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The effect of experimentally induced hyperglycemia by alloxan on some hematological parameters in rabbits

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تأثير ارتفاع سكر الدم المستحث تجريبيا بواسطة ألألوكزان على بعض معايير الدم في الأرانب

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الخلاصة

هدف هذه الدراسة هو فحص وتقييم تأثير ارتفاع نسبة سكر الدم على بعض معا يير الدم ونسب الدهون في الأرانب خلال سبعين يوما وهي الفترة التي استغرقتها التجربة.اجريت هذه الدراسة في قسم الامراضظ كلية الطب البيطري ظ جامعة القادسية من الفترة ٢٠٠٨/١٠/٣١ حتى ٢٠٠٩/٠٢/١٠ وتشمل تقييم معايير مصل الدم والدهون وكريات الدم الحمراء و البيضاء.

وتبين النتائج أن فرط سكر الدم أحدث انخفاضاً كبيراً (P<٠.٠٥)في حجم الخلايا المرصوصة وخلايا الدم الحمراء عند مقارنتها مع مجموعة السيطرة، و أيضاً أحدث فرط سكر الدم انخفاضاً كبيراً (P<0.05) في خلايا الدم البيضاء عند مقارنتها مع مجموعة السيطرة، وقد تسبب فرط سكر الدم بزيادة مؤثرة بشـــكل كبيـر (P<0.05) في تركيز الكوليسترول في

مصل الدم بينما كان تاثيرها أقل أهمية (P<0.05) علىنسبة الدهون البروتينية الثقيلة الى كوليسترول مصل الدم عند مقارنتها مع مجموعة السيطرة ومع ذلك ، فإن فرط سكر الدم قد احدث زيادةً كبيرةً (P<0.05)في تراكيز كوليسترول مصل الدم (triacylglycerol).
نستنتج من هذه الدراسة أن فرط سكر الدم قد يكون طردي الأثر على تركيز الكولسترول في المصل ، على الرغم من أنه ذو تأثير سلبي على بعض مؤشرات الدم .

Abstract

The study was designed to investigate the effects of hyperglycemia on some hematological and serum lipid parameters in rabbits during a period of seventy days. This study was achieved in the university of Al-Qadisyiah / college of veterinary medicine college department of pathology from 31/10/2008 till 10/02/2009. The parameters evaluated include serum lipids, red and white blood cell indices. The results show that the hyperglycemia significantly reduced (P<0.05) packed cell volume and red blood cell count when compared with control groups. Also, the hyperglycemia significantly reduced (P<0.05) white blood cell count when compared with control. Moreover, the hyperglycemia significantly elevated (P<0.05) total cholesterol concentration in the serum while it had less significant effect (P<0.05) on serum HDL-cholesterol concentration when compared with controls. Whereas, hyperglycemia significantly increased (P<0.05) serum triglycerol concentration. The results of this study suggest that hyperglycemia may have extrusive effect on serum cholesterol concentration, although it possibly possesses the potential of adversely affecting some hematological indices.
Key Words: alloxan, hyperglycemia, hematological parameter, rabbits.

INTRODUCTION

Diabetes mellitus, a leading non communicable disease with multiple etiologies, affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world **(1)**. The World Health Organization (WHO) 2002, reported that 300 million people would suffer from diabetes mellitus by the year 2025 **(2)**. Diabetes mellitus is characterized by an increased concentration of blood glucose due to derangement in carbohydrates metabolism and defective secretion of insulin. These metabolic disturbances result in acute and long-term diabetic complications, which are responsible for premature death and disability **(3)**. The common denominator of diabetes

complications is hyperglycemia of any degree. Hyperglycemia affects the function of nerves and muscles acutely and possibly all other tissues as well. Therefore, erythropoietin responses to anemia in diabetes may also be disturbed. An example of such a mechanism would be the glycosylation of both low density lipoprotein (LDL) and the LDL receptor, which results in a failure of mutual recognition **(4)**. As diabetes progresses, the basement membrane of the glomeruli thickens as a result of glycosylation, leading to increase intra-renal pressure **(5)**. This damage ultimately results in chronic kidney disease (CKD), decreased production of erythropoietin, and anemia **(6)**. In patients with diabetic nephropathy, the onset of anemia can occur early in the course of CKD, in marked contrast to non diabetic patients, who do not develop anemia at the same stage of CKD **(7).**As CKD progresses, anemia typically worsens **(8).** Yun and colleagues compared the characteristics and erythropoietin levels of 35 diabetic patients with anemia but without overt renal disease to those of non diabetic patients with anemia They found that erythropoietin concentrations were significantly lower in diabetic than in non diabetic patients with similar decreases in hemoglobin (Hb) concentration (P <0.001) **(9)**. Hb concentrations in the diabetic patients were related to creatinine clearance, serum creatinine, and albumin excretion rate, suggesting that the blunted erythropoietin response in patients with diabetes but without overt renal disease may be due to early renal interstitial damage or the glycosylation mechanism.

Anemia and diabetes are risk factor for short –term mortality following an acute myocardial infarction (AMI).Anemia is prevalent in patient with diabetes **(10)**. The etiology of anemia in type 1-diabetes is diverse. Diabetic patients, who do not follow the appropriate dietary regimes, are at risk of developing nutritional deficiency anemia, especially iron and folate deficiency. The occurrence of diabetic nephropathy with ultimate renal failure is an important cause of anemia in patients **(11).**

Materials and Methods:

This study was achieved during seventy day in the university of Al-Qadisyiah / college of veterinary medicine college department of pathology from 31/10/2008 till 10/02/2009. Fifty two rabbits (White

New Zealand Rabbits) purchased from the local markets, weighing 1.5-2 kg were used in the experiment. Animals were housed in cages with dimension (130×100×70) under 12/12 h light/dark cycle at 25±2 C & 60% relative humidity with standard granulated food, & water available *ad libitium.* The animals were divided according to gender into two equal groups**:** male group that were subdivide into diabetic group (13 rabbits denoted by Dm) and control group (13 rabbits denoted by Cm), female group that were also subdivided into diabetic group (13 rabbits denoted by Df) and control group (13 rabbits denoted by Cf).

Induction of Diabetes Mellitus:-

Diabetes mellitus was induced in overnight fasting rabbits by a single injection of **alloxan** (alloxan monohydrate) at dose 100 mg /kg into marginal ear vein .Each 100 mg of alloxan was diluted in 1 ml of 0.9% normal saline **(12; 13)**. Immediately, after alloxan injection, 10 ml of 20% glucose I.V & 5 ml of 20% glucose I.P was given to the rabbits in order to overcome sudden decrease in blood glucose level (hypoglycemia).The rabbits were prevented from feeding for 12 hours and the drenching water replaced by 5% glucose for 24h. The control groups were injected intravenously with 1 ml of 0.9 % of normal slain.

Estimation of Fasting Serum Glucose:-

After 3 days of alloxan injection the animals were fasting overnight and bled for checking the hyperglycemia .Fasting serum blood glucose (FSG) was measured by using special kit prepared by (SPINREACT, S.A.Ctra.Santa Coloma, 7E-17176SANT ESTEVE DE BAS (GI) SPIN), then the (FSG) concentrations were checked every ٢٠ days .

Determination of hematological and serum lipid parameters

At the end of the experiment period, the rabbit's venous blood was collected into sample bottles containing no anticoagulant. The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 rpm for 5 minutes **(14)**. The clear serum was removed by pipetting and the serum lipid variables were then determined. The total cholesterol concentration in the serum was estimated by using special kit prepared by (SPINREACT, S.A.Ctra.Santa Coloma, 7E-17176SANT ESTEVE DE BAS (GI) SPIN), while serum HDL -cholesterol concentration was determined by the method of (**15)**. The serum

triacylglycerol concentration was estimated by (SPINREACT, S.A.Ctra.Santa Coloma, 7E-17176SANT ESTEVE DE BAS (GI) SPIN). Hematological parameters of the blood samples were then estimated using routine hematological methods and kits. The packed cell volume (PCV), red blood cell count (RBC) and white blood cell count (WBC).

Statistical analysis

The data presented as mean ±SD were analyzed using the Duncan Multiple Range test and differences at $P< 0.05$ were considered significant **(16)**.

RESULTS AND DISCUSSION

The effect that hyperglycemia leaves on some serum lipid indices is presented in figure number 1 (a, b and c). Hyperglycemia significantly ($P<0.05$) increased serum total cholesterol concentration and posed a significant effect ($P<0.05$) on serum HDL-cholesterol concentration when compared with controls. However, hyperglycemia significantly increased ($P<0.05$) serum triacylglycerol concentration.

High blood cholesterol concentration is one of the important risk factors for cardiovascular disease **(10)**. The most important gross appearance at necropsy after the experimental inductions of diabetes in rabbits was Cardiac hypertrophy **(17)**. Thus the elevation in serum total cholesterol concentration induced by diabetes mellitus increased the risk of cardiovascular disease **(18;26)**.The increase in serum total cholesterol concentration is as a result of increase in serum HDL-cholesterol concentration. It thus implies that elevation in other fractions of serum total cholesterol concentration other than HDL-cholesterol may be responsible for the elevation of serum total cholesterol concentration observed **(19)**.

As observed in figure 2(a, b and c), diabetes significantly ($P<0.05$) reduced RBC counts and PCV, when compared with controls. RBC and PCV relate to the total population of erythrocytes in the blood, it thus imply that the diabetes mellitus may neither affect the incorporation of hemoglobin into erythrocytes nor the morphology and osmotic fragility of erythrocytes produced **(20)**. However, the reduction in RBC and PCV implies that the hyperglycemia reduce the population of erythrocytes produced from the bone marrow. The hyperglycemia may reduce the oxygen- carrying capacity of the whole blood because of the reduced

population of erythrocytes in the blood **(21)**. Reduction in RBC and PCV observed in this study suggests anemia which may result from impaired erythrocytes production **(22)**.

Hyperglycemia significantly reduced ($P<0.05$) WBC counts when compared with control (Figure 2C). This suggests that the hyperglycemia may lead to formation of some bioactive agents that could cause destruction or impaired production of white blood cells **(22)**. It has been reported that granulocyte-macrophage colony stimulating factor (GmCSF), macrophage colony stimulating factor, interleukins IL-2, IL-4 and IL-5 regulate the proliferation, differentiation, and maturation of committed stem cells responsible for the production of white blood cells **(23)**. It may be that hyperglycemia reduced the production of these regulatory factors or interfered with the sensitivity of the committed stem cells (responsible for the production of white blood cells) to these factors **(24)**. The reduction of WBC at all periods of hyperglycemia and that of RBC at the highest period without corresponding reduction in platelet count at all periods, suggest that the hyperglycemia may possess the potential of causing a progressive but selective bone marrow depression with increasing time **(25)**. This is because the bone marrow is responsible for the production of red blood cells, white blood cells and platelets. Also the production of these components of blood may be susceptible to modulation by the hyperglycemia in an order having white blood cell production as the most susceptible and platelet production as the least susceptible **(20)**.

From the above result, it may be concluded that **hyperglycemia** leaves, may adversely affect some hematological indices, especially those relating to erythrocytes and white blood cells as well as special effect on serum cholesterol concentration.

Figure -1(a): The effect of hyperglycemia on Triacylglycerol concentration(mmol/L)

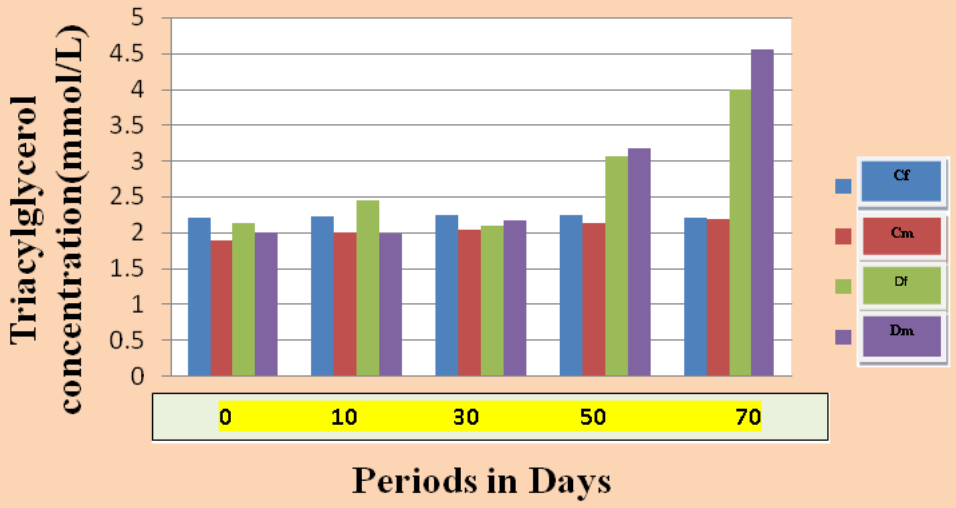


Figure -1(b): The effect of hyperglycemia on Total cholestrolconcentration(mmol/L)

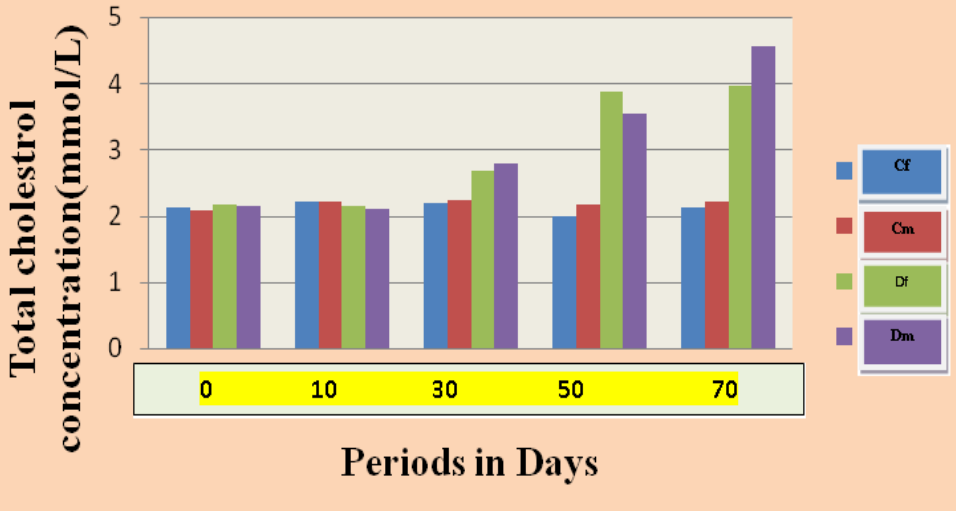


Figure-1(c): The effect of hyperglycemia on HDL-cholestrol concentration(mmol/L)

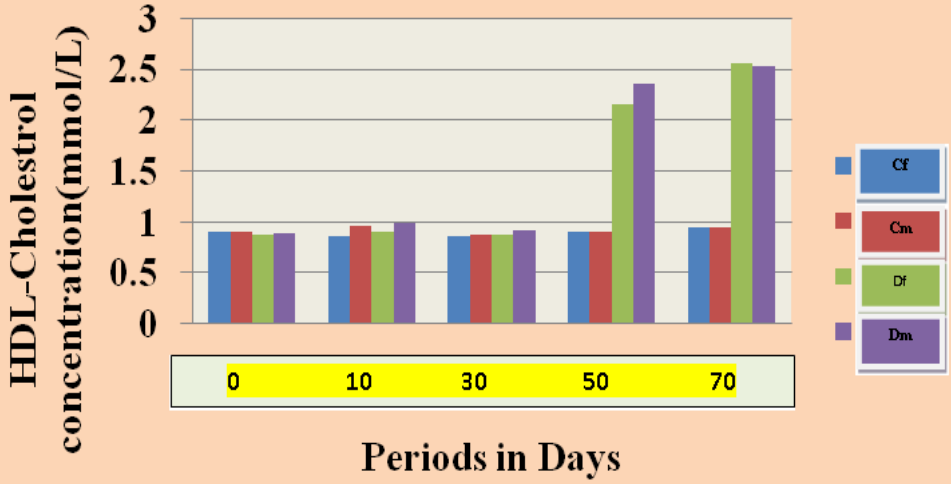
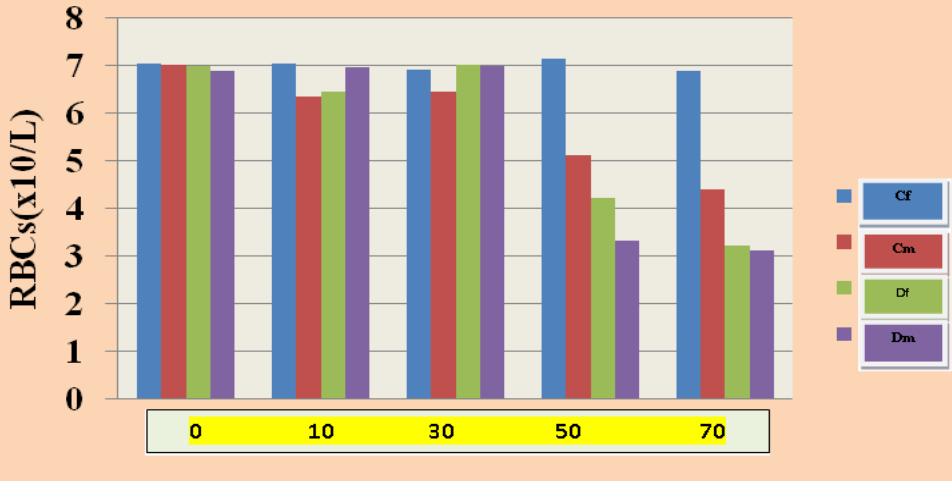


Figure-2(a): The effect of hyperglycemia on RBCs(x10/L)



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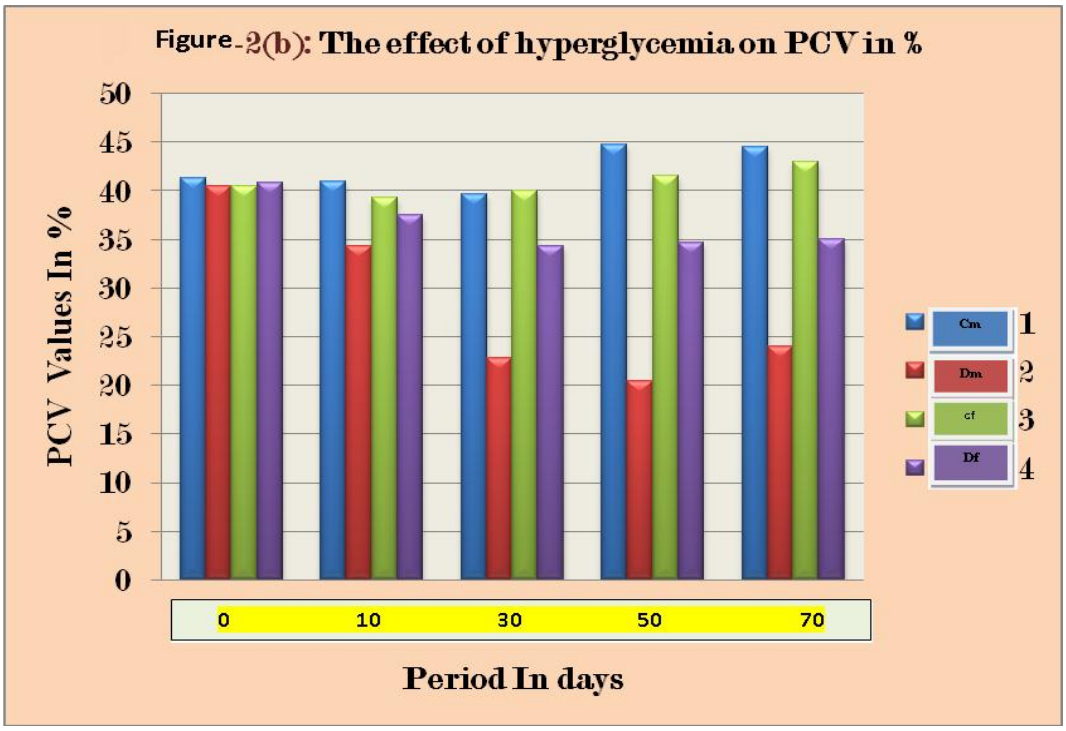
Figure-2(b): The effect of hyperglycemia on PCV in %
PCV Values In %
50
45
40
35
30
25
20
15
10
5
0
0
10
30
50
70
Period In days
Cm
Dm
cf
Df
1
2
3
4

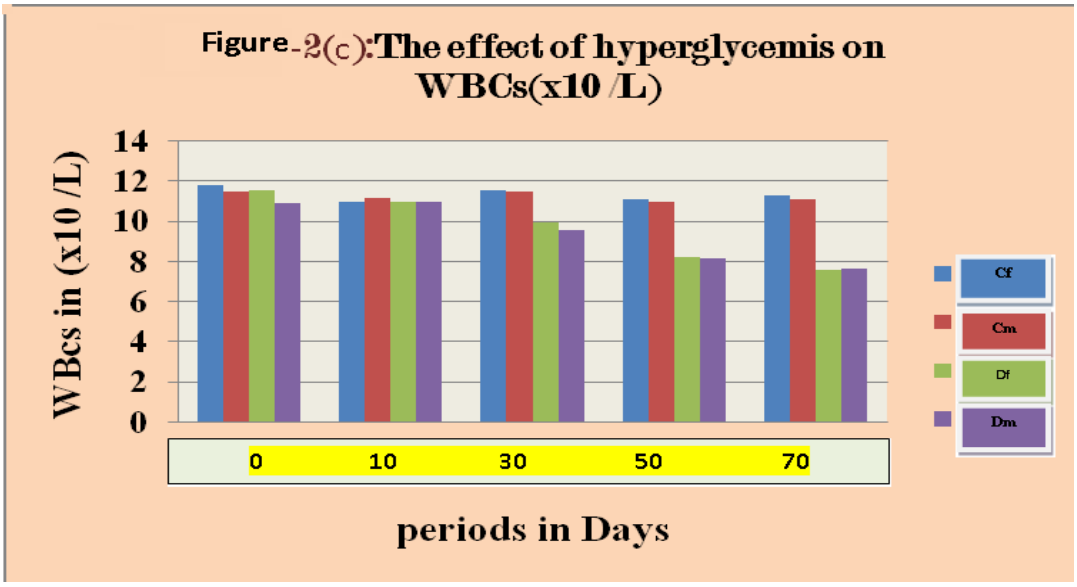
Figure-2(c):The effect of hyperglycemis on WBCs(x10 /L)
WBcs in (x10 /L)
14
12
10
8
6
4
2
0
0
10
30
50
70
periods in Days
Cf
Cm
Df
Dm

Table (1): mean ±SD. values with different superscripts across the row are significantly different at P<0.05.

	Blood Parameter				
Group	**Total Cholestrol concentration**	**Triacyl-glycerol concentration**	**HDL-Cholestrol Concentration**	**RBCs(X$10^{12}/L$)**	**PCV%**
Cf	**2.1±0.1^{a}**	**2.2±0.0^{a}**	**0.74±0.05^{a}**	**6.98±0.08^{a}**	**41.2±21.64**
Cm	**2.18±0.1^{a}**	**2.02±0.13^{a}**	**0.8±0.1^{a}**	**5.92±1.06^{a}**	**42.4±2.07**
Df	**2.94±0.86^{b}**	**2.72±0.80^{a}**	**1.36±0.90^{a}**	**5.58±1.76^{ba}**	**36.4±2.79**
Dm	**3.04±1.01^{b}**	**2.8±1.22^{b}**	**1.46±0.86^{a}**	**5.42±2.11^{ab}**	**28.4±8.61**

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