

THE ROLE OF ALOE VERA ON HAEMATOLOGICAL PARAMETERS FOLLOWING PHENYLHYDRAZINE (PHZ)-INDUCED ANAEMIA IN WISTAR RATSElemi J. Ani^{*1}, Akaninyene U. Ime², Augustine L. Udefa³ and Udemeobong E. Okon⁴¹⁻⁴Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria.***Corresponding Author: Elemi J. Ani**

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ABSTRACT

The effect of Aloe vera on some haematological indices in phenylhydrazine-induced anaemic rats was studied. Fifteen female wistar rats weighing 180-220grams were used for this study. They were randomly divided into three groups (n=5) thus: Group A: Control; Group B: Induced with anaemia using PHZ at a dose of 0.5ml/100g body weight on days 1, 3, 5 and 7. Group C received the same treatment as B and additionally treated daily with aloe vera gel administered orally at a dose of 0.6ml/kg b.w., one week after induction of anaemia. All groups received normal rat chow and water *ad libitum*. The feeding and treatment lasted for 28days and thereafter, the rats were subjected to sacrifice following standard methods and blood collected via cardiac puncture into EDTA sample bottles for analysis. Aloe vera treated group showed a significant increase in the mean food and water intake compared to B. A marked reduction in body weight was seen in group C compared to control while aloe vera treated group showed a significant increase in body weight compared to group B. A marked decrease in Hb, RBC count was seen in group B compared to control. Conversely, Aloe vera treated group had a significant increase in Hb and RBC count when compared to group B. Total white cell count was significantly increased in group B compared to control and Aloe vera treated groups. This trend was also observed for platelet count. From these results, Aloe vera treatment showed potent ameliorative effect following anaemia.

KEYWORDS: Anaemia; Phenylhydrazine; Aloe vera; Red blood cell; white blood cell; platelet.**INTRODUCTION**

Before the advent of modern Medicine for the treatment and management of ailments, man relied on therapeutic plants. Medicinal plants still provide effective health protection for a large percentage of humans mostly in the developing world.^[1, 2] Up till date, it is estimated that about 80% of human population still depend on these natural remedies.^[3] Among them, aloe vera (*Aloe barbadensis*) has captured great interest due to its curative and therapeutic effects. About 75 bioactive compounds comprising of primary and secondary metabolites with several health benefits have been found in this plant.^[4, 3] Aloe vera has been reported to ameliorate several adverse effects associated with the chronic consumption of thermoxidized palm oil.^[5, 6] *Aloe barbadensis* has been reported to increase antioxidant enzymes^[7], potent in wound healing.^[8] Ani *et al.*^[9] reported that in rats fed thermoxidized palm oil diet with consequent derangements in some haematological parameters, treatment of these rats with aloe vera gel ameliorated this effect. Aloe vera extract has been reported to modulate and stimulate proliferation of murine pluripotent haematopoietic stem cells.^[10]

Anaemia is a blood disorder characterised by a decrease in red blood cell count, haemoglobin concentration, packed cell volume and other related indices. It results from multiple causative factors and has been one of the leading causes of death globally.^[11] Conventional drugs employed in the treatment of this blood disorder are usually expensive and some may have side effects. Following the reported efficacy of Aloe vera in ameliorating haematologically related derangements^[9], improvement in some haematological parameters^[12] amongst others, there is need to investigate if this plant, which is readily available and affordable, can reverse phenylhydrazine-induced anaemia.

MATERIALS AND METHODS**Experimental Animals/Protocol**

Fifteen (15) female wistar rats weighing 180-220grams were used for this study. They were obtained from the animal house of the Department of Pharmacology, University of Calabar, Calabar. This was done after permission was granted by the Ethical Committee of the Faculty of Basic Medical Sciences, University of Calabar, for the use of experimental animals for scientific purposes with approval number 02/18. They

were kept in clean cages in the animal house of the Department of Physiology throughout the period of the experiment. They were randomly divided into three groups (n=5), Groups A, B and C. Group A (Control) were allowed free access to normal rat chow and water *ad libitum*. Group B (anaemic control) induced with anaemia and untreated. Group C (test group) was also induced with anaemia but treated daily with aloe vera gel administered orally at a dose of 0.6ml/kg b.w. from the 8th day (that is one week from the first day of induction of anaemia). The feeding and treatment lasted for 28days.

Induction of Anaemia

Anaemia was induced using PHZ diluted with distilled water (at 0.5ml of PHZ to 2ml of distilled H₂O) and intraperitoneally administered at a dose of 0.5ml/100g body weight on days 1, 3, 5, 7 and thereafter remain untreated till day 28th.

The value used for aloe vera gel (0.6ml/kg b.w) was derived from LD50 study by Ime *et al.*^[5, 6] while 0.5ml/100g bw for PHZ was in line with a study by Ani *et al.*^[9]

Preparation of Aloe vera gel

Aloe Vera plants were bought from botanical garden, Calabar. The plant was authenticated by a Botanist in the Department of Botany, University of Calabar. Following the method previously used by Ani *et al.*^[13], Aloe vera leaves were plucked from the nodes and thoroughly washed with clean water. With the aid of a knife, the leaves were cut open from the base and sliced along the margins to reveal the transparent mucilage. The transparent mucilage was carefully scooped into a beaker with the aid of a spatula. The gel was homogenized and further processed using an electric blender for 20minutes. The homogenized juice was left for 20minutes and afterwards filtered using Whatman filter paper to obtain a particulate-free gel. This extract was preserved in a refrigerator in the research Laboratory of the Department of Physiology, University of Calabar, after each day's use.

Collection of Blood Sample and Analysis

After 28 days of the experimental protocol, the animals were allowed an overnight fast and in the morning were anaesthetized with chloroform by inhalation. Anaesthetized animals were immediately cut along the linea alba to the thorax to expose the heart from which blood was collected via cardiac puncture with sterile needle and syringe and then transferred into EDTA sample bottles and kept for analysis. Blood sample was analysed using Automated Blood Cell Analyser (Model PCE 210, Japan) for whole blood count.

Measurement of food, water intake and body weight

Food and water intake was measured daily by subtracting the food and water left every morning from that administered the previous day. Body weight was

measured at two days intervals with the aid of animal weighing balance.

Statistical Analysis

The results are presented as mean \pm SEM. The data was analysed using one-way analysis of variance (ANOVA), followed by the post hoc multiple comparison test (LSD). Values of $p < 0.05$ were considered significant. Computer software SPSS (version 17.0) and excel analyser (Microsoft office, 2010 version) were used for the analysis.

RESULTS

Comparison of mean food/water intake and body weight change in the different groups

As seen in table 1, there was a significant decrease in food intake in PHZ-induced anaemic untreated group (Group B) when compared to Control and Aloe vera treated groups. Conversely, water intake was significantly increased in anaemic untreated group than control and aloe vera treated group. Body weight change was greatly and significantly reduced in anaemic untreated group when compared to control and Aloe vera treated group.

Comparison of Mean values of Haematological Parameters in the different groups.

The result of red blood cell count as seen in table 1 revealed a significant decrease in RBC count of anaemic untreated group compared to control. However, aloe vera treated group greatly reversed this effect to values that were not significantly different from control.

Haemoglobin concentration was significantly reduced in anaemic untreated group but was significantly increased following treatment with aloe vera. The results for packed cell volume (PCV) and mean corpuscular haemoglobin concentration (MCHC) followed the same trend as seen for Hb concentration. However, no significant difference was seen across the groups for mean corpuscular volume.

There was a marked significant increase in white blood cell count of anaemic untreated group when compared to control and aloe vera treated group. The same trend was presented for platelet count.

Table: Food/water intake, weight changes and haematological parameters in the different groups

VARIABLES	GROUPS		
	Control (A)	Negative Control (B)	Test (C)
Food intake (g)	107± 10.09	78.34 ± 9.10*	101.98 ± 6.68 ^a
Water intake (ml)	70.93 ± 9.66	120.07 ± 5.23*	73.44 ± 8.57 ^a
Body weight change (g)	215.65 ± 3.70	155.35 ± 6.45*	202.65 ± 6.92 ^a
RBC count (x1million cels/µl)	8.35 ± 0.13	2.78 ± 0.04*	7.12 ± 0.16 ^a
Hb conc. (g/dl)	17.70 ± 1.20	11.23 ± 0.70*	17.24 ± 1.60 ^a
PCV (%)	47.04 ± 0.45	32.21± 0.76*	44.21± 1.02 ^a
MCV (fL)	89.68± 2.00	82.20 ± 4.50	87.88 ± 3.20
MCHC (%)	29.68 ± 0.49	19.22 ± 0.65*	27.58 ± 0.94 ^a
Platelet Count (x10 ⁹ /l)	393.6±7.80	606.7±22.1*	433.7±8.31 ^a
WBC (x1000 cells/µl)	6.62 ± 1.11	9.82 ± 0.52*	7.34 ± 0.23 ^a

Values are Mean±SEM, *= VS control (At P<0.01), a=VS Group B (At P<0.01)

DISCUSSION

Water intake was significantly increased in anaemic untreated group when compared to control and aloe vera treated group. Anaemia is known to result in hypoxia thereby leading to generation of oxygen derived free radicals.^[14, 15, 16] More so, Anaemia and consequent hypoxia gives rise to anaerobic respiration which results in generation of lactic acid and acidosis. Increased ROS and acidosis with resultant stimulation of the thirst center may explain the increased water intake seen in anaemic untreated group. On the other hand, food intake was markedly reduced in anaemic untreated group when compared to control and aloe vera group. However, there was a great improvement in food intake following aloe vera treatment. The mechanism of this effect by aloe vera is not very clear. However, Aloe vera is known to be very rich in antioxidants necessary for sound health and hence, good appetite. Similarly, body weight change followed the same trend as food intake. This result is in contrast with the work carried out by Nna *et al.*^[17] who reported a decrease in food intake by Aloe vera following its use in treatment of Streptozocin-induced diabetes mellitus rats. Obviously, the increase in food intake seen in aloe treated group may be responsible for the increase in body weight change seen in aloe vera treated group.

Except mean corpuscular volume which showed no significant difference across the groups, RBC, PCV, MCHC and Haemoglobin concentration were significantly decreased in anaemic untreated group compared to control and aloe vera treated group. This decrease caused by PHZ is consistent with several works that reported a decrease in some haematological indices in PHZ-induced acute anaemia.^[18, 19, 20, 21, 22] Conversely, Aloe vera treatment following PHZ-induced anaemia markedly ameliorated these effects. These results may be due to the presence of vitamins, iron and other active ingredients found in aloe^[23] which acts to improve the haemopoietic process.

White blood cell count was significantly increased in PHZ-induced anaemic untreated group when compared to control and aloe vera treated group. The reason for this

increase is not clearly understood but could be an immune response to cellular injury common in anaemia.

Platelets are produced in the bone marrow by cytoplasmic fragmentation of megakaryocytes which arise by a process of cell differentiation from the haemopoietic stem cell.^[24] Aloe vera has been seen in this study to maintain platelet count not significantly different from control despite an increase in anaemic untreated group. This result is consistent with a study by Ime *et al.*^[6] who reported that aloe vera treatment reversed effect in platelet increase caused by thermoxidized palm oil. More so, it is likely that PHZ may negatively affect the haemostatic process thereby causing an increase in platelet count.

CONCLUSION

From these results, Aloe vera treatment showed potent ameliorative effects in some haematological parameters (RBC, Hb, PCV, MCHC) following PHZ-induced anaemia in rats. Additionally, aloe vera improves food intake and body weight in PHZ-induced anaemia in rats. These effects could be attributable to the rich antioxidants, vitamins, iron as well as other important constituents present in aloe vera and their resultant role in haemopoietic process. Therefore, if this study is to be applicable to humans, the use of aloe vera in the treatment or management of anaemia should be encouraged. More so, research should be encouraged on revealing the possible mechanism of action in this regard.

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AUTHORS CONTRIBUTION

This study was carried out by four authors. EJA designed the study and did the laboratory work with the assistance of AUI, ALU and UEO. AUI carried out the analysis and interpretation of the data and also wrote the initial draft which was corrected by EJA. The final manuscript was read and approved by all authors.

CONFLICTS OF INTEREST

The Authors declare that there is no conflict of interest regarding the publication of this article

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