International Journal of Pharmaceutical Science and Research
www.pharmacyjournal.net
ISSN: 2455-4685
Received: 10-01-2022, Accepted: 25-01-2022, Published: 10-02-2022
Volume 7, Issue 1, 2022, Page No. 1-6

Antianaemic effect of lyophilized red beetroots *(Beta vulgaris L.)* juice on anaemic rats induced by phenyl hydrazine

Haneen H Mouminah, Maha A Althaiban
Department of Food and Nutrition, College of Human Science and Design, King Abdulaziz University, Kingdom, Saudi Arabia

Abstract
Anti-anaemic effect of lyophilized red beet juice in anemia rats were induced rats by phenyl hydrazine at the dose of 40 mg / kg / day for two days were evaluated. Forty (40) Wister albino male and female rats weighing between 100-150g. Groups B, C, D and E were injected twice into the intraperitoneally for two days with 40 mg / kg of phenyl hydrazine hydrochloride induce anemia in the rats, while group one served as a healthy rats without anemia. Group A rats without anemia were treated with 0.5ml of normal saline, Group B served as aneamic rats treated with 0.5ml of normal saline, Group C rats with anemia was treated with 50 mg/kg body weight of lyophilized red beet juice, Group D rats with anemia was treated with 100 mg/kg body weight of lyophilized red beet juice. Group E served as anemia rats was treated with 150 mg/kg body weight of lyophilized red beet juice. The feeding lasted for 21days after which the blood samples were collected from each rats via the ocular region for analysis. The parameters analyzed were packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC) and hemoglobin (HB) concentrations. From the results of present work indicate that, the aneamic rats untreated with lyophilized red beet juice (group B) showed were decreased in hemoglobin concentration, Packed Cell Volume (PCV), red blood cells count (RBC) and increased in white blood count (WBC mm-3/L) compared with healthy rats group A after 7, 14 and 21 days respectively. The aneamic rats for group C, group D and group E were treated with lyophilized red beet juice orally at 50,100 and 150 mg / kg of body weight per day for three weeks were increased in hemoglobin concentration, Packed Cell Volume (PCV), red blood cells count (RBC) and decreased in white blood count (WBC) compared with aneamic rats untreated with lyophilized red beet juice (group B) respectively. Treated with lyophilized red beet juice for groups C, D and E were improved. The results of this study showed that lyophilized red beet juice improved the Haematological parameters of the treated rats thereby increasing the hemoglobin (Hb), packed cell volume (PCV) and red blood cell (RBC) levels of the rats. This is suggestive that lyophilized red beet juice may be implored in the treatment of anaemia.

Keywords: anaemia, red beetroots juices, hematological parameters, bioactive components, packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC) and hemoglobin (HB)

Introduction

Anemia is a blood condition characterized by a low concentration of hemoglobin accompanied by a low number of circulating red blood cells. The main function of red blood cells is to transport oxygen to the tissues of the body and in anemia there is a decrease in the oxygen carrying capacity which is harmful to the body. Vamsee *et al.,* (2015) [35]. Anemia is a common blood disorder that affects people of all ages, although the most vulnerable people are the elderly, young women of childbearing age, and infants. According to the World Health Organization, the global prevalence of anemia is 24.8% and an estimated 1620 million people de Benoist *et al.,* (2008) [7]. In anemia there is a decrease in the level of diffuse hemoglobin, less than 13 g / dl in males and 12 g / dl in females Okochi *et al.,* (2003) [22]. In the tropics, due to the endemic of malaria, between 10 and 20% of the population represents less than 10 g / dl of hemoglobin. Diallo *et al.,* (2008) [8]. Iron deficiency anemia is common in poor countries because most people are malnourished. It occurs when the body does not have enough iron, which leads to decreased red blood cell production because iron is the main factor in the synthesis of hemoglobin Al-Zabedi *et al.,* (2014) [2]. In women, anemia of childbearing age is caused by menorrhagia and pregnancy, due to the increased need for iron Ramesh *et al.,* (2010) [26] and Rajarathinam *et al.,* (2013) [25]. Iron supplements are mainly used to anemia Sembulingam and Prema (2007) [29]. Traditional medicines in our regions contain many plants to manage anemia. Red beet is the most important food product that contains this category of coloring. Besides, red beets contain other health promoting ingredients such as phenols (phenolic acids, phenolic esters and flavonoids) and folic acid. Jastrebova *et al.,* (2003) [16] and Kujala *et al.,* (2002) [18] High antioxidant activities of betalain extracts from both beet roots and stems have been also reported. The red beet (Beta vulgaris L.), which is a vegetable traditionally consumed worldwide, is a source of antioxidant compounds. Lee *et al.,* (2014) [20]. Red beetroot is one of the most effective vegetables in terms of antioxidant activity due to

the presence of many active compounds such as carotenoids, glycine, saponins, betacyanines folate, betaenin, polyphenols, and flavonoids. Sheila *et al.,* (2017) [31]. Beetroot is a rich source of nutrients. It is very rich as regards B vitamins (B1, B2, B3, and B6), folic acid, vitamin A and vitamin C. The mineral content are iron, magnesium, selenium, potassium, calcium, zinc, phosphorous and sodium Strauss *et al.,* (2013). The bioactive components include phenolic compounds, saponins, and especially Betaline, responsible for the distinctive color of this tuber Zielinska-Przyjemska *et al.,* (2009) [36]. The presence of different phenolic compounds in beets adds antioxidant properties to these vegetables Krajka *et al.,* (2012) [17]. It was concluded that beet supplementing could have positive effects on various aspects of health and disease, and thus could represent an economical, practical and important product from the point of view of natural nutritional intervention. Clifford *et al.,* (2015) [5]. Plants are a rich source of medicinal components because they produce a group of biologically active molecules, most likely acting as a chemical defense against predators or infectious agents. Pathirana *et al.,* (1990) [23]. Phytochemical analysis revealed the presence of large chemical groups such as: alkaloids, tannins, flavonoids, polyphenols, quinones, sterols, turbines, cardiac glycosides, saponins and leucanthocyanins. They have anti-oxidant strength, promote tissue regeneration, reduce capillary permeability in the blood and increase their resistance to hemolysis Singh *et al.,* (1991) [32], Saimak, 2009 [28] and Kushwaha *et al.,* (2017) [19].
The aim of the study is to investigate the anti anaemic effect of lyophilized red beet juice of *Beta vulgaris L. var. conditiva* on phenyl hydrazine induced anaemic rats.

Material and Methods
The Red beets (*Beta vulgaris* L. *var. conditiva*) were obtained from local market in during February 2021.

Preparation of lyophilized red beet juice
Red beets were washed, peeling and cut in pieces of 1 cm. About 400 g of peeled red beet was mixed in blender (Moulinex juice extractor) with 200 ml of deionized water (acidified with 2% citric acid) for 15 min at room temperature and homogenized for 4 min. The mixture was pressed and filtrate through cheese cloth for obtaining red beet juice Lyophilized of beetroot juice is obtained after the freeze-drying of this juice at -52°C, 0,044 mbar and during 48 h, using Chris ALPHA 1-2 LD plus instrument. The lyophilized ref beet juice was dissolved in deionized water to a concentration of 150 mg/ml before administration in anemic rats.

Animals: Forty (40) Wister albino male and female rats weighing between 100-150g were obtained and kept in well aerated laboratory cages in the Animal house, Food and Nutrition Department, Human Science and Design, King Abdulaziz University, Jeddah, Saudi Arabia. The animals were allowed to adapt to the laboratory environment for 7 weeks before starting the experiment. They were fed with water and grower mash from Vital Feed ad libitum.

Induction of anemia
Induction was done by modified the method of Iwalewa *et al.,* (2005) [15]. Rats were injected twice into the intraperitoneally for two days with 40 mg / kg of phenyl hydrazine hydrochloride. Anemia was allowed to be established within 24 hours after the second induction. Packed Cell volume <35% was considered an indicator of anemia.

Experimental design
Animal classification: The experimental rats were randomly divided into five (5) groups, with eight animals per group and treated for twenty-one (21) days. They were fed with commercial rats feed and water *ad libitum* daily. With the exception of the first group, all other groups were anemia-induced.
Group A: healthy rats (normal control).
Group B: Anemic rats without treatment. Group C: Anemic rats treated with 50mg/kg body weight of lyophilized ref beet juice orally. Group D: Anemic rats treated with 100mg/kg body weight of lyophilized ref beet juice orally. Group E: Anemic rats treated with 150mg/kg body weight of lyophilized ref beet juice orally. Determination of hematological parameters. Blood was collected in sample bottles containing EDTA on days 7, 14 and 21 from experimental animals for hematological analysis as recommended by Malomo *et al.,* (2002) [21]. Red Blood Cells (RBC) and White Blood Cells (WBC) counting was done with the help of Neubaur's chamber. Packed cell Volume (PCV) with Wintrobe hematocrit tubes, Hemoglobin (Hb) by Sharma, (2007) [30].
Cardiac glycosides, polyphenols, sterols, terpenes, tannins, flavonoids, quinones, anthocyanins, Betaline, reducing compounds, leucoanthocyanins, saponins and alkaloids were determined by the methods described by Houghton and Raman (1998) [14], Sofowora (1993) [33], Evans (1989) [10], Harborne 1979 and Pavlov *et al.,* (2005) [24].

Statistical analyses: Results are presented as mean ± standard error of mean (SEM). Within and between groups, comparisons were performed by the analysis of variance (ANOVA) (using SPSS 17.0 for windows Computer Software Package). Significant differences were compared by Duncan‟s new Multiple Range test; a probability level of less than 5% ($P< 0.05$) was considered significant Duncan, (1955) [9].

Results and Discussion
Bioactive components in lyophilized red beetroots juice are shown in Table 1.The results indicated the presence of cardiac glycosides, polyphenols, sterols, terpenes, tannins, flavonoids, quinones, reducing compounds,

anthocyanins, betalins, leucoanthocyanins and alkaloids. Red beet is the most important food product that contains this category of coloring. Besides, red beets contain other health promoting ingredients such as phenols (phenolic acids, phenolic esters and flavonoids) and folic acid Jastrebova *et al.,* (2003) [16] and Kujala *et al.,* (2002) [18]. High antioxidant activities of betalins extracts from both beet roots and stems have been also reported.

Table 1: Bioactive components in lyophilized red beetroots juice

Bioactive components	Detection
Cardiac Glycosides	+
Flavonoids	+
Alkaloids	+
Tannins	+
Leucoanthocyanins	+
Saponins	-
Polyphenols	+
Sterols and Terpenes	+
Quinones	+
Anthocyanins	+
Betaline	+
Reducing compounds	+

The effects of lyophilized red beet juice on the hemoglobin concentration (Hb g/dl) in anaemic rats induced by phenyl hydrazine hydrochloride during the experimental periods for three weeks are presented in Fig 1. Results were showed that, anaemic rats induced by phenyl hydrazine hydrochloride (Group B) were decremented in hemoglobin concentration (Hb g/dl) by 34.06, 36.62 and 38.72 % compared to the healthy rats (group A) after 7,14 and 21 days respectively. These results were confirmed with Riordan *et al.,* (1995) [27], Criswell *et al.,* (2000) [6] and Agbor *et al.,* (2005) [1]. Intraperitoneal intake of phenyl hydrazine has been shown to reduce hemoglobin concentration. Aneamic rats treated with lyophilized red beet juice at 50, 100 and 150 mg/kg of body weight were increased in hemoglobin concentration (Hb g/dl) after 21 days by 62.68, 68.97 and 72.04 % in for anemic rats treated with 50 mg of lyophilized red beet juice (Group C), anaemic rats treated with 100 mg of lyophilized red beet juice (Group D) and anaemic rats treated with 150 mg of lyophilized red beet juice (Group E) respectively compared with Hyperglycemic control rats (Group 2). Treated with lyophilized red beet juice for groups C, D and E were improved the hemoglobin concentration during the experimental periods compared to anemic rats (Group B). It has also been reported that phenyl hydrazine causes oxidative damage to red blood cells by increasing the formation of reactive oxygen species Clemens *et al.,* (1984) [4]; Hill and Thornalley, (1982) [13]. However, alkaloids and flavonoids protect cells as powerful antioxidants that prevent or repair damage to red cells due to free radicals or highly reactive oxygen types.

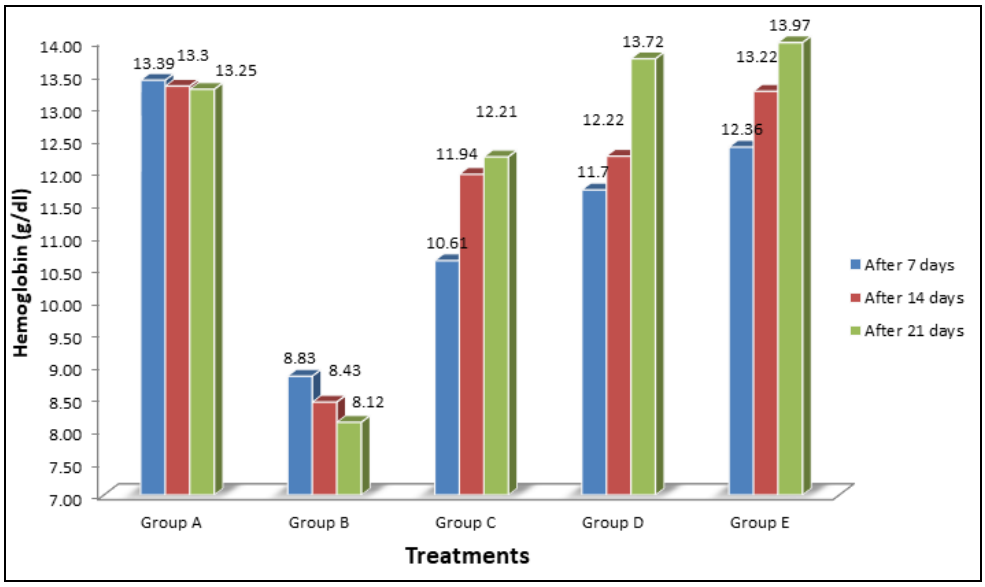

Fig 1: The effects of lyophilized red beet juice on the hemoglobin concentration (Hb g/dl) in anaemic rats induced by phenyl hydrazine hydrochloride.

The effects of lyophilized red beet juice on Packed Cell Volume (PCV %) anaemic rats induced by phenyl hydrazine hydrochloride during the experimental periods for three weeks are presented in Fig 2. The results revealed that, Packed Cell Volume (PCV) in anaemic rats in (Group B) (without treated with lyophilized red beet

juice) were decreasing by 47.63, 41.05 and 33.80% compared to the normal control (Group A) after 7,14 and 21 days respectively. anaemic rats treated with 50 mg of lyophilized red beet juice (Group C), anemic rats treated with 100 mg of lyophilized red beet juice (Group D) and anaemic rats treated with 150 mg of lyophilized red beet juice (Group E) were increased in the serum level of packed cell volume by 30.18, 43.39 and 48.55% compared to anemic rats (Group B) after three weeks respectively. These results were confirmed with Riordan *et al.*, (1995) [27], Criswell et al., (2000) [6] and Agbor *et al.* (2005) [1]. Intraperitoneal intake of phenyl hydrazine has been shown to reduce Hb concentration, red blood cell count and hematocrit. Also Agbor *et al.*, (2005) [1], showed that consuming 10 mg / kg phenyl hydrazine for 8 days reduced hematological indicators by 50%. This decrease in packed cell volume, hemoglobin, and red blood cells confirms the induction of haemolytic anaemia.

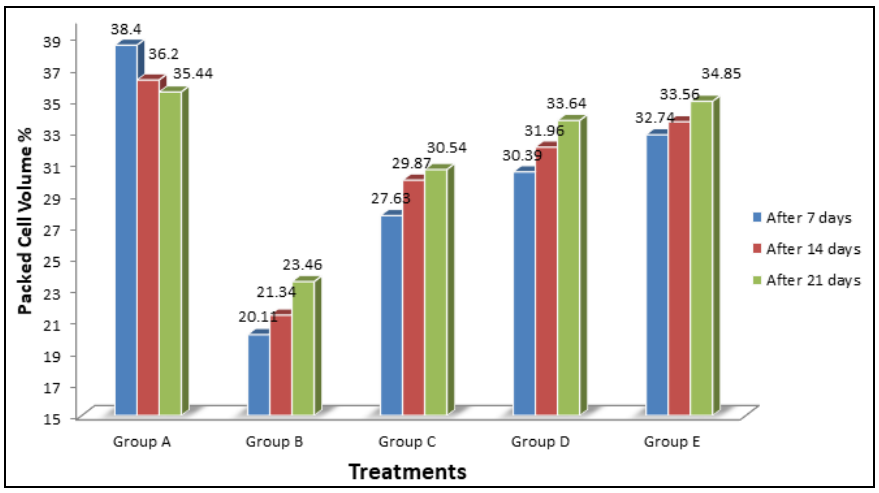

Fig 2: The effects of lyophilized red beet juice on Packed Cell Volume (PCV %) in anaemic rats induced by phenyl hydrazine hydrochloride

The effects lyophilized red beet juice on red blood cells count (RBC) of anaemic rats induced by phenyl hydrazine hydrochloride during the experimental periods for three weeks are presented in Fig 3. The results revealed that the red blood cells count (RBC) were decreased in anaemia rats Group B (positive control group) by 28.84, 10.65 and 7.21% compared to healthy rats (Group A) after 7,14 and 21 days respectively. These results were confirmed with Riordan *et al.*, (1995) [27], Criswell *et al.*, (2000) [6] and Agbor *et al.*, (2005) [1]. Intraperitoneal intake of phenyl hydrazine has been shown to reduce red blood cell count. Anaemic rats treated with 50 mg of lyophilized red beet juice (Group C), anaemic rats treated with 100 mg of lyophilized red beet juice (Group D) and anaemic rats treated with 150 mg of lyophilized red beet juice (Group E) were increased in red blood cells count (RBC) by 4.93, 8.67 and 11.21% compared with anemic rats (Group B) after 21 days respectively. Red blood cells, hemoglobin concentration and Packed Cell Volume in anaemic rats (Group B) were decrease when compared with healthy rats (Group A). This could be due to toxicity caused by phenyl hydrazine by the involvement of aryl and hydroxyl radicals it generates. It could also be due to poor affinity of oxygen to hemoglobin molecules since the tendency of hemoglobin to bind to oxygen enhances blood flow to the tissues Ganong, (2005) [11]. In the rats treated with the lyophilized red beetroots extract, there was also a increase in these parameters when compared to the anaemic rats (Group B). This can be due to the phytochemical components in the extract as well as the presence of minerals and vitamins. These components are known hematopoietic agents that have a direct effect on blood production in the bone marrow

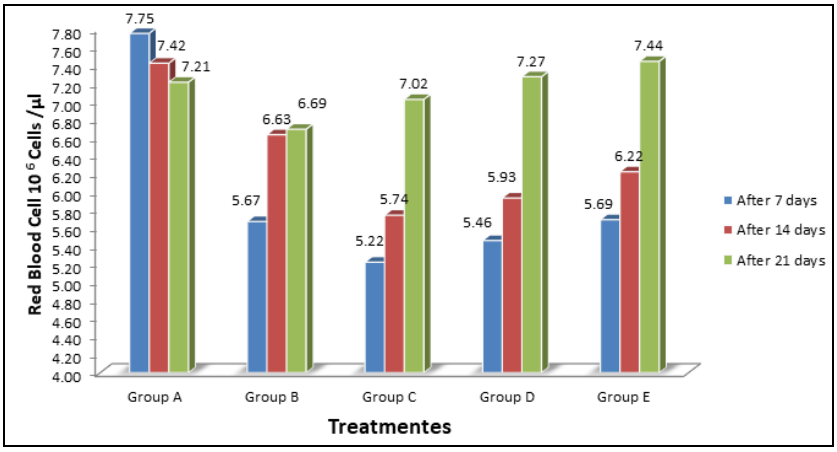

Fig 3: The effects lyophilized red beet juice on red blood cells count (RBC) of anaemic rats induced by phenyl hydrazine hydrochloride

Effect of lyophilized red beet juice on the white blood count (WBC) of anaemic rats induced by phenyl hydrazine hydrochloride during the experimental periods for three weeks is shown in Fig.4. Results of present work indicate that, the white blood cells count (WBC) were increased in anaemia rats Group B (positive control group) by 141.15, 131.4.65 and 108.68% compared to healthy rats (Group A) after 7,14 and 21 days respectively. The anaemic rats induced by phenyl hydrazine hydrochloride treated with lyophilized red beet juice at 50, 100 and 150 mg/kg of body weight were decreased in white blood count by 26.54, 35.19 and 48.07 % for (Group C), (Group D) and (Group E) after 21 days respectively compared with anaemia rats (group B). Increase of the white red cells in anaemic rats (Group B) and anaemic rats treated with lyophilized red beetroots juice compared with the healthy rats (Group A). Phenyl hydrazine was reported to induce the development of Heinz bodies on RBC membranes Bowman and Rand, (1980) [12] and Gordon-Smith, (1980) [12]. It has been also reported that phenyl hydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species Clemens *et al., (*1984) [4] Hill and Thornalley (1982) [13]. However, alkaloids and flavonoids protect cells as powerful antioxidants that prevent or repair damage to red cells due to free radicals or highly reactive oxygen types.

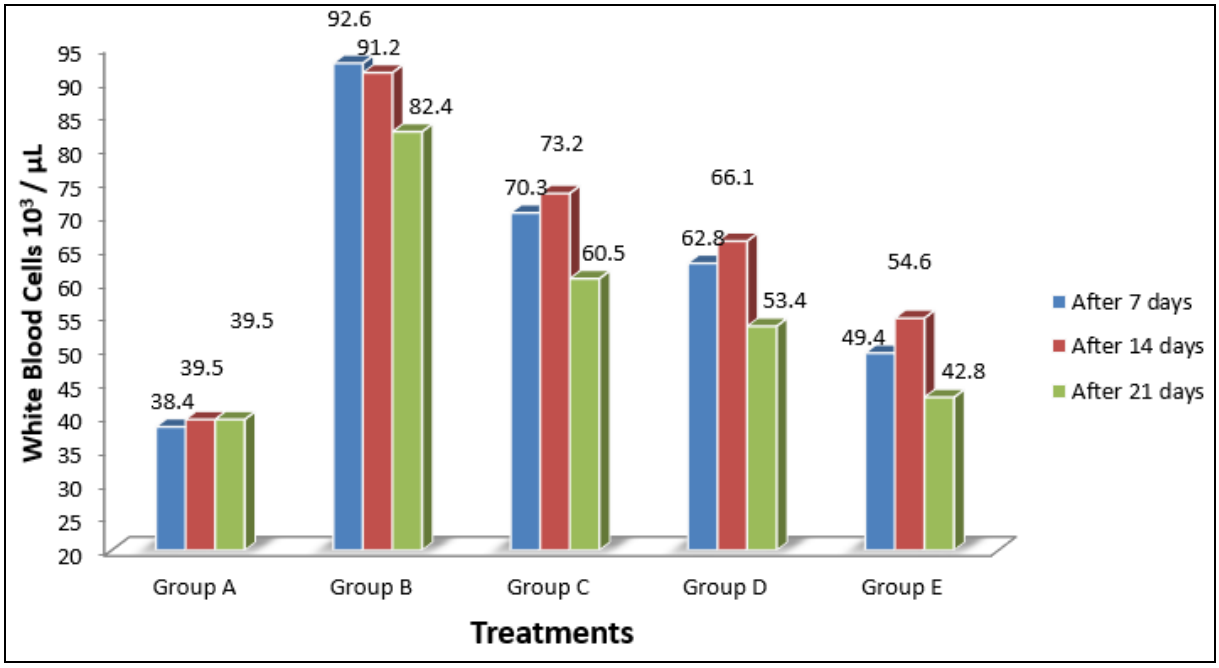

Fig 4: Effect of lyophilized red beet juice on the white blood count (WBC 10^3 Cells / µl) of anaemic rats induced by phenyl hydrazine hydrochloride

References

1. Agbor GA, Oben JE, Ngogang JY. Haematinic activity of Hibiscus cannabinus. African Journal of Biotechnology,2005:4(8):833-837.
2. Al-Zabedi EM, Kaid FA, Sady H, Al-Adhroey AH, Amran AA, Al-Maktari MT. Prevalence and risk factors of iron deficiency anaemia. American journal of health research,2014:2(5):19-326.
3. Bowman WC, Rand MJ. Textbook of Pharmacology. Blackwell Scientific publication, New York, 1980, 21-31.
4. Clemens MR, Remmer H, Waller HD. Phenyl hydrazine-induced lipid peroxidation of red blood cells: in vitro and *in vivo* monitoring by the production of volatile hydrocarbons. Biochem. Pharmacol,1984:33:1715-1718.
5. Clifford T, Howatson G, West D, Stevenson E. The Potential Benefits of Red Beetroot Supplementation in Health and Disease. *Nutrients*,2015:7:2801-2822.
6. Criswell KA, Sulkanen AP, Hochbaum AF, Bleavins MR. Effects of phenylhydrazine or phlebotomy on peripheral blood, bone marrow and erythropoietin in Wistar rats.Journal of Applied Toxicology,2000:20(1):25-34.
7. de Benoist B, McLean E, Egli I, Cogswell M. Worldwide prevalence of anaemia 1993-2005. WHO Global Database on Anemia, 2008.
8. Diallo A, Gbeassor M, Vovor A, Eklu-Gadegbeku K, Aklikokou K, Agbonon A et al. Effect of *Tectonagrandis*on phenylhydrazine induced anaemia in rats. Fitoterapia,2008:79:332-336.
9. Duncan DB. Multiple ranges and multiple F-test. *Biometrics*,1955:11:1-42.
10. Evans WC. Trease and Evans Pharmacognosy, 13th edition. Balliere Tindall, flavouring agents. Global Journal of Pure Applied. Science,1989:7(3):455-459.
11. Ganong WF. Review of Medical Physiology 22nd ed. Lange medical books/Mc Graw-Hill New York, 2005, 515-517.

12. Gordon-Smith EC. Haematological effect of drug therapy. Journal of Clinical Haematology,1980:9:572-576.
13. Hill HAO, Thornalley PJ. Free radical production during phenyl hydrazine-induced haemolysis. Can. J. Chem,1982:60:1528-1531.93.
14. Houghton PJ, Raman A. Laboratory Handbook for the Fractionation of Natural Extracts. London: Chapman and Hall, 1998, 22-52.http://dx.doi.org/10.1007/978-1-4615-5809-5.
15. Iwalewa EO, Adewumi CO, Omisore NO, Adebanji OA, Azike CK. Proantioxidant effects and cytoprotective potentials of nine edible vegetables in Southwest. Nigeria. Journal of Medical Food,2005:8:539-544.
16. Jastrebova J, Wittho¨ft C, Grahn A, Svensson U, Ja¨gerstad M. HPLC determination of folates in raw and processed beetroots. Food Chemistry,2003:80:579-588.
17. Krajka-Ku´zniak V, Szaefer H, Ignatowicz E, Adamska T, Baer-Dubowska W. Beetroot juice protectsagainst N-nitrosodiethylamine-induced liver injury in rats. Food Chem. Toxicol,2012:50:2027-2033.
18. Kujala TS, Vienola MS, Klika KD, Loponen JM, Pihlaja K. Betalains and phenolic compositions of four beetroot (Beta vulgaris) cultivars. European Food Research and Technology,2002:214:505-510.
19. Kushwaha H, Ankur J, Sapna M, Anil K. Anti-Anemic activity of Hydro-alcoholic extract of *Calotropis procera* flower on phenyl hydrazine-induced anaemic rats. International Journal of Comprehensive and Advanced Pharmacology,2017:2(1):6-10 7.
20. Lee EJ, An D, Nguyen CTT, Patil BS, Kim J, Yoo KS. Betalain and betaine composition of greenhouseor field-produced beetroot (*Beta vulgaris* L.) and inhibition of HepG2 cell proliferation. *J. Agric. Food Chem*,2014:62:1324-1331.
21. Malomo SO, Adebayo JO, Olorunniji FJ, Modulatory effect of vitamin E on some hematological parameters in dihydroartemi sinin-treated rats. Trop. J. Health Sci.2002:9:15-20.
22. Okochi YI, Okpuzor J, Alli LA. Comparison of an African herbal formula with commercially available haematinics. Afr. J. Biotechnol,2003:2(8):237-240.
23. Pathirana C, Gibney MJ, Taylor TG. Atherosclerosis,1990:36:595-599.
24. Pavlov A, Kovatcheva P, Tuneva D, Ilieva M, Bley T. Radical scavenging activity and stability of betalains from *Beta vulgaris* hairy root culture in simulated conditions of human gastrointestinal tract. Plant Foods for Human Nutrition,2005:60:43-47.
25. Rajarathinam J, abel R, asokar JS. Prevalence of anaemia among adolescent girls in rural Tamil Nadu,Indian paediatrics,2013:37:532-6.
26. Ramesh chellan, lopamudrapaul; prevalence of iron deficiency anaemia in India, journal of population and social studies, 2010, 19.
27. Riordan O, ODK, Sharp P, Sykes RM, Epstein O, Debnam ES. Response to Phenlyhydrazine -induced haemolytic anaemia. European Journal of Clinical Investment,1995:25:722.
28. Saimak TN. MD, MPH. Editor Melissa Conrad Stoppler MD, 2009.
29. Sembulingam K and Prema Sembulingam, Iron and Haemoglobin metabolism, Essentials of MedicalPhysiology, fourth edition 2007, 72.
30. Sharma S. Experiments and techniques in Biochemistry, Haematology determination Galgotia, (New Delhi), 2007, 90-100.
31. Sheila John1, Sarah Jane Monica, Priyadarshini S, Sivaraj C, Arumugam P. Antioxidant and Antibacterial Activities of *Betavulgaris* L. Peel Extracts Int J Pharma Res Health Sci,2017:5(6):1974-79.
32. Singh N, Verma P, Mishara N, Nath R. Indian J. Pharmacol,1991:21:9.
33. Sofowora A. Medicinal and Traditional Medicine in Africa, Spectrum Books Limited, Second edition, 1993, 130.
34. Straus S, Bavec F, Turinek M, Slatnar A, Rozman C, Bavec M. Nutritional value and economic feasibility of red beetroot (*Beta vulgaris L. ssp. vulgaris Rote Kugel*) from different production systems. African Journal of Agricultural Research,2012:7(42):5653-5660.
35. Vamsee Veena A Jyothi Y, Rina Pokhrel, Rajdwip Ghosh, Ronak Patidhar, Vijay Yadav. Comparative anti-anemic activity of Azadirachta indica leaves and its combination with Emblica officinalis in phenyl hydrazine induced anemia using rats Journal of Chemical and Pharmaceutical Research,2015:7(8):1019-1022.
36. Zielinska-Przyjemska M, Olejnik A DobrowolskaZachwieja A, Grajek W. In vitro effects of beetroot juice and chips on oxidative metabolism and apoptosis in neutrophils from obese individuals. Phototherapy Research,2009:23(1):49-5.