhatryadi Ghrita was formulated in Pharmacognosy lab of PSIT, Kanpur. The herbal drugs were dried and powdered to be utilized for the pharmacognostical investigation. Ghrita was prepared according to the technique mentioned in Ayurvedic formulary. The fresh fruits of Amla,,Benincasa fresh tuberous roots of Pueraria and Sugarcane, as well as fresh roots of Shatavari were rinsed, shade dried, and extracted for fresh juice. Stolons and roots of Liquorice and heartwood powder of white sandalwood were cleaned, dried and powdered. Resin was admixed with the above powdered ingredients in order to sweeten the formulation. Then, adequate amount of purified water was added to make homogenous composition. After this, Go-Ghrita and milk was admixed in equal quantity, while heating with continuous stirring for about 3 hours. It was then kept overnight, filtered next day, and heated and cooled afterwards. After cooling, sugar was admixed and stirred vigorously. After this, ethanolic extract of Ghrita was obtained. [18-21]

Figure 1. Prepared Dhatryadi ghrita

Toxicity studies

The acute toxicity investigations of the experiment dealing with various doses as varying from 1000-4000 mg/kg did not result in death of animals till day 14 of observation in the experimentation period. Dhatryadi Ghrita is safe in rodents and mice. Thus, the extract is safer for being the part of different pharmaceutical formulations. Ghrita in varying concentrations were revealed to be safe and non-toxic under acute toxicity investigations. [22-24] The Institutional Ethical Committee of Pharmacy department, 1273/PO/Re/S/09/CPCSEA for analysis for education purpose on little animals, PSIT, Kanpur, India verified the protocol for these investigations. [25]

Animals:

Wistar male rats, weighing 100–150 g were chosen for the research. The animals were kept separately in polypropylene cages under hygienic and standard environmental conditions as temperature 22 ± 3°C, humidity 30–70%, 12 h light/dark cycle. The animals were allowed to have prescribed diet and water ad libitum. They were acclimated to the environment for one week prior to research. All the animal experiments were based on the protocols of Institutional Animal Ethical Committee (IAEC). [26-28]

Housing and Nutrition

The animals were kept in cages with wood litter, under optimum temperature, and light:dark cycle of 12:12 hours. Every cage was assigned a separate card, showing the number of the cage, weight of the animals, details of the administered drug, route of administration, and the dose. The animals were given animal food, along with water as needed. [29-31]

Anti-anemic activity

Phenyl hydrazine

Anemia was induced by intra-peritoneal injection of phenyl hydrazine at 60 mg/kg for 2 days. Following the injections, rats were divided into five groups of six rats each. [32] Group I-anemic control received distilled water from day 2 to 14. Group II-positive control treated with 1 mL/day Vitamin B12 syrup from day 2 to 14. Group III-test group treated with Dhatryadi

Ghrita at 100 mg/kg/day from day 2 to 14. Group IV-test group treated with Dhatryadi Ghrita at 200 mg/kg/day from day 2 to day 14. Group V-test group treated with Dhatryadi Ghrita at 300 mg/kg/day from day 2 to day 14.

The blood was collected in EDTA-coated tube by tail puncture under phenobarbitone (45 mg/kg, ip) anesthesia and the estimation of different biochemical parameters like hemoglobin and RBC values were performed. [33, 34]

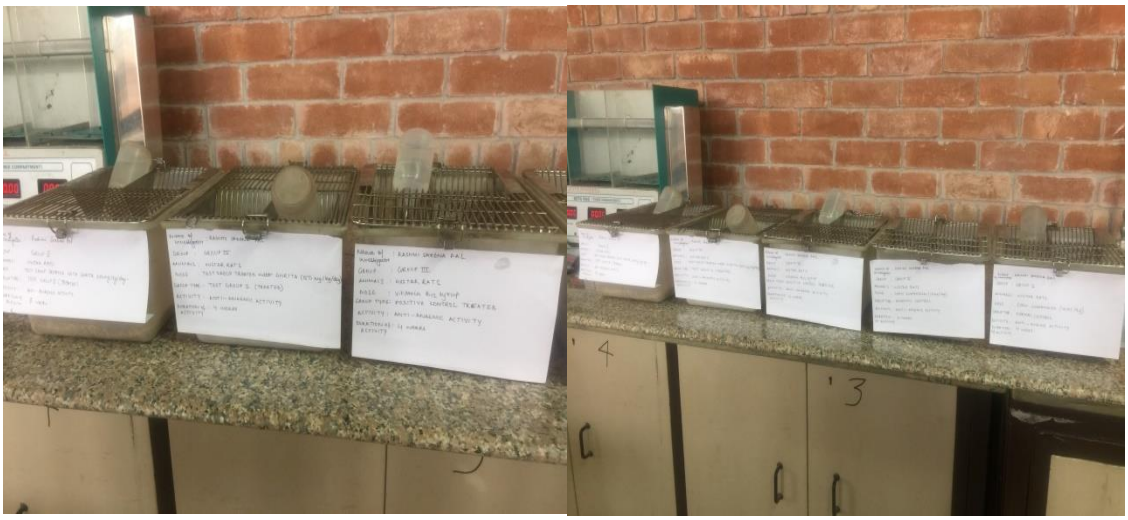

Figure 2: The animals were caged according to their groups.

Statistical Analysis

Data were expressed as mean±SEM. The data were analyzed by using one-way analysis of variance (ANOVA) followed by t-test, where P-values < 0.05 were considered significant. [35, 36]

At first, anemia was induced in rats (except normal control or Group I) by intraperitoneal administration of 40 mg/kg of phenyl hydrazine (PHZ) for 2 days (D0 and D1).

Table 1: The classification of groups on the basis of the treatment provided to them as per the experimental design constructed for the research work.

S. No.	Group Number	Group
1.	Group I	Anemic control received distilled water from day 2 to 14.
2.	Group II	Positive control treated with 1 mL/day Vitamin B12 syrup from day 2 to 14.
3.	Group III	Test group treated with Dhatryadi Ghrita at 100 mg/kg/day from day 2 to 14
4.	Group IV	Test group treated with Dhatryadi Ghrita at 200 mg/kg/day from day 2 to day 14.
5.	Group V	Test group treated with Dhatryadi Ghrita at 300 mg/kg/day from day 2 to 14

Experimental Design

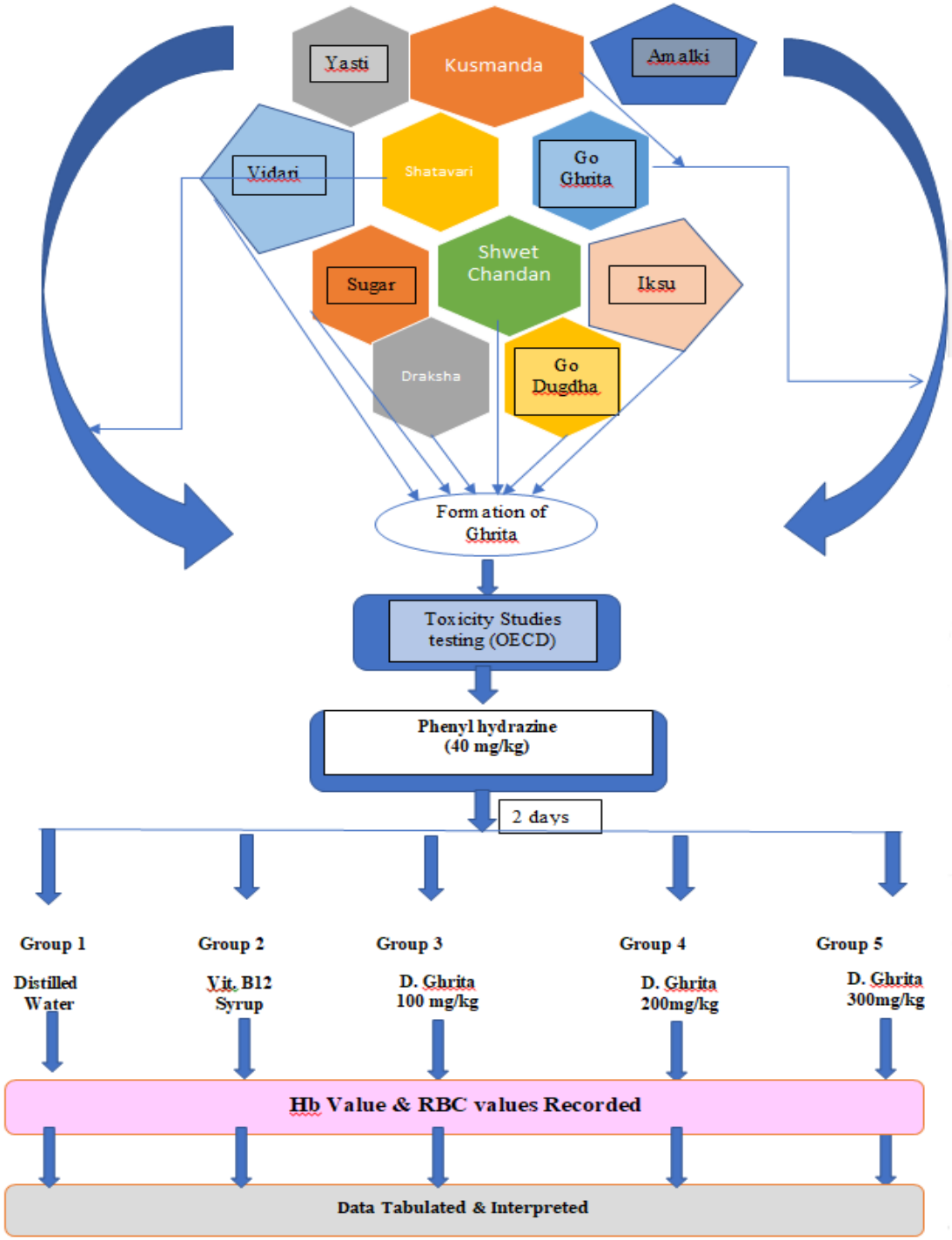

Table 2: Hb levels in wistar rats before and after PHZ-induced anemia and after 14 days of treatment by Dhatryadi Ghrita.

S. No.	Group Number	Hb levels Before PHZ Administration	Hb levels after 2 days of PHZ-induced Anemia	Hb levels post 7 days of respective treatment	Hb levels post 14 days of respective treatment
1.	Group I	12.71±0.353	8.69±0.542	8.93±1.250	9.41±0.231
2.	Group II	13.10±0.212	8.32±0.212	10.12±1.346*	12.52±0.143*
3.	Group III	12.93±0.136	8.91±1.151	9.32±0.314	10.27±0.221*
4.	Group IV	13.79±0.296	8.37±0.814	10.01±0.192*	11.5±1.521*
5.	Group V	13.94±0.631	8.34±0.521	11.12±1.812*	13.56±0.731*

The results are mean ± SEM; N=6; *P<0.05 (control group vs. extract)

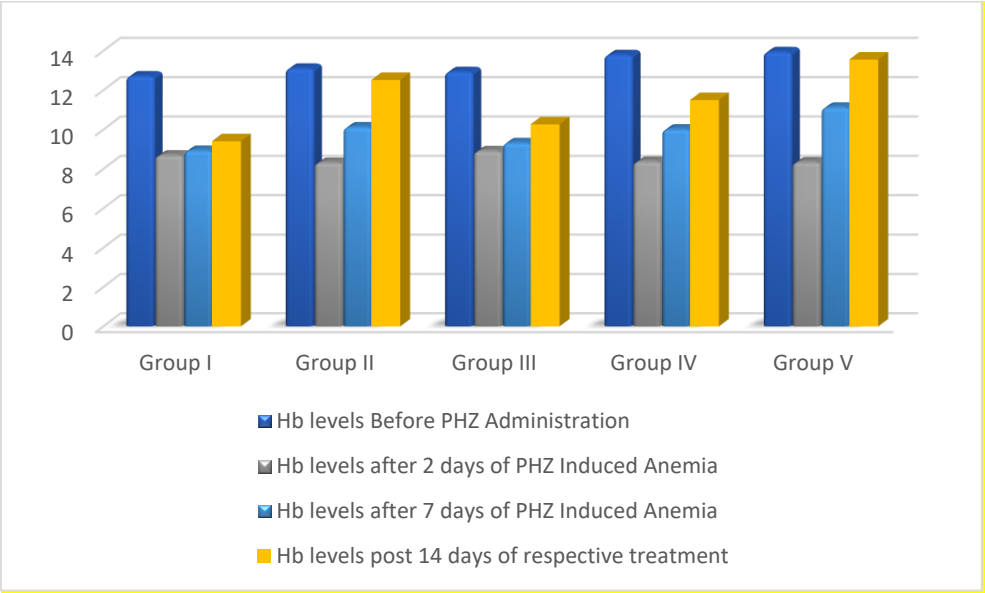

Figure 3: Graphical representation of the Hb levels in all groups on respective days of study.

Table 3: Effect of Dhatryadi Ghrita on red blood cell number in anemia induced by phenylhydrazine in wistar rats on Days 0, 2, 7, and 14.

S. No.	Group Number	Values of RBC on Day 0 of treatment (10^6 /µL)	Values of RBC on Day 2 of treatment (10^6 /µL)	Values of RBC after 7 days of treatment (10^6 /µL)	Values of RBC after 14 days of treatment (106 /µL)
1.	Group I	7.52±1.19	3.5±0.32	4.24±0.4	5.34±0.14
2.	Group II	7.93±1.36	3.54±0.2	5.36±0.1 *	7.32±0.121*
3.	Group III	7.86±0.62	3.41±0.13	5.12±0.24 *	6.35±0.321*
4.	Group IV	7.69±1.3	3.7±0.2	6.22±0.14 *	7.18±0.732*
5.	Group V	7.69±0.46	4.12±0.18*	6.14±0.02*	7.42±0.312*

The results are mean ± SEM; N=6; *P<0.05 (control group vs. extract)

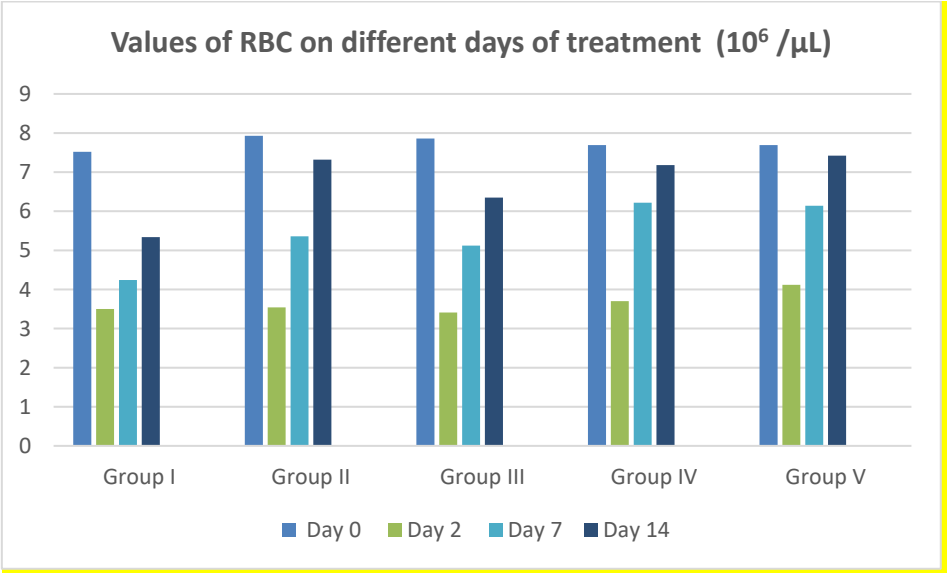

Figure 4: Graphical representation of RBC values on different treatment days (10^6 /µL)

Statistical analysis

Data were expressed as standard error of mean (SEM). Statistical comparisons were performed by one-way ANOVA, followed by t-test, and the values were considered statistically significant when p-value was less than 0.05 (p<0.05). [37, 38]

Discussions

PHZ is a non-immunogenic drug that causes alterations in the red cell membrane, leading to oxidative denaturation of hemoglobin. The effect of the denaturation is the reduction in the life span of the erythrocytes. [39] Altered erythrocytes are eradicated by the spleen and liver, resulting in compensated hemolytic anemia. PHZ-induced anemia is a model, helping out for the investigation of hematinic impacts. [40-43] Ghrita reigned the number of RBC and amount of hemoglobin and raised myeloid:erythroid cell ratio and normalized cathepsin D activities by counteracting the action phenyl hydrazine. The results confirm the claims of Ayurveda that these drugs possess the potency to cure anemia through protection of RBCs from hemolysis and simultaneously lowering cathepsin D activities from the spleen. [44-46] The Hb concentration was found to be higher than the positive control animals. This indicates presence of some bioactive agents that prevent or repair the damage to the cells by free radicals or highly reactive oxygen species. This attributes to the presence of raisins and emblica in the formulation. Dhatri, which is emblica, is a very good source of micro-nutrients. It is the richest source of Vitamin C. The nutritive value of fresh, mature fruits of amla is 100 gr, with 0.4 gr proteins, and 14 gr carbohydrates. It is rich in Vitamin B1, B3, C, calcium, iron and phosphorus. It has a high antioxidant value due to the presence of tannins as well. [47] From our study, it can be established that the anti-anemic potential of Dhatryadi Ghrita can be explored for further research in developing a novel herbal delivery system.

Conclusion

The collective results of the above study confirmed that Ghrita has considerable anti-anemic activity as revealed in PHZ-induced anemia in experimental rat model. Further studies are required to precisely define its bioactive compounds and to ensure maximum bioavailability and therapeutic efficacy. The injection of phenyl hydrazine to rats caused a hemolytic anemia characterized by reducing hematological parameters. The oral administration of ethanol extract of Dhatryadi Ghrita in the dose of 300 mg/kg/day significantly increased hemoglobin level in the first week of treatment. The anti-anemic effect of the extract was more pronounced in the dose of 300 mg/kg/day as compared to 150 mg/kg/day. The anti-anemic potential of the plant could come from phytochemicals and also the possible vitamin and mineral constituents. [48-50]

References

1. Gupta D, Kushwah C, Joshi A, Malviya S, Kharia A. Anti-Anemic Activity of Hydro-Alcoholic Extract of Fruit of Allium tricoccum in Phenylhydrazine Induced Anemic Rats. PharmaTutor. 2018 Jul 1;6(7):5-7.
2. Jurenka J. Therapeutic applications of pomegranate (Punica granatum L.): a review. Alternative medicine review. 2008 Jun 1;13(2).
3. Powers A, Silberstein LE. Autoimmune haemolytic anaemia. In: Hoffman R, editor. Haematology: Basic Principles and Practice. 5th ed. Philadelphia, PA., USA: Elsevier/Churchill Livingstone. 2009.
4. Beutler E. Hemolytic anemia due to chemical and physical agents. Williams Hematology, 6th edition, New York. 2001:629-32.
5. Kozlov VA, Zhuravkin IN, Coleman RM, Rencricca NJ. Splenic plaque-forming cells (PFC) and stem cells (CFU-s) during acute phenylhydrazine-induced enhanced erythropoiesis. Journal of Experimental Zoology. 1980 Aug;213(2):199-203.
6. De Benoist B, Cogswell M, Egli I, McLean E. Worldwide prevalence of anaemia 1993-2005; WHO Global Database of anaemia.
7. De Mayer EM., M Tegman. The prevalence of anaemia in the world. World health statistics quarterly. 1985; 38: 302-316.
8. Yeshoda KM. Phenylhydrazine anaemia in rats. Current Science. 1942 Sep 1;11(9):360-3.
9. Smith JR. The clinical and economic burden of anemia. The American journal of managed care. 2010 Mar;16:S59-66.
10. Silja VP, Samitha VK, Mohanan KV. Ethnomedicinal plant knowledge of the mullu kuruma tribe of Wayanad district of Kerala. Indian Journal of Traditional Knowledge. 2008; 7(4): 604-612
11. Sanjeev K, Kumar AR. Synonyms and therapeutic review of Mulethi (GlycyrrhizaglabraLinn.) commonly known as Licorice: from Kosha and Nighantus, International journal of ayurvedic & herbal medicine. 2015; 5(4): 1868-1874.
12. Balasubramanian A, Ramalingam K, Krishnan S, Ajm C. Anti-inflammatory activity of Morus indica Linn. Iranian journal of Pharmacology and Therapeutics. 2005 Jun 15;4(1):13-0.
13. Baker FJ, Silverton RE, Pallister CJ. Introduction to medical laboratory technology. 7th ed., Elvis Publishers. 1998; 356-360.
14. Saeed MA, Sabir AW. Effects of Fagonia cretica L. constituents on various haematological parameters in rabbits. Journal of ethnopharmacology. 2003 Apr 1;85(2-3):195-200.
15. Adebajo AC, Ayoola OF, Iwalewa EO, Akindahunsi AA, Omisore NO, Adewunmi CO, Adenowo TK. Anti-trichomonal, biochemical and toxicological activities of methanolic extract and some carbazole alkaloids isolated from the leaves of Murraya koenigii growing in Nigeria. Phytomedicine. 2006 Mar 13;13(4):246-54.

16. Bumah VV, Essien EU, Agbedahunsi JM, Ekah OU. Effects of Khaya grandifoliola (Meliaceae) on some biochemical parameters in rats. Journal of ethnopharmacology. 2005 Dec 1;102(3):446-9.
17. Burkill HM. The useful plants of West Tropical Africa. 2nd ed. (Families A-D) Royal Botanical Gardens Kew, London. 1985; 20-29.
18. Pal RS, Mishra A. Standardization of Dhatryadi Ghrita: a herbal Ghee Based Ayurvedic Medicinal Preparation. Open Medicine Journal. 2018 Aug 31; 5: 47-55.
19. Pal RS, Mishra A. A review on dhatryadi ghrita. Int J Res Ayurveda Pharm. 2017;8(2):190-5.
20. Ayurvedic Pharmacopoeia of India Part 1,1st edition, published by Gov. of India, The controller of publications civil lines, Delhi – 2011.
21. Lala PK. Lab manuals of Pharmacognosy. CSI Publishers and Distributors, Calcutta. 1993;5:38-48.
22. Pal RS, Mishra A. Evaluation of Acute Toxicity of the Methanolic Extract of Dhatryadi Ghrita in Wistar Rats. The Open Pharmacology Journal. 2019 Mar 15;9(1).
23. Schoepfer AM, Engel A, Fattinger K, Marbet UA, Criblez D, Reichen J, Zimmermann A, Oneta CM. Herbal does not mean innocuous: ten cases of severe hepatotoxicity associated with dietary supplements from Herbalife® products. Journal of hepatology. 2007 Oct 1;47(4):521-6.
24. Ansari SH. Essential of pharmacognosy 1st ed. Birla Publications (Regd) Pvt Ltd, 2007.
25. Antman EM. ST-elevation myocardial infarction: pathology, pathophysiology, and clinical features. Heart disease. 2008:1207-32.
26. Ankur J, Priyanka S, Narendra V, Javed K, Sapna M, Anil K. Anti-anemic activity of hydro alcoholic leaf extract of Aegle marmelos in phenylhydrazine induced anemic rats. International Journal of Current Research. 2017;9(4):48928-31.
27. Ankur J, Deepanshu G, Priyanka S, Sapna M, Deenanath J, Anil K. Anti-anemic activity of hydro alcoholic leaf extract of Tamarindus Indica in phenylhydrazine induced anemic rats. Journal of Harmonized Research in Applied Sciences. 2017; 5(3): 132-135.
28. Gupta D, Kushwah C, Joshi A, Malviya K, Malviya S. Anti-anemic activity of hydro-alcoholic leaf extract of brassica oleracea var in phenylhydrazine induced anemic rats, Asian Journal of Pharmaceutical Education and Research. 2018; 7(2): 12-18.
29. Arakawa H. Age dependent effects of space limitation and social tension on open-field behavior in male rats. Physiology & Behavior. 2005 Mar 16;84(3):429-36.
30. Armario A, Castellanos JM, Balasch J. Chronic noise stress and insulin secretion in male rats. Physiology & behavior. 1985 Mar 1;34(3):359-61.
31. Armstrong KR, Clark TR, Peterson AR. Use of corn-husk nesting material to reduce aggression in caged mice. Journal of the American Association for Laboratory Animal Science. 1998 Jul 1;37(4):64-6.
32. Shafie EH, Keshavarz SA, Kefayati ME, Taheri F, Sarbakhsh P, Vafa MR. The effects of nanoparticles containing iron on blood and inflammatory markers in comparison to ferrous sulfate in anemic rats. International journal of preventive medicine. 2016;7.
33. Alaarg A, Schiffelers R, van Solinge WW, Van Wijk R. Red blood cell vesiculation in hereditary hemolytic anemia. Frontiers in physiology. 2013 Dec 13;4:365.
34. Ashenden M, Clarke A, Sharpe K, d'Onofrio G, Plowman J, Gore CJ. Stability of athlete passport parameters during extended storage. International journal of laboratory hematology. 2013 Apr;35(2):183-92.
35. Emerson JD, Colditz GA. Use of statistical analysis in the New England Journal of Medicine. New England Journal of Medicine. 1983 Sep 22;309(12):709-13.
36. Feinstein AR. XXV. A survey of the statistical procedures in general medical journals. Clinical Pharmacology & Therapeutics. 1974 Jan;15(1):97-107.
37. Godfrey K. Statistics in practice. Comparing the means of several groups. New England Journal of Medicine. 1985 Dec 5; 313: 1450-1456.
38. Livingston EH. Who was student and why do we care so much about his t-test? 1. Journal of Surgical Research. 2004 May 1;118(1):58-65.
39. RIFKIND RA, Danon D. Heinz body anemia—an ultrastructural study. I. Heinz body formation. Blood. 1965 Jun 1;25(6):885-96.
40. Suzuki Y. The development of a sensitive micronucleus test: an in vitro method using cultured bone marrow cells. Jikeikai Med. J. 1985;100:709-19.
41. Adamson JW, Longo DL. Hematologic alterations. In: Braumwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL. Harrison's Principles of Internal Medicine. New York: McGraw Hills, 2001.
42. Waghmare AN, Tembhurne SV, Dinesh MS. Anti-Anaemic Potential of Murraya koenigii Fruit Extracts In Phenylhydrazine Induced Anaemic Rats. International Journal of Advances in Pharmaceutical Research. 2015 May;6(05):124-7.
43. Ghosh MN. Toxicity Studies, Fundamentals of Experimental Pharmacology. 2nd ed. Calcutta: SC and RC Book Agencies, 1984.

44. Mir AH, Sexena M, Malla MY. An acute oral toxicity study of methanolic extract from Tridex procumbens in Sprague Dawley's Rats as per OECD guidelines 423. Asian Journal of Plant Science and Research. 2013;3(1):16-20.
45. Itano HA, Hosokawa K, Hirota K. Induction of haemolytic anaemia by substituted phenylhydrazines. British journal of haematology. 1976 Jan;32(1):99-104.
46. Berger J. Screening of toxic-haemolytic anaemia in laboratory rats: a model of phenylhydrazine-induced haemolysis. Haematologia. 1985;18(3):193-200.
47. Pal RS, Mishra A. A review on dhatryadi ghrita. Int J Res Ayurveda Pharm. 2017;8(2):190-5.
48. Biswas S, Bhattacharyya J, Dutta AG. Oxidant induced injury of erythrocyte—Role of green tea leaf and ascorbic acid. Molecular and cellular biochemistry. 2005 Aug 1;276(1-2):205-10.
49. Turaskar AS, More SA, Sheikh RI, Gadhpayle J, Bongade SI. Inhibitory potential of Picrorrhiza kurroa Royle ex. Benth extract on phenylhydrazine induced reticulocytosis in rats. As J Pharm Clin Res. 2013;6(2):215-6.
50. Ndem JI, Otitoju O, Akpanaiabiatu MI, Uboh FE, Uwah AF, Edet OA. Haematoprotective property of Eremomastax speciosa (Hochst.) on experimentally induced anaemic Wistar rats. Annals of Biological Research. 2013;4(6):356-60.