Ibrahim Card Med J 2023; 13 (1&2): 40-46

ORIGINAL ARTICLE

Effects of *Aloe Vera* Gel on Red Blood Cell-parameters in Phenylhydrazine-induced Anaemic *Wistar albino* Rats

Shameema Akhter,[1] Dilruba Begum,[2] Ishrat Jahan,[3] Aliya Hossain Sharna,[4] Sumaiya Khan,[5] Israt Azmin[6]

ABSTRACT

Background & objective: Anaemia is the most significant disorder of the blood. Many researchers have developed the concept of using medicinal plants as an alternative source of treatment for anaemia with fewer side effects. *Aloe vera*, being a medicinal plant, is popularly used as a natural drug in haematological disorders. The present study was intended to observe the effects of *Aloe vera* gel on red blood cell parameters in phenylhydrazine (PHZ)-induced anaemic *Wistar albino* rats.

Methods: This Experimental study was conducted in Dhaka Medical College, Dhaka from July 2021 to June 2022. A total of 24 healthy *Wistar albino* rats were selected for the study. After procurement, the rats were initially kept in a standard laboratory condition on a 12/12-hour light/dark cycle for 14 days of acclimatization before 21 days of the experiment. All the rats had free access to basal diet and normal saline during the period of acclimatization and experiment. After acclimatization for 14 days, the rats were divided into three groups-Group A (baseline control group, n = 8), Group B (PHZ-treated group, n = 8), and Group C, the Experimental Group (PHZ-induced and *Aloe vera* gel treated group, n = 8). To induce anaemia, Group B and Group C received intraperitoneal injection of PHZ at a dose of 0.5 ml/100 g body weight on days 1, 3, 5 and day 7. Moreover, the experimental group (Group C) received *Aloe vera* gel orally at a dose of 0.6 ml/kg body weight from Day 8 to Day 21. On day 22, rats were sacrificed to test their blood samples for haematologic variables (RBC count, Hb conc., and PCV) and haematological indices (MCV, MCH and MCHC).

Result: All the haematological parameters and indices were almost similar among the study groups at baseline (on Day 1). On Day 8, rats of groups B and C demonstrated a drastically reduced count of RBC (2.66 ± 0.53 and 2.69 ± 0.52 million/µl respectively) compared to the rats of group A (8.16 ± 0.76 million/µl). On Day 22, the RBC count in rats of Group C staggeringly increased to 7.63 ± 0.64 million/µl; however, the RBC count in rats of Group B remained almost the same as was found on Day 8. A significant decrease in Hb conc. and PCV in PHZ-induced control on Day 8 compared to that on Day 1 was also observed. The mean MCV in all the study groups on Day 1 was around 50 fl, which abnormally increased to 85 and 87 fl in Groups B and C respectively on Day 8. On Day 22, Group B further exhibited an increase in MCV to > 87 fl. Meanwhile, the MCV in Group C (*Aloe vera*-treated group) favourably decreased to 55 fl. On the 8th Day of intervention, the MCH level significantly decreased in Groups B and C. Although no significant change in MCH was observed in Group B from Day 8 to Day 22, group C demonstrated a significant increase in the mean MCH to nearly 18 pg during the same period. The mean MCHC significantly dropped to < 23% in Groups B and C on Day 8 from the baseline figure of 34%. While no significant change in MCHC was evident in Group B from Day 8 to Day 22, it significantly improved to about 28% in Group C during the same period.

Conclusion: From the study, it can be concluded that *Aloe vera* has significant erythropoietic potentials which result in the improvement of red blood cell parameters (RBC count, Hb conc., PCV, MCV, MCH and MCHC) in PHZ-induced anaemic rats.

Key words: *Aloe Vera* gel, red blood cells, phenylhydrazine, anaemia, *Wistar albino* rats etc.

Authors' information:

[1] ***Dr. Shameema Akhter,*** *MBBS, M. Phil (Physiology), Lecturer (Physiology) Dhaka Medical College, Dhaka, Bangladesh*

[2] ***Professor Dr. Dilruba Begum,*** *M. Phil (Physiology), Head, Department of Physiology, Dhaka Medical College, Dhaka*

[3] ***Dr. Ishrat Jahan,*** *MBBS, M. Phil (Physiology), Lecturer (Physiology), Bangabandhu Skeikh Mujib Medical College, Faridpur, Bangladesh.*

[4] ***Dr. Aliya Hossain Sharna,*** *MBBS, M. Phil (Physiology), Lecturer (Physiology),Shaheed Suhrawardy Medical College, Dhaka, Bangladesh.*

[5] ***Dr. Sumaiya Khan,*** *MBBS, M. Phil (Physiology), Lecturer (Physiology), Magura Medical College, Magura.*

[6] ***Dr. Israt Azmin,*** *MBBS, M. Phil(Biochemistry), Medical Officer(Biochemistry), National Institute of Cardiovascular Disease and Hospital Sher-e-Bangla Nagar, Dhaka, Bangladesh*

Correspondence: ***Dr. Shameema Akhter,*** *Phone: +8801714084960 E-mail: shah6209@gmail.com & shameema_rumee@yahoo.com*

INTRODUCTION:

Anaemia is a serious worldwide public health problem (when the prevalence of low Hb concentration exceeds 5% of the population),[1] associated with an increased risk of morbidity and mortality in most South-Asian countries like Bangladesh.[2] It is a haematological disorder, characterized by a decrease in RBC count or Hb concentration or PCV.[3] Before the advent of modern medicine, people used to depend on therapeutic plants for the treatment of ailments. *Aloe vera*, is commonly known as "Ghrit Kumari" in our country. Native to the hot and dry areas of Middle East Asia, the Southern Mediterranean, North Africa, and the Canary Islands[4], it is an inseparable part of folk medicine, for its curative and therapeutic potentials. *Aloe vera* gel is rich in active ingredients like anthraquinones, vitamins, minerals, enzymes, amino acids, natural sugars, and fatty acids. These active ingredients perhaps improve anaemia by neutralizing free radicals, synthesizing haem and globin, acting as co-enzymes, and maturation of red blood cells which ultimately stimulate erythropoiesis. However, limited studies have been done in the country related to its erythropoietic effects on animals.

The haematological analysis is considered a commonly used diagnostic tool in any disease, particularly for the diagnosis of haematological disorders. The commonly used haematological parameters are RBC, WBC, Hb concentration, PCV, and values of MCV, MCH and MCHC.[5] It gives information about the production of all blood cells and recognizes a patient's oxygen-carrying capacity through the evaluation of RBC indices, Hb concentration and haematocrit. The RBC indices estimate the size, shape, physical characteristics of the RBC and also help diagnosing the cause of anaemia.[6] The phenylhydrazine (PHZ) is termed as an ingredient of organic dye and a selective herbicide.[7] Exposure to PHZ may cause anaemia because of its powerful oxidant nature. It causes the destruction of RBCs by oxidative stress and many changes at the cellular level leading to haemolytic anaemia.[8]

In the last few decades, there has been an expanding growth in the field of herbal drugs. Several plants[8,9-16] have been found to have therapeutic and curative potentials. Several researchers observed the effects of Aloe Vera gel on hematological parameters in Wistar albino rats and other laboratory animals. Persistent consumption of Aloe vera gel increases Hb concentration, PCV, RBC count, platelet count, WBC count as well as lymphocyte count in rats.[17] Ani and associates[18] stated that Aloe vera improves some haematological parameters like RBC count, Hb concentration, PCV, MCHC in PHZ-induced anaemic rats. In India, Navathej et al[19] revealed that Fluoride induction caused a decrease in RBC count, Hb concentration, and PCV in rats. However, these levels were significantly improved in the *Aloe vera*-treated group. Furthermore, Nku et al[17] also studied that persistent consumption of *Aloe vera* gel increases Hb concentration, PCV, RBC count, platelet count, WBC count as well as lymphocyte count in rats. Though several studies have been conducted to assess the haematopoietic effects of *Aloe Vera* on *Wistar albino* rats and other animals in different countries, no published data have been found in the context of our country. The present study was therefore conducted to evaluate the effects of *Aloe Vera* gel on red blood cell parameters and indices in PHZ-induced anaemic Wistar rats.

METHODS:

This Experimental study was conducted in the Department of Physiology, Dhaka Medical College, Dhaka from July 2021 to June 2022. A total of 24 healthy *Wistar albino* rats, weighing 150-200 gm and ages ranging from 90-120 days were selected for the study. Ethical permission was taken from the Ethical Review Committee of Dhaka Medical College, Dhaka to carry out this study. The animals were purchased from the Animal Resource Facility, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), and were kept in metallic cages in the animal house of the Institute of Nutrition and Food Science, University of Dhaka. The rats were kept in a standard laboratory condition on a 12/12-hour light/dark cycle for 14 days of acclimatization before 21 days of the experiment. All the rats had free access to basal diet and normal saline during the period of acclimatization and experiment. After acclimatization for 14 days, all rats were divided into three groups Group A (baseline control group, n=8),

Group B (PHZ-treated group, n = 8), and Group C (PHZ-induced & *Aloe vera* gel-treated Experimental Group, n = 8). After grouping, the body weights of all rats were measured on Day 1, and then on Day 22. While Group A received a basal diet and normal saline (1 ml/kg body weight orally) for 21 days,[18] Group B received a basal diet for consecutive 21 days. In addition, they received PHZ (0.5 ml of PHZ was diluted with 2 ml of distilled water) intraperitoneally at a dose of 0.5 ml/100 kg body weight and remained untreated for 21 days.[18] Group C received a basal diet and PHZ as Group B received and then *Aloe vera* gel daily at a dose of 0.6 ml/kg body weight from Day 8 up to Day 21.[18]

On day 22, rats were anaesthesized by inhalation of 30% chloroform and were sacrificed. Blood samples (approximately 0.5 ml) were drawn via cardiac puncture with a sterile needle and syringe and were kept in EDTA sample test tubes with proper identification numbers for analysis. The samples were analyzed using an Automated Hematology Analyser (Sysmex XE-5000) for whole blood count. The following laboratory investigations were done in the Department of Laboratory Medicine, Dhaka Medical College Hospital, Dhaka.

Data were collected using a semi-structured questionnaire containing the key variables of interest and were processed and analyzed with the help of SPSS (Statistical Package for Social Sciences), version 25.0. While the data presented on a continuous scale were expressed as mean ± SD, the data presented on a categorical scale were presented as frequency with corresponding percentages. The test statistics used to analyze the data were ANOVA with the post-hoc Hochberg test for multiple comparisons. The level of significance was set at 5% and a p-value < 0.05 was considered significant.

RESULTS:

The mean RBC counts in Groups A, B and C at baseline (on Day 1) were quite similar (p = 0.829). On Day 8, rats of groups B and C demonstrated a drastically reduced count of RBC (2.66 ± 0.53 and 2.69 ± 0.52 million/µl respectively) compared to the rats of group A (8.16 ± 0.76 million/µl) (p < 0.001). On Day 22, the RBC count in rats of Group C staggeringly increased to 7.63 ± 0.64 million/µl; however, the RBC count in rats of Group B remained almost the same as was found on Day 8 (Table I). Multiple comparisons of RBC count by Post-hoc Hochberg test revealed that there was a significant difference between A and B groups in terms of RBC count on Day 8 and Day 22 compared to Day 1 (p < 0.001). A significant difference was also noted between groups A and C on day 8 (p < 0.001), but not on Day 22 (p = 0.353). However, no difference was found between groups B and C on Day 8, although a wide difference was evident on Day 22 (Table II). The mean haemoglobin level in all the study groups was > 13 g/dl at baseline (p = 0.829), which on Day 8 significantly dropped to < 9 g/dl in Group B and Group C, compared to Group A (p < 0.001). On day 22, the Hb level of Group C improved almost to its baseline level, although no change was noted during the same period in Group B (p < 0.001). Multiple comparisons of Hb levels among different study groups using the post hoc Hochberg test are shown in Table IV. The mean PCV in all the study groups on Day 1 was around 40%, which reduced to < 23% on Day 8 in Groups B and C (p < 0.001). While Group C demonstrated a modest increase in PCV on Day 22, Group B did not experience any further change in PCV level from Day 8 to Day 22 (p < 0.001) (Table V). Multiple intergroup comparisons using the post-hoc Hochberg test are shown in Table VI.

The mean MCV in all the study groups on Day 1 was around 50 fl, which increased to 85 and 87 fl in Groups B and C respectively on Day 8 (p < 0.001). On Day 22, Group B experienced an increase in MCV to > 87 fl, although Group C experienced a sharp decrease in MCV to 55 fl (p < 0.001) (Table VII). Multiple intergroup comparisons of MCV using the post hoc Hochberg test are shown in Table VIII. The mean MCH in all the study groups on Day 1 was around 17 pg. Following the intervention, it fell to 13 and 14 in Group B and C respectively on Day 8 (p < 0.001). While no significant change in MCH was observed in Group B from Day 8 to Day 22, group C had a significant increase in the mean MCH to 17.7 pg during the same period (p < 0.001) (Table IX). Multiple intergroup comparisons of MCH using

the post hoc Hochberg test are depicted in Table X. The mean MCHC in all the study groups at baseline was about 34%. After the intervention, it dropped to < 23% in Groups B and C on Day 8 ($p < 0.001$). While no significant change in MCHC was evident in Group B from Day 8 to Day 22, it significantly improved to about 28% in Group C during the same period ($p < 0.001$) (Table XI). Multiple intergroup comparisons of MCHC are illustrated in Table XII.

Table I. RBC Count in different groups of rats and on different days of evaluation

Group	RBC Count (million/µl)		
	Day 1	Day 8	Day 22
A (n = 8)	8.11 ± 0.71	8.16 ± 0.76	8.28 ± 0.74
B (n = 8)	8.09 ± 0.68	2.66 ± 0.53	2.69 ± 0.52
C (n = 8)	8.32 ± 0.80	2.69 ± 0.52	7.63 ± 0.64
p-value	0.829	< 0.001	< 0.001

Data were analyzed using **ANOVA statistics (F)** and were expressed as **mean ± SD.**
Group A: Normal control group; Group B: PHZ-induced anaemic control group
Group C: PHZ-induced anaemic rats treated with *Alo Vera*

Table II. Multiple comparisons of RBC count by Post-hoc Hochberg test

Group	Intergroup difference of RBC count (million/µl)		
	Level of significance (p-value)		
	Day 1	Day 8	Day 22
A vs B	1.000	<0.001[S]	<0.001[S]
A vs C	1.000	<0.001[S]	0.353
B vs C	1.000	1.000	< 0.001[S]

S = Significant

Table III. Hb conc. in different groups of rats and on different days of evaluation

Group	Hb conc. (gm/dl)		
	Day 1	Day 8	Day 22
A (n = 8)	13.95±1.14	13.75±0.94	13.93±1.10
B (n = 8)	13.75±0.94	8.41±0.73	8.51±0.73
C (n = 8)	13.68±0.92	8.51±0.73	12.05±1.15
p-value	0.860	<0.001	<0.001

Data were analyzed using **ANOVA statistics (F)** and were expressed as **mean ± SD.**
Group A: Normal control group; Group B: PHZ-induced anaemic control group
Group C: PHZ-induced anaemic rats treated with *Alo Vera*

Table IV. Multiple comparisons of Hb level by Post-hoc Hochberg test

Group	Intergroup difference of Hb conc. (million/µl)		
	Level of significance (p-value)		
	Day 1	Day 8	Day 22
A vs B	1.000	<0.001	<0.001
A vs C	1.000	<0.001	0.005
B vs C	1.000	1.000	<0.001

Table V. PCV in different groups of rats and on different days of evaluation

Group	PCV (%)		
	Day 1	Day 8	Day 22
A (n = 8)	40.74±3.70	40.65±3.83	40.40±2.75
B (n = 8)	39.55±2.68	22.46±4.41	22.98±5.08
C (n = 8)	40.22±3.91	22.98±5.08	41.80±2.71
p-value	0.879	<0.001	<0.001

Data were analyzed using **ANOVA statistics (F)** and were expressed as **mean ± SD.**
Group A: Normal control group; Group B: PHZ-induced anaemic control group
Group C: PHZ-induced anaemic rats treated with *Alo Vera*

Table VI. Multiple comparisons of PCV level by Post-hoc Hochberg test

Group	Intergroup difference of PCV (%)		
	Level of significance (p-value)		
	Day 1	Day 8	Day 22
A vs B	1.000	<0.001	<0.001
A vs C	1.000	<0.001	1.000
B vs C	1.000	1.000	<0.001

Table VII. MCV in different groups of rats and on different days of evaluation

Group	MCV (fl)		
	Day 1	Day 8	Day 22
A (n = 8)	50.14 ± 5.71	49.81 ± 5.60	48.48 ± 6.58
B (n = 8)	49.08±4.56	84.58±17.75	87.39±21.02
C (n = 8)	48.48±6.59	87.39±21.02	55.38±5.85
p-value	0.840	< 0.001	< 0.001

Data were analyzed using **ANOVA statistics (F)** and were expressed as **mean ± SD.**
Group A: Normal control group; Group B: PHZ-induced anaemic control group
Group C: PHZ-induced anaemic rats treated with *Alo Vera*

Table VIII. Multiple comparisons of MCV by Post-hoc Hochberg test

Group	Intergroup difference of MCV (fl)		
	Level of significance (p-value)		
	Day 1	Day 8	Day 22
A vs B	1.000	<0.001	<0.001
A vs C	1.000	<0.001	1.000
B vs C	1.000	1.000	<0.001

Table IX. MCH in different groups of rats and on different days of evaluation

Group	MCH (pg)		
	Day 1	Day 8	Day 22
A (n = 8)	17.39±1.48	17.05±1.13	16.90±1.13
B (n = 8)	17.05±1.14	13.66±1.41	14.06±1.61
C (n = 8)	16.70±1.13	14.06±1.61	17.70±0.95
p-value	0.730	< 0.001	< 0.001

Data were analyzed using **ANOVA statistics (F)** and were expressed as **mean ± SD.**
Group A: Normal control group; Group B: PHZ-induced anaemic control group
Group C: PHZ-induced anaemic rats treated with *Alo Vera*

ORIGINAL ARTICLE

Table X. Multiple comparisons of MCH by Post-hoc Hochberg test

Group	Intergroup difference of MCH (pg)		
	Level of significance (p-value)		
	Day 1	Day 8	Day 22
A vs B	1.000	0.001	<0.001
A vs C	1.000	0.001	1.000
B vs C	1.000	1.000	<0.001

Table XI. MCHC in different groups of rats and on different days of evaluation

Group	MCHC (%)		
	Day 1	Day 8	Day 22
A (n = 8)	33.82±3.77	33.44±3.35	33.89±3.82
B (n = 8)	33.42±3.35	22.30±4.95	22.89±5.16
C (n = 8)	33.89±3.83	22.89±5.16	27.90±2.57
p-value	0.965	< 0.001	< 0.001

Data were analyzed using **ANOVA statistics (F)** and were expressed as **mean ± SD.**
Group A: Normal control group; Group B: PHZ-induced anaemic control group
Group C: PHZ-induced anaemic rats treated with *Alo Vera*

Table XII. Multiple comparisons of MCHC by Post-hoc Hochberg test

Group	Intergroup difference of MCHC (%)		
	Level of significance (p-value)		
	Day 1	Day 8	Day 22
A vs B	1.000	< 0.001	< 0.001
A vs C	1.000	< 0.001	0.008
B vs C	1.000	1.000	0.043

DISCUSSION:

The present study attempted to observe the effects of *Aloe Vera* gel on red blood cell parameters in PHZ-induced anaemic *Wistar albino* rats. The study revealed that there was a significant difference in mean total RBC count between PHZ-induced negative control and baseline control group on Day 8 compared to Day 1. These results were in agreement with several experimental studies[14-16]. The RBC count was significantly increased in the PHZ-induced and *Aloe vera*-treated group than the PHZ-treated negative control on Day 22 compared to Day 8. These findings might be due to the presence of vitamins, and iron in *Aloe vera*. A similar finding was observed in a study conducted by Ani et al[18] and Ofem et al[20] in Nigeria. The study showed that there was a significant decrease in Hb conc. and PCV in the PHZ-treated anaemic control on Day 8 compared to that on Day 1. Ali and Eleby[21] found a significantly decreased level of Hb conc. and PCV in the anaemic control group than in the normal control group, which bears consistency with the present study. Ani et al[18] observed that these levels were also significantly increased in the PHZ-induced *Aloe vera*-treated group than in the PHZ-treated group. Navathej and associates[19] in a similar study in India observed that the *Aloe vera*-treated group had a significant increase in Hb and PCV levels.

In our study, the mean MCV in all the study groups on Day 1 was around 50 fl, which abnormally increased to 85 and 87 fl in Groups B and C respectively on Day 8. On Day 22, Group B further exhibited an increase in MCV to > 87 fl. Meanwhile, the MCV in Group C favourably decreased to 55 fl. Similar to this study, Akara et al.[9] also observed that MCV was initially significantly increased in the PHZ-treated anaemic control group than in the normal control group. However, on Day 22, MCV was significantly decreased in the PHZ-induced *Aloe vera*-treated group than in the PHZ-treated negative control group. Conversely, several studies[18,20] reported no meaningful statistical difference among different groups in terms of MCV. On the 8th Day of intervention, the MCH level significantly decreased in Groups B and C. Although no significant change in MCH was observed in Group B from Day 8 to Day 22, Group C demonstrated a significant increase in the mean MCH to nearly 18 pg during the same period indicating that *Aloe vera* helped improve the haemoglobin concentration in PHZ-treated rats. Kolawole and associates[22] showed that the MCH level was significantly decreased in the PHZ-treated only group than that in the normal control group which is consistent with the findings of the present study. Then, this level was increased on Day 22 compared to that on Day 8 in the PHZ-induced *Aloe vera*-treated group compared to PHZ-treated only anaemic rats. Consistent results were found in some studies.[23,24] Laboratory analysis of *Aloe vera* shows that the plant contains plenty of vitamins like riboflavin, thiamine, folic acid and essential amino acids required for haemoglobin synthesis.

The mean MCHC significantly dropped to < 23% in Groups B and C on Day 8 from the baseline figure of 34%. While no significant change in MCHC was evident in Group B from Day 8 to Day 22, it

significantly improved to about 28% in Group C during the same period indicating that the PHZ-induced anaemic rats responded well to the *Aloe vera* treatment. Increased MCHC showed that immature red cells were present in circulation which was the indication of initial stimulation of the haematopoietic system by *Aloe vera*. Similar results were also observed in some investigations.[25,26]

CONCLUSION:

This study revealed that all the haematological parameters like RBC, Hb and PCV and haematological indices like MCV, MCH and MCHC in the phenylhydrazine (PHZ)-induced rats become abnormal, which are reversed back to normal following 2 weeks of treatment with aloe vera. The haemopoietic activities observed in the PHZ-induced rats could be due to the presence of rich antioxidants, vitamins, and iron as well as other important constituents in *Aloe vera*. As this was an animal model study, an experiment on human subjects is recommended before its application on human beings as part of the treatment of anaemia.

REFERENCES:

1. World Health Organization (WHO) 2006. Guidelines on Food Fortification with Micronutrients. Available at: https://www.who.int/publications/i/item/9241594012
2. Rahman MA, Rahman MS, Aziz Rahman M, Szymlek-Gay EA, Uddin R, et al. Prevalence of and factors associated with anaemia in women of reproductive age in Bangladesh, Maldives and Nepal: Evidence from nationally-representative survey data. *PLOS ONE* 2021;16(1): e0245335. https://doi.org/10.1371/journal.pone.0245335
3. Turner J, Parsi M, Badireddy M. Anemia. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499994/
4. Sanchez M, Gonzalez-Burgos E, Iglesias I, Gomez-Serranillos MP. Pharmacological Update Properties of Aloe Vera and its Major Active Constituents. *Molecules* 2020;25(6):1324.
5. Etim NN, Enyenihi GE, William ME, Udo MD. Haematological parameters: indicators of the physiological status of farm animal. *British Journal of Science* 2013;10(1):33-45. Available at: http:// ajournal. co.uk/pdfs/BSvolume10 (1)/BSVol.10%20(1)%20Article%204.pdf
6. Weatherspoon D. RBC Indices. Healthline 2017. Available at:https://www.healthline.com/health/rbc-indices. (Accessed:23 February 2022).
7. Bystricky L, Vesela Z, Sohler E, A Study of the side-reaction products of phenylhydrazine production by gas chromatography-mass spectrometry. *Journal of Chromatography* 1988,447(1):202-207.
8. Meena Ak, Patidar D, Singh RK. Ameliorative Effect of Hibiscus rosa sinensis on Phenylhydrazine - Induced Haematotoxicity. *International Journal of Innovative Research in Science, Engineering and Technology* 2014;3(2):8678-8683.
9. Akara EU, Emmanuel O, Ude VC, Uche-Ikonne C, Eke G, Ugbogu EA. Ocimum gratissimum leaf extract ameliorates phenylhydrazine -induced anaemia and toxicity in Wistar rats. DE GRUYTER 2021;36(4): 311-320. Available at: https://www.degruyter.com/document/doi/10.1515/dmdi-2020-0185/html (Accessed: 08 March 2022).
10. Umoren EB, Kolawole TA, Wopara I, Adebayo OG, Ben-Azu B, Uzokwe JI, Obembe AO. Hibiscus sabdariffa Aqueous Leaf Extract Reverses Haematological Alterations in Phenylhydrazine Anemic Wistar Rats. *International Journal of Biochemistry Research & Review* 2020;29(4):30-38.
11. Ezeigwe OC, Nzekwe FA, Nworji OF, Ezennaya CF,Lloanya EL, Asogwa KK. Effect of Aqueous Extract of F. capensis Leaves and Its Combination with C. acontifolius Leaves on Essential Biochemical Parameters of Phenylhydrazine - Induced Anemic Rats. *Journal of Experimental Pharmacology* 2020;12(1): 191-201.
12. Kutshik RJ, Jiyil MK, Mafuyai CE, Ichor LL. Anti - Anemic Effect of Cnidoscolus Chayamansa (Mc Vaugh) Leaf Extract on Phenylhydrazine- Treated Rats. *Journal of Advances in Biology & Biotechnology* 2020;23(11): 23-32.
13. Ekweogu CN, Ude VC, Nwankpa P, Emmanuel O, Ugbogu EA. Ameliorative Effects of aqueous Leaf Extract of Solanum aethiopicum on phenylhydrazine - Induced anaemia and toxicity in rats. Toxicological Research 2019. Available at: https://doi.org/10.1007/ s43188-019-00021-5.(Accessed: 10 March 2022).
14. Pandey S, Ganeshpurkar A, Bansal D, Dubey N. Hematopoietic Effect of Amaranthus cruentus Extract on Phenylhydrazine - Induced Toxicity in Rats. *Journal of Dietary Supplements* 2016;13(6):607-615. Available at: https://doi.org/10.3109/19390211.2016.1155685. (Accessed: 25 February 2022).
15. Chauhan SP, Sheth NR, Suhagia BN. Hematinic effect of fruits of Opuntia elatior Mill. on phenylhydrazine- induced anemia in rats. *Pharmacological Study* 2015;36(2): 208-213.

16. Agustin MKA, Balines MC, Bitong KCC, Godoy JLL, Dumaoal OSR, Cabanela RA. Effect of Diplaziumesculentum (Retz) Sw (Paco) Extract against Phenylhydrazine - Induced Anemia on Sprague Dawley Rats, *The Steth* 2014;8(1):80-108.

17. Nku CO, Ofem OE, Okon UA. Effect of prolong consumption of crude Aloe barbedensis (Aloe vera) gel on haematological indices in rats. *Asian journal of medical sciences* 2014;6(2):57-61.

18. Ani EJ, Ime AU, Udefa AL, Okon UE. The Role of Aloe vera on haematological parameters following phenylhydrazine (PHZ) - induced anaemia in Wistar rats. *European Journal of Pharmaceutical and Medical Research* 2018;5(6):140-143.

19. Navathej A, Sujatha K, Adhlilakshamamma k. Haematobiochemical and antioxidant evaluation of Aloe vera whole leaf extract on Fluoride induced toxicity in Wistar albino rats. *Symbiosis* 2016;2(1):1-5.

20. Ofem OE, Ani EJ, Archibong AN. Effect of masfon aloe vera gel on some blood parameters in high salt loaded rats. *Scholars research library* 2015;7(8):26-34.

21. Ali JB, Elaby SM. The anti-anemic effect of dried beet green in phenylhydrazine treated rats. Archieves of Pharmaceutical *Sciences Ain shams University* 2018; 2(2):54-69.

22. Kolawole TA, Oluwatayu BO, Umoren EB, Iiochi ON, Dapper DV, Igbokwe UV. Hematinic Effect of Locust Bean (Parkia biglobosa) Seeds on Phenylhydrazine-Induced Anaemia in Rats. *Journal of African Association of Physiological Sciences* 2021;9(1).

23. Iji OT, Oyagbemi AA, Azeez OI. Assessment of chronic administration of Aloe vera gel on haematology, plasma biochemistry, lipid profiles and erythrocyte osmotic resistance in Wistar rats. *Physiological Society of Nigeria* 2010;1(1):107-113.

24. Channa AA, Qazi IH, Soomro SA, Shah AH, Jameel AG, Korejo RA, Shah IA, Kalhoro NA, Khaskeli B. Effects of oral supplementation of Aloe vera extract on haemalogy indices and immune cells of blood in rabbit. *African Journal of Pharmacy and Pharmacology* 2014; 8(19):497-501.

25. Ekande B, Oripuda OA, Oyeyemi MO. Assessment of the safety of aqueous extract Aloe vera on haematology of Wistar rats. *African journal of Biotechnology* 2015; 14(30):2395-2399.

26. Bansode FW, Ayra KR, Meena AK, Singh RK. Haematinic and Anti-Anemic Effects of the Methanol Extract of Saraca indica stem bark against Phenylhydrazine –induced Hemolytic Anemia in Rats. *World Journal of Pharmaceutical Resaerch* 2019;8(9):1176-1201.