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EXPERIMENTAL STUDY OF CHRONIC IRON DRUGS TOXICITIES IN ANEMIC EWES

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ABSTRACT
The experiment conducted to investigate the effects of chronic iron toxicity on some hematological parameters. Twenty anemic Iraqi local breed ewe sat (2– 4) year old were intra muscular injected with 10 mg/ kg of body weight iron dextran every week for 90 day. Blood and bone marrow smear of treated groups show proliferation of leukocyte especially eosinophil and accumulation of excessive iron in bone marrow. Erythrocyte count of treated group increased significantly ($p<0.05$) 8.307 $\times 10^{12}$/L at 45 day, then remained without change to the90 day after iron dextran injection, packed cell volume and hemoglobin of treated group increased significantly ($p<0.05$) to reach 23.123%, 106.50 g/L at 45 day respectively, then remained stable to the 90th day, blood indices of treated group showed no significant different in the mean corpuscular volume and mean corpuscular hemoglobin but mean corpuscular hemoglobin concentration showed significant increasing ($p<0.05$) at 90 day. Total and differential leukocyte counts of treated group show significant increasing ($p< 0.05$) at (15th– 30th) day. The increase was continued up to reach the peak at 90 day. Thus iron dextran could be considers as a good treatment for anemia but the continuous treating for a long time could cause accumulation in liver, kidney and brain.

KEYWORDS: Iron dextran, hemogram, hemoatotoxic, anemia.

INTRODUCTION

Iron is an essential component not only for hemoglobin synthesis and erythropoiesis but also for many enzymes and hormones, iron deficiency had negative effect on the sheep flock, deficient iron causes anemia in all animals (Gutteridge and Halliwell, 1994).

Iron deficiency is common finding in ruminant, where low dietary intake, starvation, gastrointestinal parasites, blood parasite infection increased incidence of infectious disease, inadequate gastrointestinal absorption, hemorrhage, effect of pregnancy and lactation, all these causes influenced on the level of essential blood constituents especially iron, cobalt, copper and many of biochemical functions such as iron utilization and hemoglobin synthesis, (Weiss and Wardrop, 2010).

Parenteral iron therapy is indicated in situations such as intolerance, contraindications or inadequate response to oral iron, however, parenteral iron is now a useful treatment in cases where there is a short time to surgery, severe anemia, especially if accompanied by significant ongoing bleeding, use of erythropoiesis-stimulating agents *etc*. (Beris *et al*., 2008).

Iron's toxicity is largely based on its ability to catalyze the generation of radicals, which attack and damage cellular macromolecules and promote cell death and tissue injury, where excessive iron accumulation results in tissue damage and organ failure, pathological iron accumulation in the liver has also been linked to the development of hepatocellular cancer (Papanikolaou and Pantopoulos, 2004).

MATERIALS & METHODS

Twenty Iraqi local breed anemic ewes at (2-4) year old were identified by ear tags. All animal monitored clinically before and during the experiment according(Jackson and Cockorft, 2002), red blood cells (RBC) count (10^{12}/l), packed cell volume (PCV) (%), hemoglobin (Hb) (g/dl), platelets (PLT) count (10^9/l),total white blood cells (WBC) count (10^9/l), granulocyte (GRA) count (10^9/l), lymphocyte(LYM) count (10^9/l), monocyte (MID) count (10^9/l),mean corpuscular volume (MCV) (fL), mean corpuscular hemoglobin (MCH) (PG), mean corpuscular hemoglobin concentration (MCHC) (g/l) by automated hematological analyzer (Abacus Vet Junior, Hungary)at zero day and (15,30,45,60,75,90,105) days before iron dextran or PBS injection.

The ewes divided into two groups: G1 which was considered as untreated group: consist of 10 ewes injected with 0.2ml/kg of body weight PBS, but subjected to all tests mentioned above at the same time of the second group. G2 which considered as treatment group: consist of 10 ewes injected with iron dextran 10 mg/k of body weight intra muscular every week for 90 day.

Statistical analysis

Analysis of Variance (ANOVA) was used and means were compared by using t-test according to Snedecor and Cochran (1989).

RESULTS

RBCs Count

Ewes of the treated group showed a range of (6 – 8) × 10^{12}/L of RBCs count (Fig. 1). On the other hand, ewes of treated group showed gradual increase of RBC count up to

reach the peak (8.3×10^{12}/L) at 45day then remain unchanged to the 90day. In the present study there was a significant difference ($P < 0.05$) between periods of study as compared with untreated group**.**

PCV

The packed cell volume (PCV) increased in treated group. As shown in (Fig. 2) there is a gradual increasing which reached to23.5% at 45day then remained unchanged up to 90 day. On the other hand, the range of %.PCV for the ewes of the untreated group was (17-23). Results revealed a significant increase with a significant difference between periods of study as compared with untreated group ($P < 0.05$).

Hb

Untreated group showed a slight increase in Hemoglobin (Hb) (Fig.3), whereas ewes of treated group, show gradually increase in Hb (109.5 g/l) at 75 day. There were significant differences ($P < 0.05$)between periods of study as compared with untreated group.

Platelets count

Ewes of the untreated group showed unstable changes (Fig. 4), while, treated group showed sharp decrease up to 172×10^9/l at 40day.In the present study there was a significant difference **(**$P<0.05$)between periods as compared with untreated group.

Total WBC count

Figure 5 illustrated that the ewes of the untreated group showed range (6.04 - 7.2) $\times10^9$ /l but remained with normal range, whereas ewes in treated group shows an increase up to 19.2×10^9/L at 90 day.

Granulocyte

Treated group showed an increase at time of iron injection to reach a peak at 90 day 8×10^9/L(Fig.6).

Lymphocyte

Ewes of treated group showed an increase at time of iron injection up to reach the peak 10.4×10^9/Lat 90 day. (Fig.7).

Monocyte

Ewes of treated group showed an increase at time of iron injection at 90day 0.094×10^9/L (Fig. 8).

These results of total and differential WBC count showed a significant difference ($P < 0.05$) throughout periods of study.

Blood indices

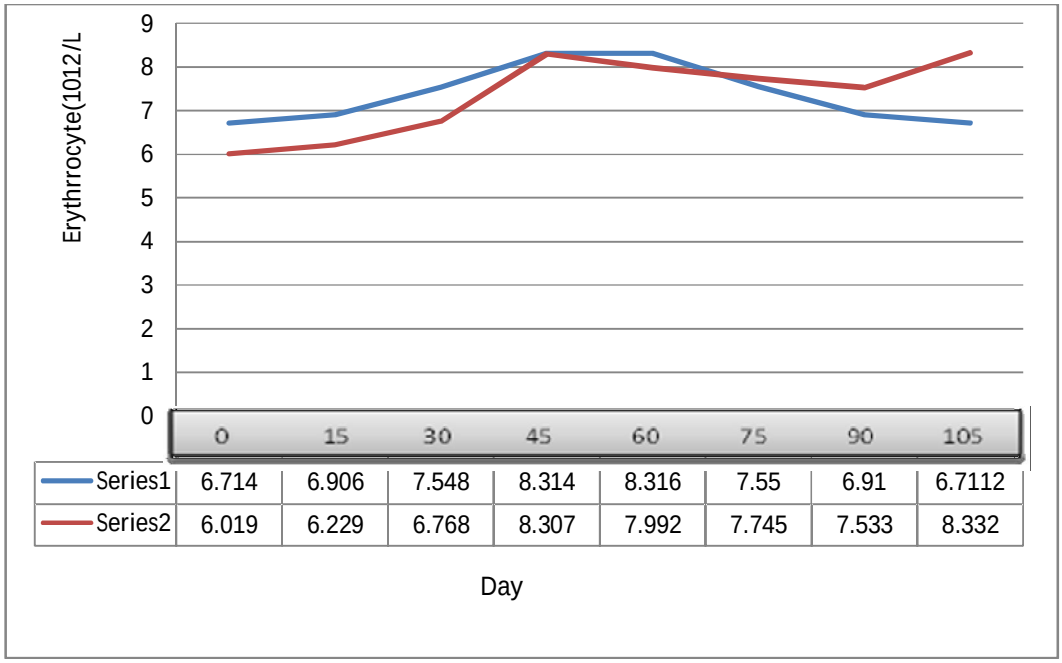
Treated group showed that there was no significant different in MCV, ranged (27 - 28.5) *f*L during a period of study (Fig.9), while the untreated group ranged (26.6 - 28.4) *f*L. These results showed no significant differences between groups.

MCH

A slightly decrease of MCH was shown at zero to30 day reached 12.7 Pg (Fig. 10), then gradual increase was noticed up to 14.3 pg. at 90day in ewes of treated groups, whereas, ewes of untreated group remind unchanged with range of (12.6 -13.2) pg. These results showed no significant differences between groups.

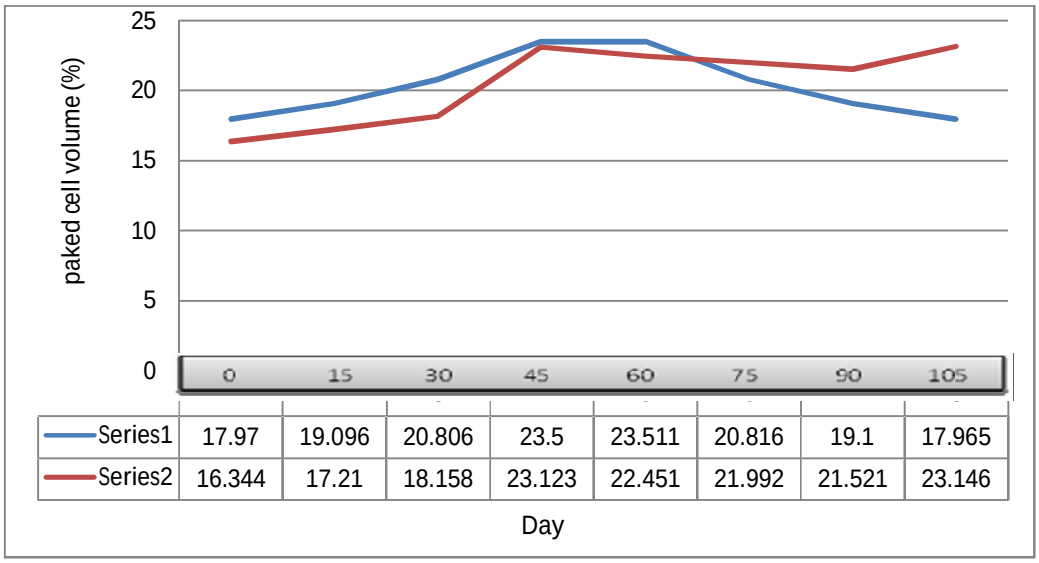
MCHC

It can be noticed different estimations of MCHC, in which these means depending on the alteration of Hb and PCV, so the ewes of the treated group showed a significant decrease at the 45and reach to 474g/l (Fig. 11), then increased at the 90day (511 g/L).Ewes of the untreated group differed with range of (456 – 477) g/l. These results showed significant differences ($P < 0.05$) between groups.



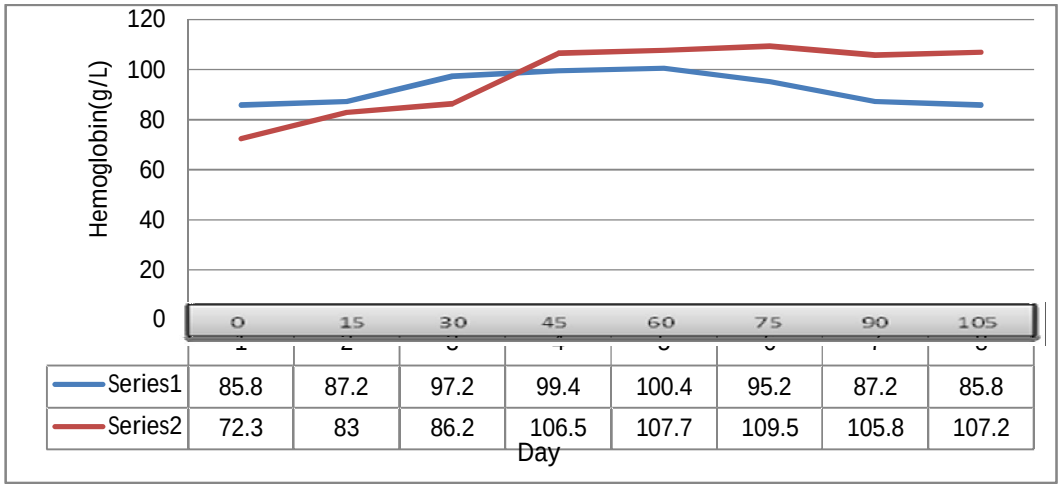
Series 1 represent the untreated group, Series 2represent the Treated group

FIGURE1: Means of RBCs count ($x10^{12}$/L) of ewes in different groups



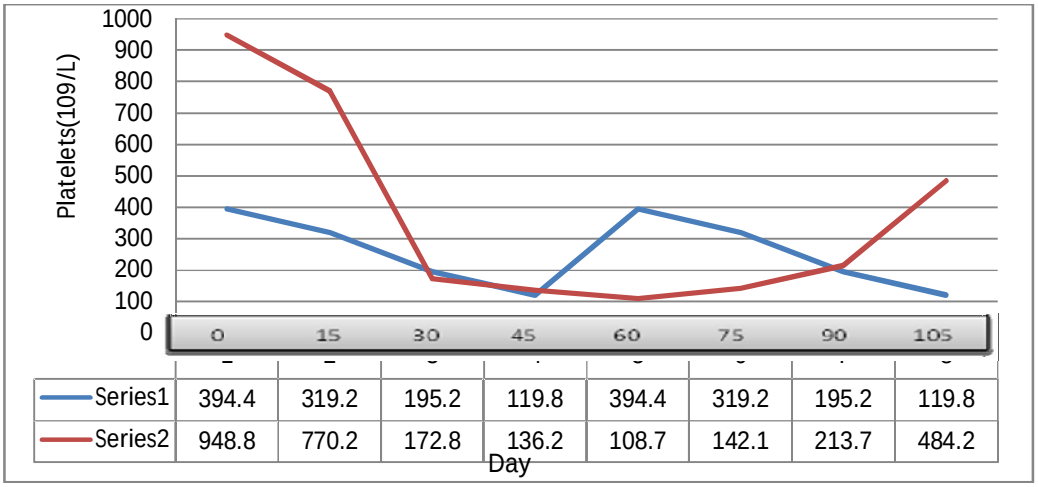
Series 1 represent the untreated group, Series 2 represent the Treated group

FIGURE 2: Means of P.C.V. (%) of ewes in different groups



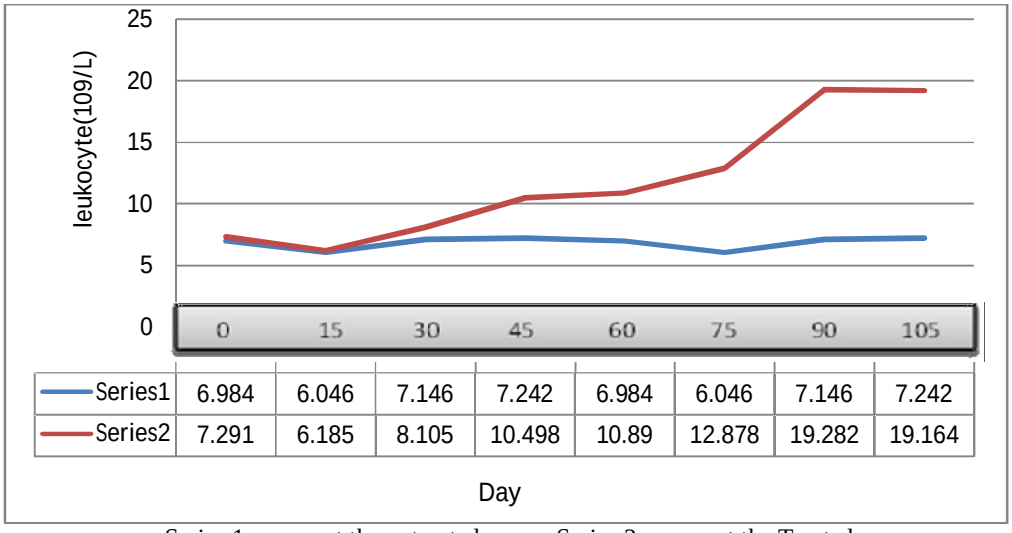
Series 1 represent the untreated group, Series 2 represent the Treated group

FIGURE 3: Means of Hemoglobin (g/dl) of ewes in different groups



Series 1 represent the untreated group; Series 2 represent the Treated group

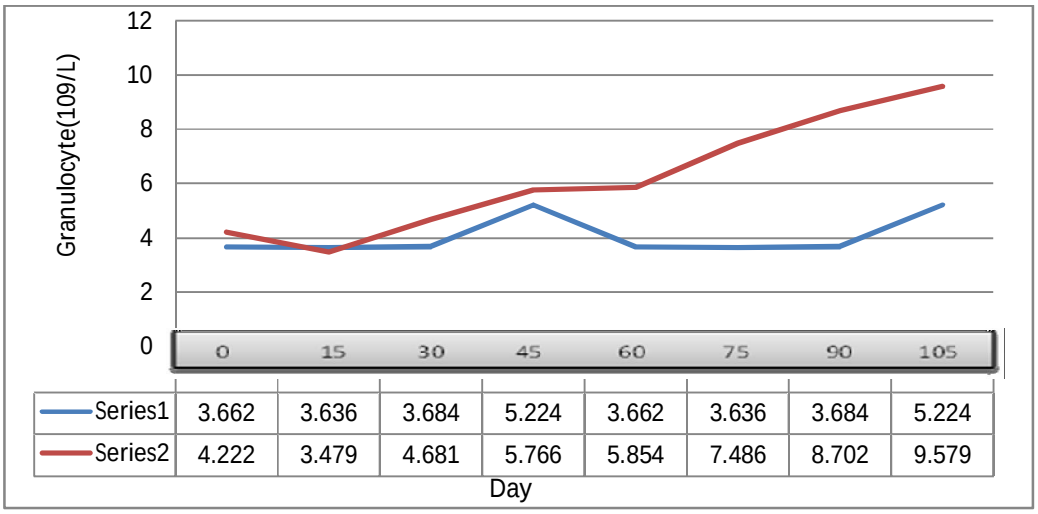
FIGURE 4: Means of platelets count ($x10^9$/L) of ewes in different groups



Series1	6.984	6.046	7.146	7.242	6.984	6.046	7.146	7.242
Series2	7.291	6.185	8.105	10.498	10.89	12.878	19.282	19.164

Series 1 represent the untreated group, Series 2 represent the Treated group

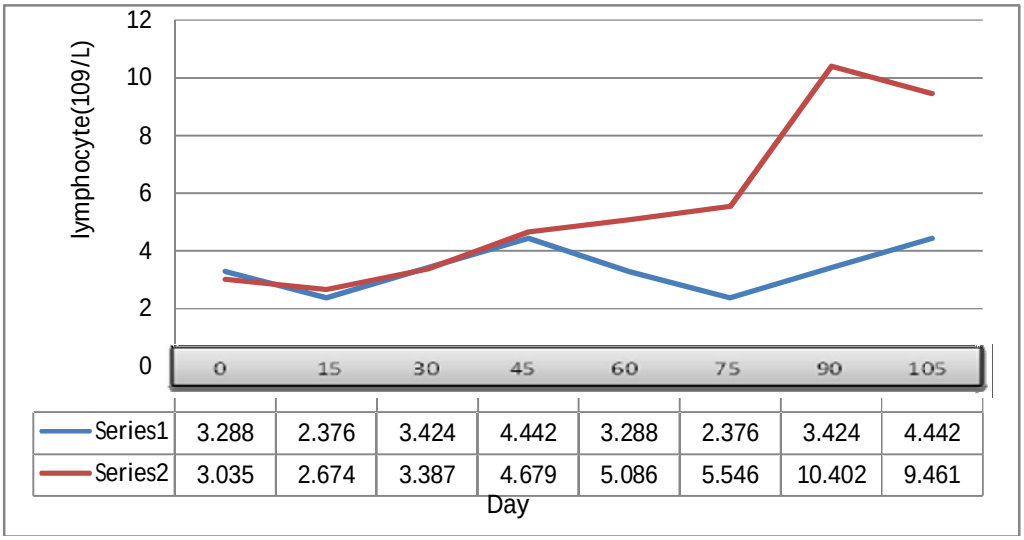
FIGURE 5: Means of WBCs count (x10^9/L) of ewes in different groups



Series1	3.662	3.636	3.684	5.224	3.662	3.636	3.684	5.224
Series2	4.222	3.479	4.681	5.766	5.854	7.486	8.702	9.579

Series 1 represent the untreated group; Series 2 represent the Treated group

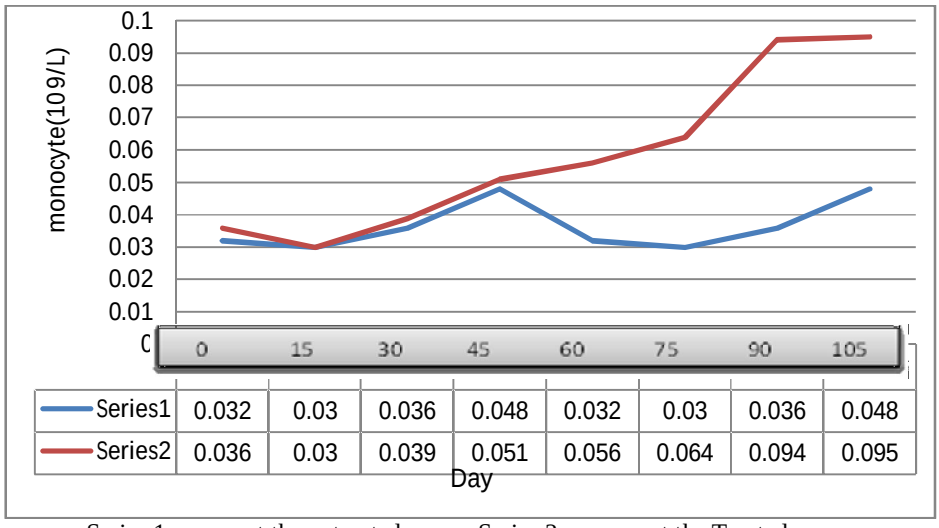
FIGURE 6: Means of granulocyte count (x10^9/L) of ewes in different groups



Series1	3.288	2.376	3.424	4.442	3.288	2.376	3.424	4.442
Series2	3.035	2.674	3.387	4.679	5.086	5.546	10.402	9.461

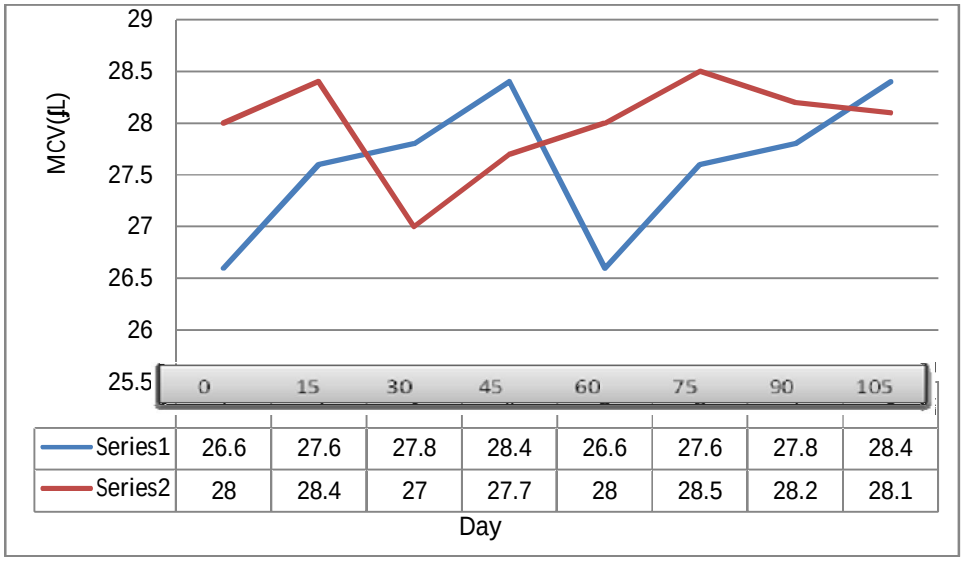
Series 1 represent the untreated group; Series 2 represent the Treated group

FIGURE7: Means of lymphocyte count (x10^9/L) of ewes in different groups



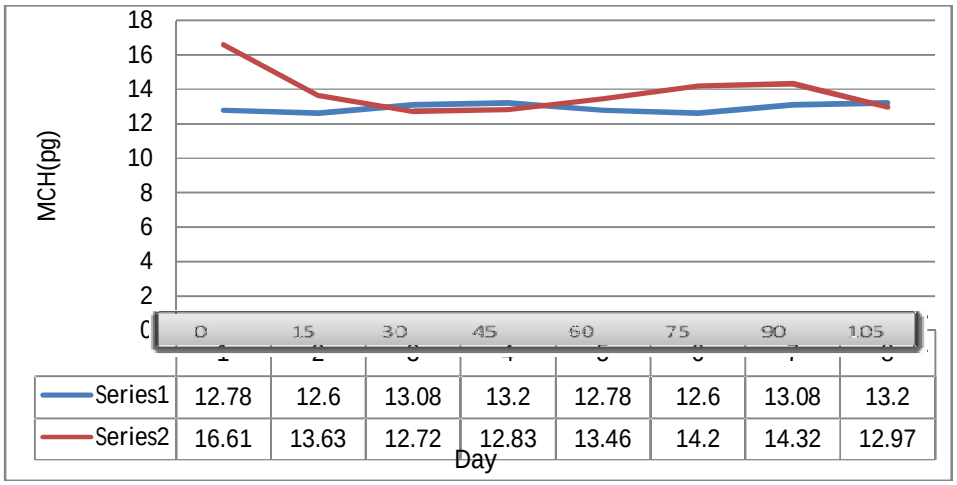
Series 1 represent the untreated group, Series 2 represent the Treated group

FIGURE 8: Means of monocyte count (x10^9/L) of ewesin different groups



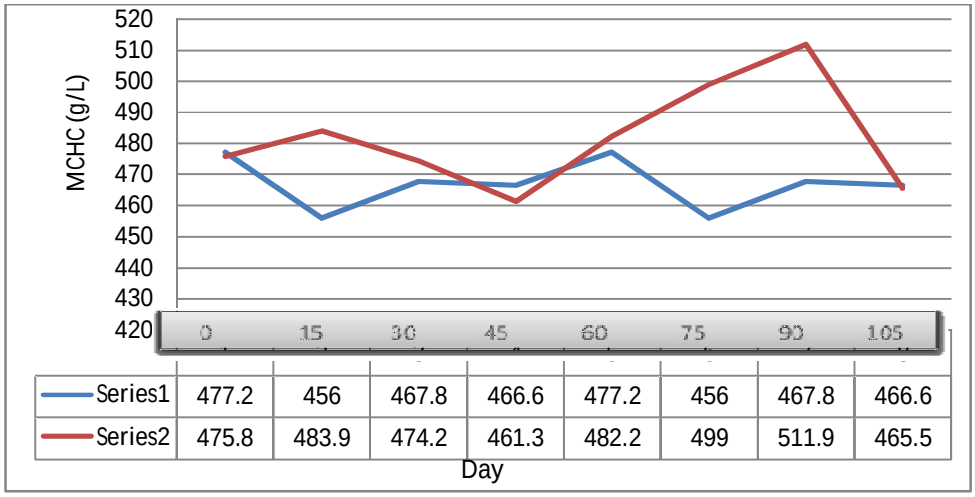
Series 1 represent the untreated group, Series 2 represent the Treated group

FIGURE 9: Means of MCV (*f*L) of ewes in different groups



Series 1 represent the untreated group; Series 2 represent the Treated group

FIGURE10: Means of MCH (pg) of ewes in different groups



Series 1 represent the untreated group; Series 2 represent the Treated group

FIGURE 11: Means of MCHC (g/l) of ewes in different groups

DISCUSSION

RBCs Count

These results illustrated in (Fig. 1) are in agreement with Steinberg and Olver (2005) and Cowgill *et al.* (1998) because iron at first supports the erythropoiesis until to reach with normal value. While, the toxicity effects appeared in blood picture which RBCs differed in shape and size due to iron(pict.1), (poikilocytosis, anisocytosis), Diffuse basophilic stippling on RBC, (Plate 2) (Edmondson *et al*., 1993 & Jain, 1986). This result is agreement with Edmondson *et al.* (1993).

PCV

These results obtained in (Fig. 2) were consistent with those reported by Gustschow *et al.* (1975) and Stohlman *et al*. (1963) the increase in PCV in this study may be due to iron injectionor feed improvement induced hematopoiesis**.** Iron represent as erythropoiesis-stimulating agents (Beris *et al.*, 2008), which effect on liver and bone marrow as they represents contraindicate with hematopoiesis.

Hb

These results shown in (Fig. 3) are coordinate with Keitt, (1985); Jain (1986) and Lavoie et al. (1987). As well as hyperchromasia which increased staining of RBCs and decrease central pallor due to increase of Hb in cell were present in this study during the time installer for the treatment of anemia but then effect on liver and bone marrow which represents contraindicate with hematopoiesis.

Platelets count

The results shown in (Fig. 4) are confirm those reported by Reddy and Singh (1995) their ameliorative effect has been ascribed to their capacity to scavenge or impair oxygen free radical generation and repair damage in endothelial tissues and that lead to decrease its number**.**

WBC

The results of this study are similar to those of, Garmo *et al*. (1986) Kaur *et al.* (2005). Hepcidin expression increases in response to inflammation and iron overdose (Weiss and Lulich, 1999)**.** While interleukin - 6 (IL - 6) plays a central role in increasing hepcidin production, other cytokines, including interleukin – 1 (IL - 1) were also likely involved. Conversely, hepcidin expression decreases in response to anemia caused by hemorrhage or hemolysis, and in conditions associated with hypoxia, and iron deficiency (Lowenstine and Munson, 1999). Hepcidin expression was increased by inflammation and iron overload. Hepcidin binds to ferroportin and causes its internalization and degradation, thus inhibiting efflux of iron into the plasma. The increase of WBC count is due to granulocyte production. Furthermore, it could be seen toxic granulation Dohle bodyin cytoplasm of eosinophil (Plate 3) some evidences indicates that the toxic granules may contain immune complexes, consisting of reactive immunoglobulin's (Jain, 1986). After 60th day, granulopoiesis increased, and granulocyte morphology is altered by maturation defects. The most common alteration is appearing of large neutrophil with hyper segmented lobules (Plate 5), and Dohle body in cytoplasm of neutrophil. All these result appeared excessive iron in blood and bone marrow (Plate 4) (Mills and Curry, 1994).

Blood indices

In regards to blood indices (MCV, MCH, MCHC) which are shown in figures 9, 10, 11 respectively, the results are to those found by Stohlman *et al.* (1963, 1985); Jain, (1986) and Lavoie *et al*. (1987) who found an increase in MCV and decline in MCHC values that reflecting macrocytic and hypochromic anemia in cows and buffaloes. Decreasing in MCV indicate a microcytosis that would be consistent with a relative or absolute iron deficiency or with an abnormality in iron utilization (Jain, 1986) when iron level is high so that mean abnormality in utilization of iron, another possibility was RBC fragmentation; furthermore there was morphologic changes appear on the blood smears which supports this hypothesis (Powers, 1989). In most anemic conditions alterations in average size of red cells (MCV) are paralleled by similar change in MCH and often the MCHC. Such alterations are specific on hemoglobin (Coles, 1986).

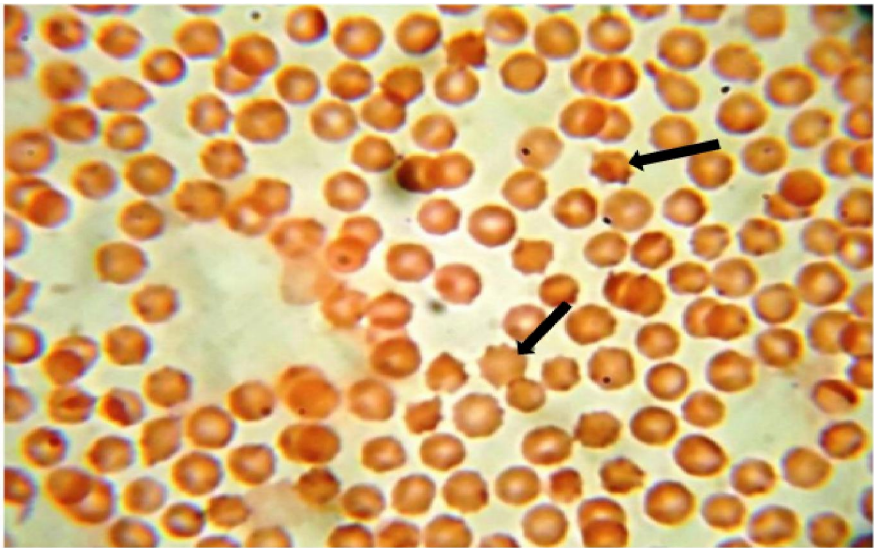


PLATE 1: Acanthocyte due to iron toxicity (Giemsa Stain)

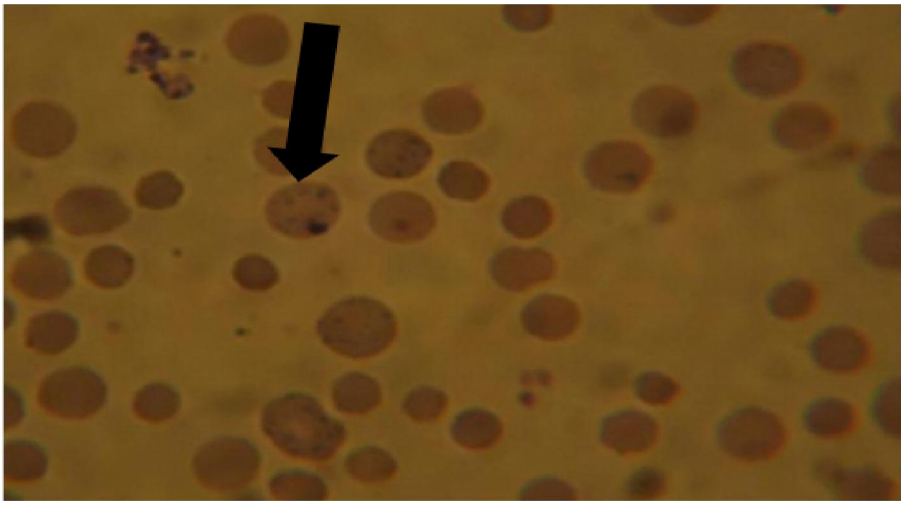


Plate 2: basophilic stippling in RBC due to iron toxicity (Giemsa stain)

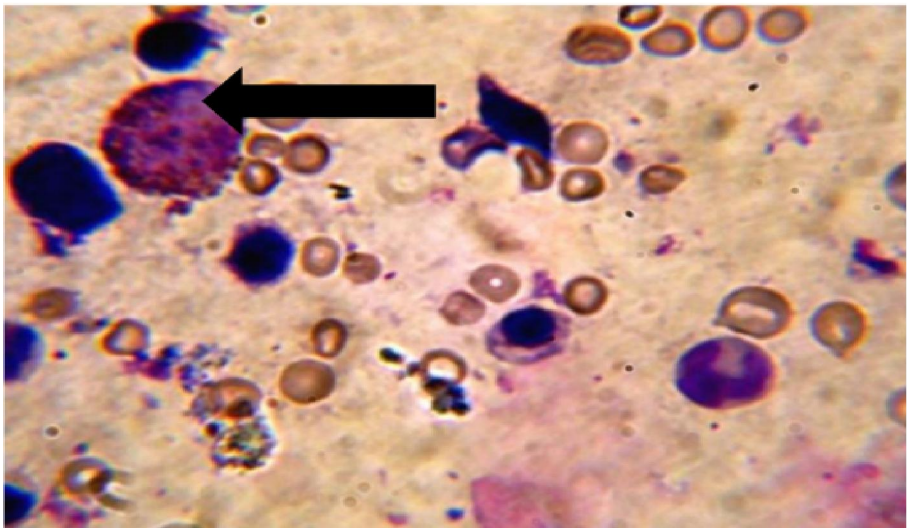


PLATE 3: (a) Toxic granulation of eosinophil in bone marrow due to iron toxicity (Giemsa stain)

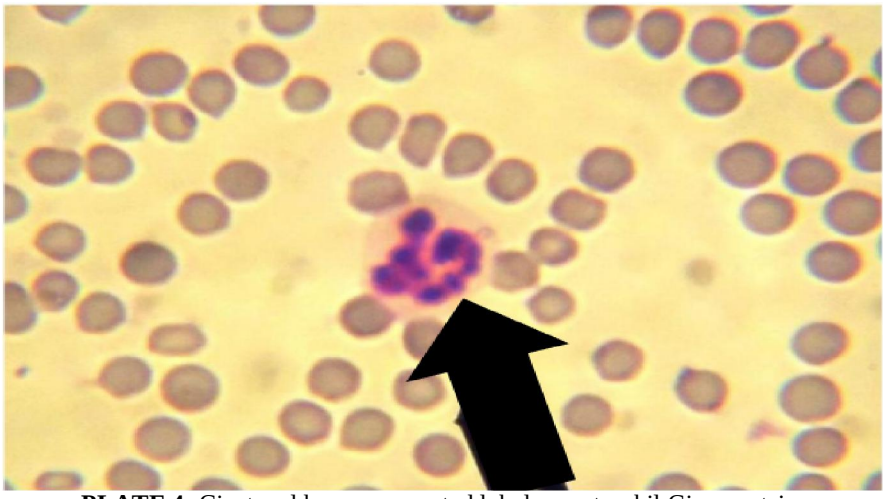


PLATE 4: Giant and hyper segmented lobules neutrophil Giemsa stain

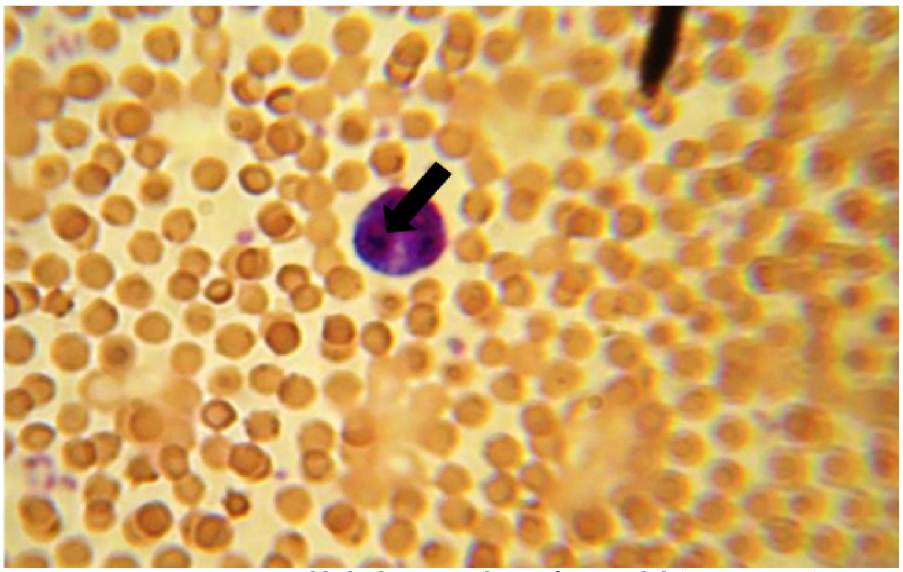


PLATE 5: Döhle body in cytoplasm of neutrophil (Giemsa stain)

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