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Physiological Study of Rats Inducing Anemia

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ABSTRACT

Anaemia is a common clinical haematological syndrome, which is characterized by a reduction of haemoglobin concentration and erythrocytes in blood and reduced oxygen delivery to tissue. A haemolytic anaemia is a condition that is characterized by a shortened life span of the erythrocyte and can be acute or chronic in form their acute form. Haemolytic anaemias can be life-threatening and thus requires rapid diagnosis and intervention. Iron deficiency (ID) and iron deficiency anaemia (IDA) are Iron disorders the most common worldwide. When iron requirements are higher than those of the amounts of its absorption, balance of negative iron and low iron storage occurs. This study was designed to induce haemolytic and blood loss anaemia in Rattus Rattus rats. Phenylhydrazine was used to produce a model of haemolytic anaemia and repeated bleeding provided a model of blood loss anaemia. In this experiment a total of forty four adult male and female rats were used. They are classified into three groups comprising of an equal number of animals of males and females during the period from September, 2015 to November, 2016. The results indicate a significant decrease ($P < 0.05$) in the body weight in the anemic group, while there were significant increases in the weights of spleen only in the group of haemolytic anaemia. The results also show a significant reduction ($p<0.05$) in number of RBCs, concentration HGB, HCT and MCHC while significant increase is observed in (MCV) and MCH of haemolytic and blood loss anaemia when compared with the normal control group. The results of the study also reveals significant decrease ($p<0.05$) in HPX level and significant increase ($p<0.05$) in lactoferrin level in PHZ (haemolytic) group whereas they demonstrate a significant elevated ($p<0.05$) in HPX level and significant reduction ($p<0.05$) in lactoferrin level in the blood loss anaemia group.

Keywords: hemopexin, Lactoferrin, PHZ, haemolytic, Blood loss.

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INTRODUCTION

Anaemia is a familiar disorder of blood common occurrence in all age and even though people are at the largest risk they are elderly and young women in childbearing stage. Most often this disorder is not a separate disease but it advanced from numerous diseases[1].It is a hematological condition described by the reduction in the haemoglobin concentration and the number of red blood cells or their oxygen-carrying capacity is insufficient[2].

Hemopexin (Hpx) is a glycoprotein produced mainly by the liver and released into plasma, can be recognized having two useful consequences, It saves heme iron when it back to the liver to prevent lost due to excretion of urine. As a result of having heme binding property, it acts to decrease free heme to avoid oxidative stress [3-4-5-6]. During intravascular haemolysis conditions, free heme liberate into the circulation, Hpx binds free heme to form Hpx–heme complex then transported to the liver via a heme-Hpx-specific receptor [7-6] .

Lactoferrin (LF), a protein binding of iron belonging to the family of transferrin, can bind and transfer two Fe3+ ions per molecule with two times higher affinity than serum transferrin [8,9,10,11,12,13]. It is playing a key role in in the host's first line of defense, it responds to a diversity of physiological and environmental variations [14]. Structural characteristics of Lf made it have other activities in addition to its importance in the Fe3+ homeostasis familiar to all transferrins: works as a strong antimicrobial against a wide spectrum of microbial, anti-inflammatory, anticarcinogenic and important in several enzymatic functions [15-16-14-12].

Daily billions of new blood cells of the human body are produced to compensate for the lost blood cells in normal cell turnover operations, as well as with respect to disease. A response happens quickly to a variety changes such as bleeding or infection by different homeostatic mechanisms to yield new cells and return to the natural levels when removing causes. Hematopoiesis is an orchestrated process to produce blood cells and occurrence homeostasis; a few number of hematopoietic stem cells (HSCs) and progenitors responsible for the production of mature blood [17-18]. In general two types of blood-forming stem cells or HSCs are distinguishable: the long-term HSCs (LT-HSCs), which have the ability to proliferate and self-renew during the entire lifetime of the organism, and a short-term subset HSCs (ST-HSCs) which show higher proliferation rates but only for a limited time, possibly a few months. HSCs lead to self-renewing progenitors is slightly ratios, which in turn lead to offspring that are more restrictive in the possibility of their own trade-offs, and the cells eventually mature and functionally [19].

The most important pathophysiological processes associated with haemolysis are acute tubular necrosis and extensive deposition of hemosiderin [20] as well as splenomegaly due to excessive eryptosis, eryptosis physiological mechanism eliminating defective erythrocytes is triggered by haemolytic anaemia[21].

MATERIALS AND METHODS

Experimental animals

Healthy adult Rattus Rattus rats weighing among 300-200 gm were used in this experiment. The rats were housed in the separated plastic cages at the Faculty of science/ University of Kufa/ Iraq, and kept in a controlled environment of 22-25 °C.

The animals were fed with commercial rodent (pellet) diet and tap water was provided to these animals during the entire period of experimentation. Rats were allowed to acclimatize for at least one week prior to experimentation. None of the rats had any clinically evident infections.

Experimental protocol

The study protocol was approved by the ethical committee of the Department of Biology- Faculty of Science - University of Kufa. A total of forty four adult from male and female rats were used in this experiment. They are classified into three groups comprising of an equal number of males and females. For the induction of the haemolytic anaemia model, Phenylhydrazine was dissolved in the saline and were injected intraperitoneally. The concentration of PHZ (40 mg/kg body weight), 480µL of PHZ were dissolved and

complemented the volume to 60 ml of normal saline. These concentrations of PHZ were determined according to (Singbrant et al., 2011). The concentrations of PHZ were calculated according to the body weight of the animals on the week before the injection. The control group received only the same volume of saline.

The dose of PHZ for each animal was placed into a medical insulin syringe that was injection under the peritoneal. Animals permitted free access to food and water immediately after ingestion.

Anaemia was induced in the blood loss group by the repeated blood sampling for a period of one month; approximately 400 µL of blood were withdrawn from the tail vein of the rats every other day for a month (Moreau et al., 2012).

Briefly, the administration of animals was classified into the following 3 groups:-

Group I: Served as the control group.
Group II: PHZ group provided a model of the haemolytic anaemia and was administrating 1 ml of Phenylhydrazine injects every other day for 18 days (an average of nine injections for 48 hours intervals).
Group III: Blood loss group, about 400 µL of peripheral blood pulled from the rats of this group.

Collection of samples

At the end of the experimental period of the PHZ group (18 days) and Blood loss group (For a month), all rats in each group were weighed. They were anesthetized by using a mixture of ketamine and xylazine i.m., and then they were sacrificed (Schiller and McNamara, 1999). The recording of body weight of each animal was done initially and at the end of the experiment.

The hemato-biomarker analysis was performed on the blood obtained from the experimental and control rats. The blood sample was obtained from the animals through the heart puncture by using a 5 ml disposable medical syringe.

The first sample of about one milliliter of fresh blood from each animal was immediately collected into a tube containing ethylene diamine tetraacetic acid (EDTA) anti coagulant and used for the assessment of the erythrocyte indices. These parameters quantified the standard hemastological measurement using an atonamic hematological analyzer. The second sample of blood was placed into Gel tubes without anticoagulant and left at the room temperature for 30 minutes for clotting then centrifuged at 3000 rpm for 10 minutes for a biomarker assessment. The blood serum was separated, transferred into Eppendorf tubes, these samples were kept in a refrigerator at -20 °C until the time of analysis.

Hematological assessment

For the estimation of erythrocytes RBC count, the haemoglobin (HGB) concentration, hematocrit (HCT) percentage, Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Corpuscular Haemoglobin and concentration (MCHC) were analyzed on a Hematologic analyzer (Sysmex).

Biomarkers assessment

After the centrifugation process of blood and then its corresponding serum were obtained and used for measurement sera concentration of total hemopexin and lactoferrin by the ELIZA (Enzyme-linked immunosorbent assay methods).This assessment employs a quantitative sandwich enzyme immunoassay technique, and is performed by Automated microtiter plate ELISA reader (HumanHsReader, HumanHswasher, Germany).

Serum haemopexin, lactoferrin estimation

This assay is executed with specific kit for the test, supplied by (Kono Biotech Co., Ltd. China).

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RESULTS AND DISCUSSION

Result of Haematological indices in anaemia and control group:

Result of Hematological indices in PHZ (haemolytic anaemia) group and control group:

The results in figure (4-1) indicated highly significant decrease ($p< 0.05$) of the hemoglobin concentration (HGB) **(7.363±0.7268 g/dL)**, number of red blood cells (RBCs) **(2.764±0.2855 10^6/uL)** and mean cell hemoglobin concentration (MCHC) **(22.34±0.5078 g/dL)** in comparison with the control group (HGB) **(11.8±0.0378 g/dL)** RBCs **(6.835±0.0926 10^6/uL)** and (MCHC) **(31.6±0.2268 g/dL)** respectively. While observed highly significant increase ($p< 0.05$) in the mean cell volume (MCV) **(120.6±3.579 fL)** and mean cell hemoglobin (MCH) **(26.79±0.4373 pg)** when compared to the control group MCV **(54.65±0.3213 fL)** and MCH (17.3±0.2268 pg) respectively. Slight decrease but not significant was showen in hematocrit (HCT) **(32.91±3.265 %)** when compared to the control group (HCT) **(37.35±0.2835 %).**

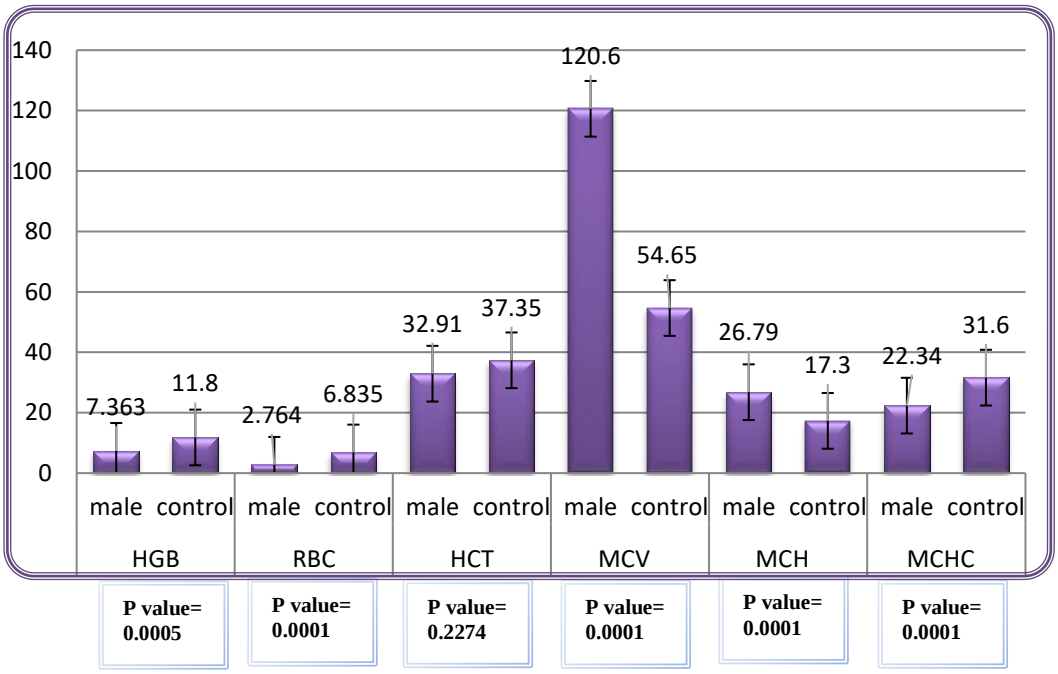

Figure (1): Illustrated Haematological indices between male PHZ (haemolytic anaemia) groups with control group.

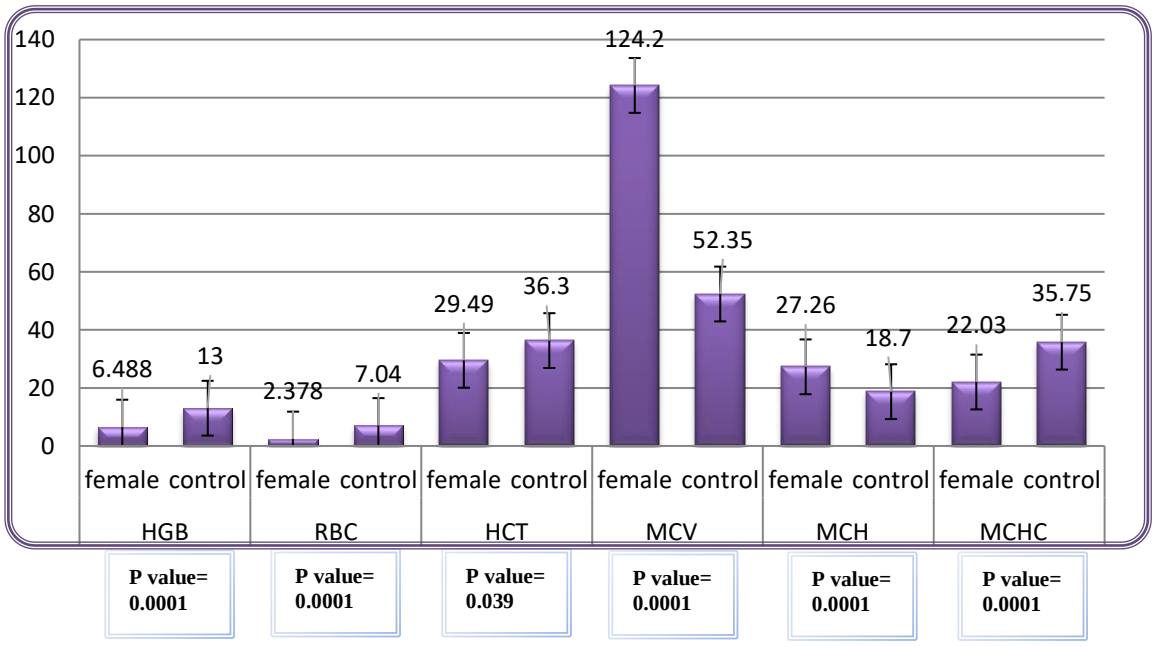

Figure (2): Illustrated Haematological indices between female PHZ (haemolytic anaemia) groups with control group.

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The results as shown in figure (4-2) revealed highly significant decrease (p< 0.05) of the haemoglobin concentration (HGB) **(6.488±0.5576 g/dL)**, number of red blood cells (RBCs) **(2.378±0.2117 106/uL)**, hematocrit (HCT) **(29.49±2.668 %)** and the mean cell haemoglobin concentration (MHCH) **(22.03±0.4507 g/dL)** in comparison with the control group (HGB) **(13±0.07559 g/dL)** RBCs **(7.04±0.06047 106/uL)**, (HCT) **(36.3±0.2268 %)** and (MHCH) **(35.75±0.0189 g/dL)** respectively .

A significant increase (p< 0.05) was observed in the mean cell volume (MCV) **(124.2±2.832 fL)** and mean cell haemoglobin (MCH) **(27.26±0.4379 pg)** when compared with the control group MCV **(52.35±0.1323 fL)** and MCH **(18.7±0.0378 pg)** respectively.

Result of Haematological indices in Blood loss anaemia group and control group:

The results in figure (4-3) indicate highly significant reduction (p< 0.05) of the haemoglobin concentration (HGB) **(6.834±0.2878g/dL)**, number of red blood cells (RBCs) **(3.915±0.1499 106/uL)** and hematocrit value (HCT) (**22.86±0.3877 %)** in comparison with the control group (HGB) **(11.8±0.0378 g/dL)** RBCs **(6.835±0.0926 106/uL)** and (HCT) **(37.35±0.2835 %)** respectively . While observed a significant increase (p< 0.05) in the mean cell volume (MCV) **(58.79±1.274 fL)** and mean cell haemoglobin (MCH) **(17.44±0.3111 pg**) in comparison with the control group (MCV) **(54.65±0.3213 fL)** and (MCH) **(17.3±0.2268 pg)** respectively. These results show no significance in the mean cell haemoglobin concentration (MHCH) **(29.81±0.7943 g/dL)** when compared with the control group (MCHC) **(31.6±0.2268 g/dL).**

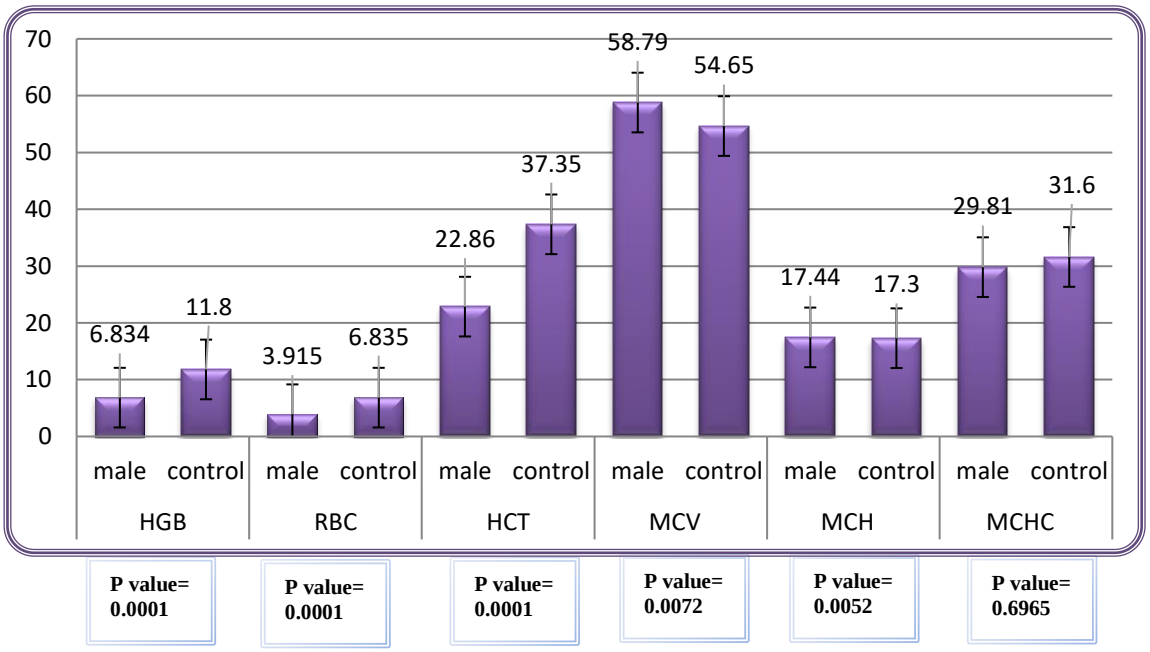

Figure (3): Illustrated Haematological indices between male Blood loss anaemia groups with control group.

The results in figure (4-4) demonstrated a significant reduction (p< 0.05) of the haemoglobin concentration (HGB) **(7.684±0.2655 g/dL)**, number of red blood cells (RBCs) **(4.025±0.1504 106/uL)**, hematocrit (HCT) **(24.43±0.6595 %)** and mean cell haemoglobin concentration (MHCH) **(31.43±0.3016g/dL)** in comparison with the control group (HGB) **(13±0.07559 g/dL)**, RBCs **(7.04±0.06047 106/uL)**, (HCT) **(36.3±0.2268 %)** and (MHCH) **(35.75±0.0189 g/dL)** respectively.

A significant increase (p< 0.05) was observed in the mean cell volume (MCV) **(60.94±1.484 fL)** and mean cell haemoglobin (MCH) **(19.11±0.4604 pg)** in comparison to the control group MCV **(52.35±0.1323 fL)** and MCH **(18.7±0.0378 pg)** respectively.

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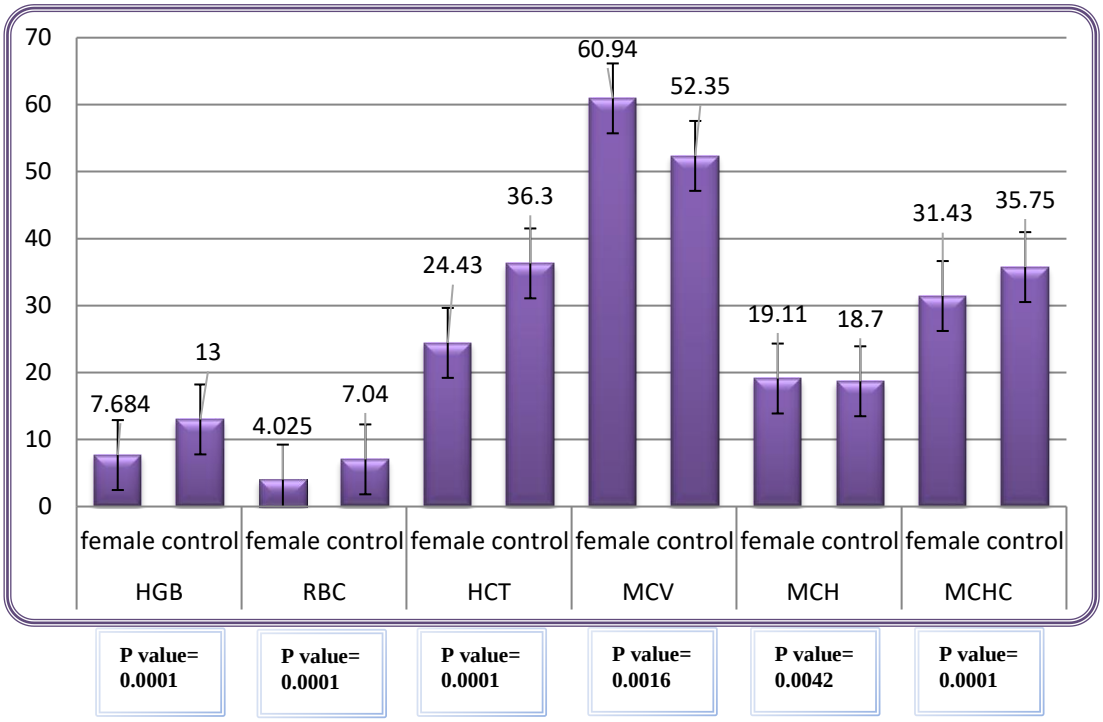

Figure (4): Illustrated Haematological indices between female Blood Loss anaemia groups with control group.

Result of Biomarker assessment in anaemia and control group:

Result of Hemopexin in PHZ (haemolytic anaemia), Blood loss and control groups:

The results of the hemopexin in figure (4-5) indicate a significant decrease (p> 0.05) in PHZ (haemolytic anaemia) female **(25.86±0.9107)** and male **(25.9±1.515)** when compared with the control groups **(32.32±1.375)** and **(30.44±1.59)** respectively. A significant increase (p> 0.05) was noticed in the Blood loss anaemia in female and male **(39.64±0.7955)** and **(37.74± 1.14)** when compared with the control groups **(32.32±1.375)** and **(30.44±1.59)** respectively.

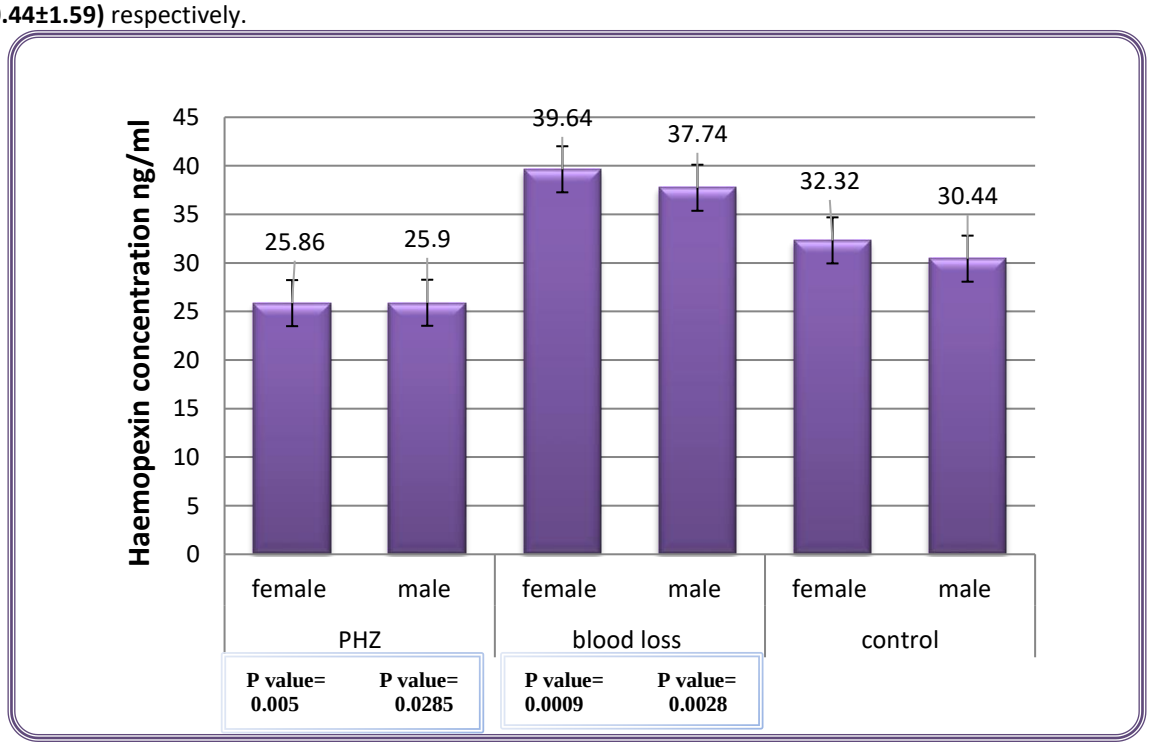

Figure (5): Hemopexin level in PHZ (haemolytic anaemia), Blood loss and control groups:

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Result of Lactoferrin in PHZ (haemolytic anaemia), Blood loss and control groups:

In figure (4-6) the results of Lactoferrin show a significant increase (p> 0.05) in PHZ (haemolytic anaemia) female **(16.57±0.5054)** and male **(17.3±0.3218)** when compared with the control groups **(11.8±1.275)** and **(12.34±0.8408)** respectively. A significant decrease (p> 0.05) was seen in the Blood loss anaemia in female **(8.375±0.2397)** and male **(9.367±0.465)** when compared with the control groups **(11.8±1.275)** and **(12.34±0.8408)** respectively.

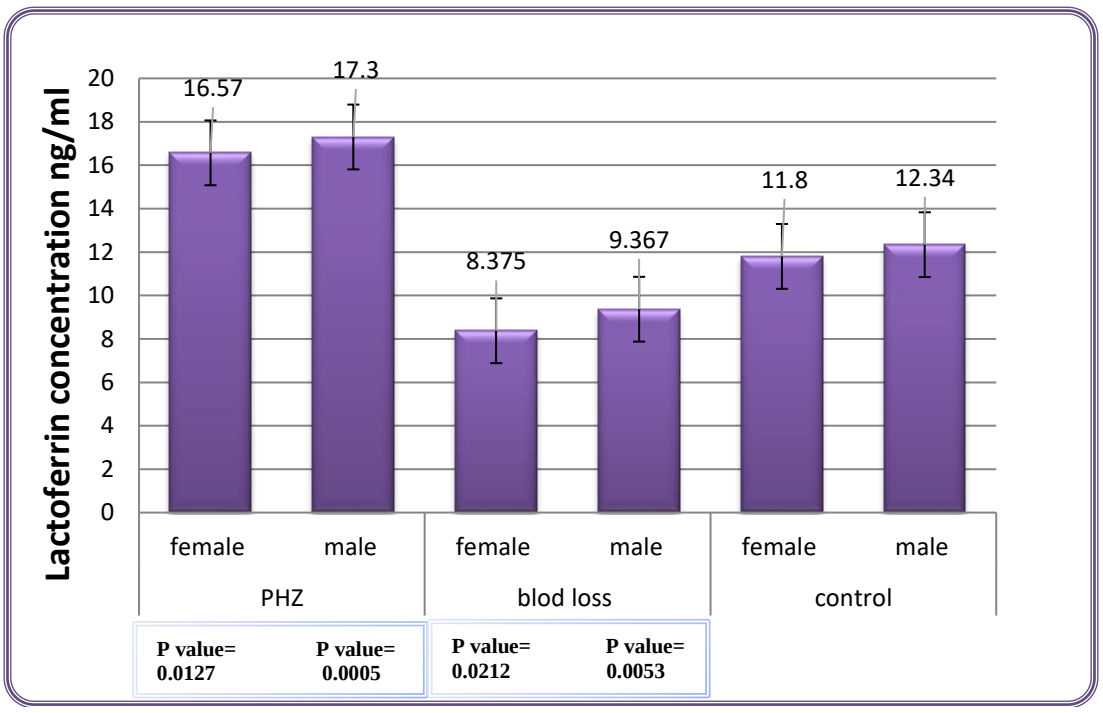

Figure (6): Lactoferrin level in PHZ (haemolytic anaemia), Blood loss and control groups.

DISCUSSION

Anaemia is very familiar and incidence is likely to increase in future, must seek to prevent the disease and work to find ways to treatment in terms of a more cost-effective and best strategies[22]. Anaemia, a condition of reduced ability of the blood for carrying oxygen, whatever the reason it is a sign of evident disorders rather than a disease[23]. In vertebrates, anaemia is a common haematological condition associated with many conditions such as, hereditary or acquired defects, blood loss, drug toxicity and parasite[24]. Anaemic individuals often experienced from fatigue, paleness, shortness of breath, edema and chilled that result from oxygen blood levels which are inadequate to maintain normal metabolism which is considered a hall mark of anaemia[25].

Hematological parameters of anemic and control groups:

Comparison of Hematological parameters in PHZ (haemolytic anaemia group) and control groups:

The results in the present study show in the group of haemolytic anaemia both males and females significant reduction (P> 0.05) in the haemoglobin concentration, number of red blood cells, hematocrit, mean cell haemoglobin concentration ; While observed a significant elevated (P> 0.05) in the mean cell volume and mean cell haemoglobin when compared with the control group.

In fact, the haemoglobin concentration is the parameter that is most commonly used as an indicator of pathophysiological consequence of anaemia. The MCV guides to diagnosis of anaemia and helps in its classification[26].

Another study conforming to the present study is that of Nadro and Modibbo (2014)[27] observed that anaemia results from the decrease in the red blood cells, haemoglobin concentration and pack cell volume under the normal range that lead to diminish in the capacity of the blood carrying oxygen[28].

Dolznig *et al.* (1995)[29] indicated through the results that elevated in the reticulocytes from chronic anaemia will lead subsequently increased in the MCVs due to the enlarged cell volumes of the reticulocytes transiently to the circulation. It also confirmed with Angermeier *et al.* (2016)[30] the MCV was higher than expected this because the calculated value includes the size of the red blood cells and immature erythrocytes.

The enhanced reticulocyte counts could also be indicative of haemolytic anaemia in mice[21]. Paterakis *et al.* (1994)[31] besides proposed that the correlation between MCV with MCH considered a method to study the influence of erythrocyte shape on the measurement of volume.

A model for research of haemolytic anaemia by using PHZ induced toxic anaemia and the influence of pathogenesis anaemia on other physiological processes [32-33]. [34] demonstrated that intraperitoneal administration of 60 mg/Kg PHZ for 2 days can reduce the hematological indices.

It has previously been shown that intraperitoneal administration of PHZ reduction HGB concentration, RBCs number and hematocrit[35] are also observed these data mimic this results reported in this study.

Haemolytic anaemia inducing by Phenylhydrazine causes the production of the reactive oxygen species (ROS) which in turn causes peroxidation of lipid and in the membrane skeleton spectrin can undergo oxidative degradation that cause damage to RBC [36] ; This free radical also works to increase the aging process of RBCs[37]. When ROS reacts with hemoglobin and change oxyhaemoglobin to methaemoglobin, hemichromes and other biodegradable products such as hemoglobin Heinz bodies [38]. Heinz bodies are formed from the altered haemoglobin may it reduce the life span of the erythrocytes[39].

When the hematotoxicity occurs macrophages capture red blood cells affected by the drug at an increasing rate in the first place, but not limited to, the spleen, resulting in a decrease in the number of red blood cells and hemoglobin low levels in the blood circulation [40].

This is often characterized by a significant increase in the incidence of micronucleated polychromated and hypochromic erythrocytes[41] resulting in increased the mean cell volume and decreases the mean cell haemoglobin concentration values[42], also decreased in the RBCs and haemoglobin. To Disposal of haemolytic products as a result of body's defense mechanism elevated WBC cells[2].

Phenylhydrazine when used for inducing destruction of red blood cells by oxidation stress and numerous joint variations at cellular levels resulted in haemolytic anaemia disease although this change in RBCs after the treatment by PHZ which is demonstrated in many published papers, little appears to be known of PHZ influences on different kinds of cells[43].

Comparison of Hematological parameters in Blood loss anaemia and control groups:

In this anaemic group, all bled animals showed a significant reduction (P> 0.05) in the haemoglobin concentration, erythrocyte number and hematocrit value ; Whereas observed a significant increase (P> 0.05) in the mean cell volume and mean cell haemoglobin when compared with the control group.

The rapid decline in RBCs and haemoglobin concentration are from the onset of the physiological response to blood loss or haemolysis then rapid recovery because of intact bone marrow functions in those animals [44-45].

This result is compatible with the previous studies which indicate that RBCs, HB, PCV, and reticulocyte count showed a significant diminution in 24 hours post bleeding in all bled animals when compared with the control group. Bled animals showed a significant increase in WBC, Platelet and lymphocyte counts within 24 hours post bleeding in comparison with control[46].

Biomarker assessment of anemic and control groups:

Hemopexin in PHZ (haemolytic anaemia), Blood loss and control groups:

The results of hemopexin refer to a significant reduction ($p > 0.05$) in females and males PHZ (haemolytic anaemia) group when compared with the control groups respectively. While show a significant increases ($p > 0.05$) in females and males Blood loss anaemia group when compared with control groups respectively.

The diminished of hemopexin level in haemolytic anaemia group is compatible with previous studies that indicate levels of hemopexin are reduced as those of heme rise, thus concentration of this protein is, indicating that good monitor for assessment of severity of intravascular haemolysis and activity in heme scavenger [47].also previous studies refers to Hpx is regarded as second line of defense against intravascular haemolysis associated with hemin release from Hb-Fe33+ [48-6-49].

Hemopexin scavenging of free heme from the plasma and limits the amounts thus preventing oxidative damage by radical formation in the circulation and contributing to the conservation of body iron [50]. The presence of heme in plasma which is produced from the oxidation of haemoglobin released through the destruction of erythroblasts in conditions intravascular haemolysis [50-51]. Intravascular haemolysis progresses as a severe medical complication in several disorders such as bleeding, haemolytic anaemia and hemoglobinopathies, polycitemia vera, malaria and ischemia reperfusion and can lead to greater increase in heme concentration [50-52].

It has been proven to form heme-Hpx complexes for the enhanced delivery of heme to the parenchymal cells in the liver by the endocytosis through heme-Hpx-specific receptor, heme degraded in the liver after extracted from heme-Hpx complexes and Hpx returned to the circulatory system [53-54-55].

Anaemia from blood loss is generally analogous to the iron deficiency anaemia that's occurred from nutritional deficiency or blood loss and this type of anaemia remains the most common[56].

Previous studies indicate that plasma levels of Hpx are regulated during numerous pathological disorders. Believed the transcriptional induction mediated by the cytokine interleukin 6 increases from plasma level of Hpx in inflammatory events [57]. Anaemia may be causing many inflammatory disorders in the body, our findings provide evidence that interactions of IL-6 family cytokines with endothelial cells that contribute to hematopoiesis. Thus, signals in the bone marrow environment enable endothelial cells to support the differentiation and proliferation of hematopoietic cells, further supporting the intimate relationship between these cell types [58].

Cytokines are a large family of extracellular ties that motivate many of the responses after binding to receptors cytokine saved structurally and functionally. Biological responses caused by cytokines cover a wide range of different biological activities, for example to survive, spread, differentiation, or maturation. In the case of hematopoietic system, cytokines most important motivating factors stimulating colony functions supportive of several lineages, as well as erythropoietin (EPO) and Trumbopuecan (TPO), which is working on single lineages [59]. Beside cytokines requirements for the organization of the basal be blood, it is also necessary to control hematopoiesis in cases of emergency in response to infection or blood loss

In the case of the hematopoietic system, the most important cytokines are interleukins and colony-stimulating factors with supportive functions for several lineages as well as erythropoietin (EPO) and thrombopoietin (TPO) that act on single lineages [59]. Beside the requirement of cytokines for regulation of basal hematopoiesis, they are also essential for controlling emergency hematopoiesis in response to infections or blood loss [59-60].

Preceding studies indicate that Hpx is binding with heme that originated from dietary sources or haemolytic events [61]. In the presence of inflammation condition, increasing Hpx level in plasma may play a marked role in inhibiting duodenal iron uptake. While confirmed that the Hpx level declines markedly in cases of severe haemolytic processes and the rate of Hpx synthesis in the liver is not adequate to replace what is consumed from it [62]. Therefore, in case of haemolysis the diminution of Hpx in the circulation best iron

uptake in the duodenum may occur and thus enhancing iron supply to other parts in the case of high demand for erythrocytes production. Additionally, Hpx deficiency combined with improved duodenal HO activity to increase the amount of iron accessible to meet requirements of body iron. [61] confirmed that the absence of hemopexin or deficiency lead to an enhanced absorption of iron from the duodenum, whereas the current study appeared elevation in the level of hemopexin, this refers to low iron absorption from the duodenum. He was stated that hemopexin probably modifies the level of iron in the body as it works to reduce or decrease of iron in the body, because hemopexin deals with iron at form heme-iron. Therefore, concludes that the lack of heme-iron it in the cause of the high level of hemopexin. [63-64] confirmed the presence of a direct association between the net synthesis of hemopexin and the quantity of heme offered to the liver. Hemopexin metabolism Parameters revealed an increase in the net rate of hemopexin synthesis after taking a low dose of heme which leads to an enhanced pool size of hemopexin in the intravascular. At the moderate dose there was an increase at the rate of synthesis hemopexin at the same time accompanied by elevate in catabolism, this resulted in the absence of any change in the rate hemopexin level. So explained in the case of a high dose of heme lead to increase in the rate of hemopexin catabolism without being a matching increase in the synthesis rate thus depletes of hemopexin level in circulation.

Lactoferrin in PHZ (haemolytic anaemia), Blood loss and control groups:

The results of lactoferrin indicate significant elevated ($p > 0.05$) in PHZ (haemolytic anaemia) female and male when compared with control groups respectively. While show significant reduction ($p > 0.05$) in Blood loss anaemia in female and male when compared with control groups respectively.

In haemolytic conditions in this model release haemoglobin into the plasma during intravascular haemolysis from the damaged erythrocyte, mechanisms protective of haemoglobin scavenging protein when become saturated level of free haemoglobin increase in the plasma, which lead to demand to other mechanics to reduce its accumulation in the tissues of the body.

This result is combatable with [65-66] who said some pathological complication such as haemolytic and sideroblastic anaemia cause accumulation of iron due to chronic haemolysis and also similar to blood transfusion cases, increased haemolysis result in release large amounts of iron from erythrocytes which accumulates in tissues. [67] indicated that lactoferrin is a member of a transferrin family formerly recognized as lactotransferrin thus belonging to those proteins that have the ability to bind iron and transferring. The receptor of lactoferrin It was found on the membrane border brush in epithelial cells of intestinal [68-69]. The vital role of these receptors in the iron absorption from intestinal and organizing in response to the amount of iron stored in the body, also biosynthesis of lactoferrin receptors in the epithelial cells of intestine can be regulated in response to the chelatable iron levels in the intracellular, and iron absorption in intestinal dependent on the levels iron stored in the body [70]. Also LfR is existed in blood vessels and nigral dopaminergic neurons and located at the surface of various tissues and types of cells to participate in absorbing Lf through specific pathway [71-72].

In the case of the low level of iron stored in the body stimulate the absorption of iron; it is considered a signal to regulate iron absorption in the proximal duodenum. There are two shapes of dietary iron: ferric Fe^{3+} (non-heme iron) prior to the uptake by duodenal enterocytes must be reduced to the ferrous ion by reductases and ferrous Fe^{2+} (heme iron) [73-74]. Whereas ferrous ions transported direct across the enterocyte apical membrane in the intestinal [75].

As well as it is proven in previous research that erythropoietin hormone of the major signal that affect absorption of iron and stimulates erythropoiesis by acting directly on intestinal epithelial cells to enhance dietary iron absorption for following utilization in the bone marrow [76].

Already has been proven in humans that the main role of Lf is the transfer iron in blood plasma [77]. Lactoferrin in the physical form relatively saturated with iron and then can be completely saturated with iron from the external environment [78-79].

Generally, rapid increased in serum lactoferrin responsible for hypoferremia through binding free iron (decrease in serum iron levels) when it is working as antimicrobial [80-81].

Anaemia of blood loss can be develop from frequent lose of blood that can lead to lose a large number of RBCs. The loss of RBCs also leads to low levels of haemoglobin and iron in the body. Iron deficiency anaemia may develop because the body's demand for iron is greater than its supply and low iron intake due to loss of RBCs through bleeding.

Many studies indicated that anaemia due to blood loss when its persistence can lead to deplete in the iron stores and increases in iron absorption to attempt for compensation of the lack [82].

Also anaemia that results from blood loss or dietary deficient describes by microcytic erythrocytes in conjunction with abnormal serum biochemical indices such as low iron, low transferrin saturation and low ferritin [56].

It has previously noted that the anaemia caused by blood loss similar to iron deficiency anaemia. [83] confirmed that Iron deficiency anemia, which described the reduction of iron stores, low serum concentrations of iron and HGB and decreased hematocrit [84] also low rate of Transferrin Saturation (TS) and a marked low serum ferritin and because of lactoferrin classified as a member of the transferrin family, because of its 60% sequence identity with serum transferrin [85] thus it seem to be the level of it decreased.

Studies have shown that iron deficiency by using at least one of these parameters, such as ferritin in serum transferrin saturation, mean corpuscular volume of erythrocyte protoporphyrin as indicator to iron status on the other hand iron deficiency without anaemia can be distinguished by normal HGB levels but abnormal values for one or several of these indicators of iron status [86].

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