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SERUM BIOCHEMICAL ALTRATIONS FOLLOWING ALLOXAN- INDUCED DIABETES MELLITUS IN THE LOCAL MALE DONKEY

(With 8 Tables and 2 Figures)

By

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التغيرات الكيميائية الحياتية الناتجة عن داء السكر المحدث بالألوكسان في السلالة المحلية لذكور الحمير

معن سمير كلّو

من المعروف أن حقن الألوكسان ينتج عنه إحداث داء السكر في النماذج الحيوية التجريبية. هدفت الدراسة الحالية إلى تقييم التغيرات الكيميائية الحياتية الناتجة عن إحداث داء السكر بواسطة الألوكسان في السلالة المحلية لذكور الحمير. تم إخضاع مجموعتين من ذكور الحمير (7 حيوانات/ مجموعة) للتجربة، اذ اعتبرت المجموعة الأولى مجموعة سيطرة سليمة بينما تم حقن المجموعة الثانية بالألوكسان (100 ملغم. كغم $^{-1}$) بجرعة منفردة. أشارت نتائج الفحوصات الكيميائية الحياتية لمصل الدم على مدى 15 يوما منذ حقن الألوكسان إلى ارتفاع كلوكوز الدم معنويا (p≤0.05) كذلك ارتفعت تراكيز كل من الكوليسترول الكلي، الكليسيريدات الثلاثية، البروتين الكلي، الألبومين، الكرياتنين، نيتروجين اليوريا، الفوسفات اللاعضوية، المالونديألديهايد في مصل الدم فضلا عن ارتفاع فعالية كل من إنزيمات الألانين ناقل الأمين، الأسبارتيت ناقل الأمين، الفوسفاتيز القاعدي، الكرياتين فوسفوكاينيز و غاما ناقل الأمين في مصل الدم لدى مقارنتها سواء بمجموعة السيطرة أو بقيمها قبل بداية التجربة بينما انخفض تركيز كل من الكالسيوم والكلوتاثيون نتيجة لإحداث داء السكر في ذكور الحمير مع الإشارة الى عدم تغير قيم تراكيز كل من البيليروبين الكلي، الهيموكلوبين، الصوديوم والبوتاسيوم. يستنتج من الدراسة الحالية أن إعطاء جرعة مفردة من الألوكسان لذكور الحمير ينتج عنه نمط تجريبي لداء السكر قابل للدراسة والبحث وهو كذلك يؤثر سلبا في تراكيز الدهون، البروتينات، إنزيمات الكبد، وظيفة الكلى، فضلا عن مؤشرات الإجهاد التأكسدي.

SUMMARY

Alloxan is known to induce diabetes mellitus in the experimental animals. The objective of this study is to evaluate the biochemical alterations resulted from alloxan- induced diabetes in the local breed of donkey males. Two groups of donkey males (n=7 each group) were subjected to the experiment, the 1st one considered as a non diabetic control whereas the 2nd injected with alloxan monohydrate (100 mg. kg^{-1}) with a single dose. Results of serum biochemical analysis along with 15 days from alloxan injection revealed in addition to the hyperglycemia, a significant (p≤0.05) elevation in total cholesterol, triglycerides, total protein, albumin, creatinine, blood urea nitrogen, inorganic phosphate, malondialdehyde serum concentration as well as increased activity of serum enzymes alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatine phosphokinase and γ-glutamyl transferase in respect to both control or baseline values. Serum calcium and glutathione concentration was declined as a result of experimental diabetes in male donkey with no significant changes in total bilirubin, hemoglobin, sodium and potassium concentrations. In conclusion, administration of a single dose of alloxan to the male donkey produced a reproducible model of diabetes mellitus which negatively affects the serum lipids, proteins, liver enzymes, kidney function and the markers of oxidative stress.

Key words: *alloxan, diabetes, biochemical parameters, donkeys.*

INTRODUCTION

Diabetes mellitus is a metabolic disease with a hallmark of tissue's inability to control the level of blood glucose resulted from a defect in insulin production (type 1), insulin response (type 2) or both with a probability to develop of some chronic complications related to the degree of glycemia (National diabetes fact sheet, 2007). These include nephropathy, retinopathy and neuropathy but arterial disease might be also developed in an unrelated manner to the degree of glycemia (Taylor and Agius, 1988). In addition to the hyperglycemia, diabetes patients show several metabolic disorders including hyperlipidemia and hypercholesterolemia (Taylor and Agius, 1988), as well as elevation of the activity of some enzymes including aminotransferases, alkaline phosphatase and gamma glutamyl transferase (McKenzie *et al.,* 2006). The markers of oxidative stress like superoxide

dismutase, catalase and total antioxidant capacity also might be elevated (Pacal *et al.*, 2011; Wang *et al.,* 2011).

Diabetes mellitus has been reported in laboratory and farm animals. Although there is a little informations about the role of genetic factors in the incidence of diabetes in animals (Kaneko *et al.,* 1997), diabetes is well-defined in several species such as dogs (Alkharfy, 2009), sheep (Carver *et al.,* 1995) and horses (Durham *et al.,* 2009), also diabetes can be induced using a specific chemicals such as alloxan and streptozotocin, alloxan (2,4,5,6- tetraoxypyrimidin; 5,6 dioxyuracil) which is an oxidized product of uric acid can induce diabetes in animals through destroying the insulin secreting β- cells in the pancreas (Medical dictionary). Alloxan exerts its diabetogenic activity when it is injected intravenously, intraperitoneally or subcutaneously in a dose dependent manner which is affected directly by animal species, route of administration, nutritional status and ambient temperature (Szkudelski, 2001).

The cytotoxic action of alloxan and it's product (dialuric acid) is mediated by enhancing of the level of reactive oxygen species causing rapid damage to the target cells (Szkudelski, 2001). Nowadays alloxan is widely used for creation of experimental diabetic status in various biological models which includes, rabbits (Okamoto *et al*., 1990; Gupta *et al*., 2010), cats (Reiser *et al*., 1987; Ladosky and Fonteles, 1988), sheep (Smith and Prior, 1984), dogs (Jelodar *et al*., 2007; Sboui *et al*., 2010), rats (Ghosh *et al*., 2010) and mice (Paul *et al*., 2011).

To our knowledge, a finite number of studies focused on deleterious effects of diabetes mellitus induction in the equine especially the local breed of donkeys in Iraq. So the aim of this study was oriented toward the induction of experimental diabetes in donkeys and the investigation about the serum biochemical consequence during a limited period.

MATERIALS and METHODS

Animals: Fourteen local breed donkey males with weight range 155-180 kg and age 2- 3.5 years were used as a biological model for the major study while another group of six donkeys were subjected to preliminary study for determining the effective dose of alloxan, donkeys emplaced in an ideal byre for large animals in the farm of the College of Veterinary Medicine, University of Mosul. Animals fed daily with a

mixture of barley and hay 3:1 with continuous providing of drinking water, All animals underwent a 32 hour of fasting period.

Induction of diabetes

Preparation and injection of alloxan: The dose of alloxan monohydrate (Molekula Ltd., UK) for each animal was weighted away from direct light as possible just before injection according to the animal's body weight, immediately dissolved in physiological saline (0.9% NaCl) in a manner to meet a final volume of injection equal to 20 ml per each animal. Alloxan injected as soon as it prepared intravenously within three minutes. Experimental design

Preliminary study: a series of alloxan doses; 75, 100 and 125 mg. kg^{-1} body weight used to fix the effective dose in local breed of donkeys. Each level of dose injected to two donkeys in a single injection. The ideal diabetogenic dose was established using serum glucose level after forty- eight hours as an indicator.

Major study: Basing on the foundations of the preliminary study, the dose of 100 mg. kg^{-1} was the ideal for the induction of diabetes in donkeys.

Two groups of donkey males was subjected to the experimental induction of diabetes as follow:

- Control: Injected with 20 ml of physiological saline intravenously.

- Diabetic: Injected with alloxan in a single dose of 100 mg. kg^{-1} body weight intravenously.

Blood specimens:

Collection of blood: Five ml of venous blood of the fasted donkeys was obtained from jugular vein in a glass centrifuge tubes at each time, an additional one ml of blood was obtained in EDTA tubes for estimation of hemoglobin concentration. Specimens collected during the major study at the times, zero, 24 hours, 48 hours, 72 hours, 7 days and 15 days respectively after alloxan injection.

Separation of serum: Blood in the centrifuge tubes allowed for coagulation then centrifuged at 1500 rpm for twenty minutes (Fox *et al*., 1997) using bench top centrifuge (Wagtech, UK). Supernatant serum aspirated using disposable Pasteur pipettes and kept in polyethylene containers at -20 °C.

Biochemical tests: All biochemical tests were performed in the Central Lab. of Researches, College of Veterinary Medicine, University of Mosul. Parameters including glucose, total cholesterol, triglycerides, albumin, total bilirubin, creatinine, blood urea nitrogen and inorganic
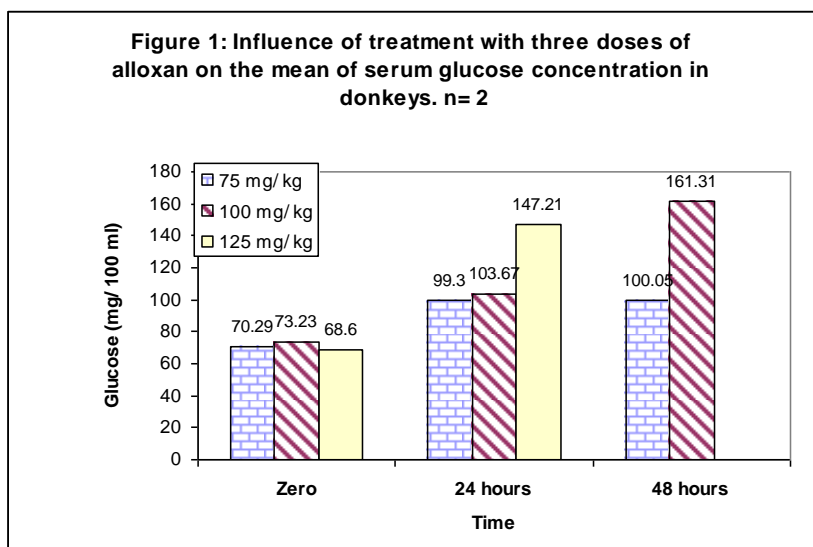
phosphate were assayed using photometric kits manufactured by Fabricant BioLabo SA. France. Activity of serum enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and creatine phosphokinase (CPK) was estimated using photometric kits manufactured by BioMerieux SA. France while gamma glutamyl transferase GGT and amylase activity was determined using specific kits of Reflotron$^{®}$ Plus chemistry analyzer, Roche. USA. The absorbance of samples was observed using UV/ Visible spectrophotometer Biotech 2601. UK. Concentrations of serum electrolytes including sodium (Na^+), potassium (K^+) and calcium (Ca^{+2}) were detected using automated electrolyte analyzer SmartLyte. Diamond. USA.

Serum total protein estimated photometrically using Biuret reagent (Burtis and Ashwood 1999) concentration of unknown serum samples assessed by application of calibration curve whereas hemoglobin (Hb) concentration was determined in the EDTA- whole blood samples using fully automated veterinary hematology coulter Abacus Junior. Diatron. Italy. Photometric analytical methods were used to determine the concentration of both serum glutathione (GSH) (Burtis and Ashwood 1999) and malondialdehyde (MDA) (Beuge and Aust, 1978). The concentration of both was calculated using extinction coefficient.
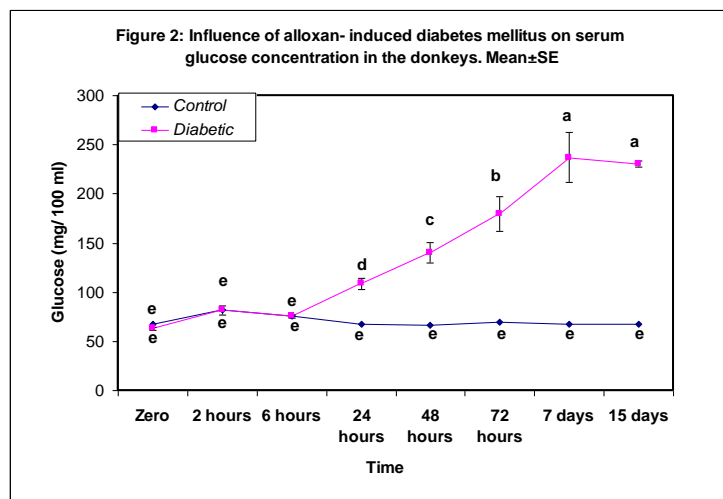
Data analysis: The quantitative data were subjected to two- way analysis of variance according to Steel and Torrie, (1980). Mean differences were statistically compared using $p \leq 0.05$ as a level of significance by Duncan multiple test (Duncan, 1955). Analysis was achieved by means of computer software, statistical package for social sciences SPSS.

RESULTS

Preliminary study: As shown in figure 1, the ideal diabetogenic dose of alloxan in the local breed of donkey males might be 100 mg.kg^{-1} which resulted in an elevation in serum glucose whereas the single dose of 75 mg.kg^{-1} was insufficient to induce a satisfactory diabetic hyperglycemia after 48 hours. The administration of dose 125 mg.kg^{-1} resulted in a high toxicity just after several hours of injection where worthy mentionable that one of the animals received alloxan dose 125 mg.kg^{-1} perished after about 17 hours and the remainder one perished after 28 hours since injection time.

Figure 1: Influence of treatment with three doses of alloxan on the mean of serum glucose concentration in donkeys. n= 2

Glucose (mg/ 100 ml)

- □ 75 mg/ kg
- ▨ 100 mg/ kg
- □ 125 mg/ kg

Zero: 70.29 73.23 68.6
24 hours: 99.3 103.67 147.21
48 hours: 100.05 161.31

Time

Major study: a significant gradual elevation ($p \leq 0.05$) in serum glucose was observed in the alloxan- treated group compared with normal control since 24 hours after alloxan injection reaching the more significance in the days 7 and 15 where there is a statistical difference of there values in comparison with control (figure 2).

Figure 2: Influence of alloxan- induced diabetes mellitus on serum glucose concentration in the donkeys. Mean±SE

Glucose (mg/ 100 ml)

- ◆ Control
- ■ Diabetic

Time: Zero, 2 hours, 6 hours, 24 hours, 48 hours, 72 hours, 7 days, 15 days

Serum total cholesterol (TC) level start to elevate after 24 hours of treatment as a result of induced diabetes relative to both zero time or control group, total cholesterol continued to elevate keeping on the same

levels throughout 48, 72 hours and 7 days. The observed value in the 15th day was slightly decreased however it stilled statistically parallel to the value of 7th day (Table 1).

Table 1: Influence of alloxan- induced diabetes mellitus on serum total cholesterol and triglycerides concentrations in donkeys.

TC (mg.100 ml^{-1})						
Time Groups	Zero	24 hours	48 hours	72 hours	7 days	15 days
Control	79.95 ±0.71 [d]	81.70 ±0.62 [d]	80.39 ±1.43 [d]	79.82 ±1.84 [d]	80.45 ±1.93 [d]	69.25 ±11.49 [d]
Diabetic	78.50 ±1.10 [d]	122.56 ±4.39 [c]	143.87 ±4.55 [ab]	155.05 ±6.84 [a]	147.47 ±7.77 [ab]	133.25 ±4.91 [bc]
TG (mg.100 ml^{-1})						
Control	49.15 ± 6.67 [de]	54.54 ± 6.56 [de]	44.14 ±5.29 [e]	57.90 ±3.19 [c-e]	56.67 ±3.75 [c-e]	56.65 ±2.44 [c-e]
Diabetic	61.56 ±1.07 [cd]	69.01 ±.67 [c]	89.7 ±3.70 [b]	115.43 ±5.75 [a]	97.62 ±4.37 [b]	93.42 ±4.04 [b]

- Values are expressed as mean± SE
- Different letters in the columns or rows refers to a significant difference p≤0.05
- n= 7

The level of serum total triglycerides (TG) also elevated after 24 hours from alloxan injection and so on reaching the peak level at 72 hours which statistically considered the highest value (p≤0.05) compared with either control group or zero hour. Triglycerides level of the diabetic animals showed significant decline outset from the 7th day to the 15th day in comparison with the value observed after 72 hours of diabetes induction (Table 1).

There was an elevation in the serum total protein (TP) in the diabetic donkeys after 72 hours of alloxan injection till the end of 15th day, the elevation was significant (p≤0.05) when compared with either control or with the baseline level. Serum albumin concentration also elevated in the same manner of that related to total protein with a notification that the value of 15th day was statistically considered the highest (Table 2).

Table 2: Influence of alloxan- induced diabetes mellitus on serum proteins concentrations in donkeys.

Time Groups	Zero	24 hours	48 hours	72 hours	7 days	15 days
TP (g.100 ml^{-1})						
Control	6.73 ±0.06 [b]	6.30 ±0.27 [b]	6.60 ±0.24 [b]	6.68 ±0.09 [b]	6.68 ±0.08 [b]	6.60 ±0.06 [b]
Diabetic	6.76 ±0.06 [b]	6.59 ±0.05 [b]	6.71 ±0.24 [b]	7.24 ±0.10 [a]	7.59 ±0.05 [a]	7.64 ±0.05 [a]
Albumin (g.100 ml^{-1})						
Control	3.08 ±0.04 [c]	3.03 ±0.04 [c]	3.13 ±0.03 [c]	3.10 ±0.02 [c]	3.07 ±0.05 [c]	3.15 ±0.03 [c]
Diabetic	3.12 ±0.03 [c]	3.16 ±0.03 [c]	3.24 ±0.02 [c]	3.79 ±0.08 [b]	3.90 ±0.19 [b]	4.21 ±0.10 [a]

- Values are expressed as mean± SE
- Different letters in the columns or rows refers to a significant difference p≤0.05
- n= 7

The activity of serum alanine aminotransferase (ALT) start to increase significantly (p≤0.05) after 24 hours of the alloxan injection. The peak activity was shown at 48 and 72 hours then it revealed a significant drop (p≤0.05) in both 7th and 15th days but it maintained more significant (p≤0.05) than those of control or baseline activity levels (Table 3).

Aspartate aminotransferase (AST) activity showed a significant (p≤0.05) elevation at 48 hours compared with control and zero hour (Table 3). The value raised significantly at 72 hours and 7 days respectively while it returns to drop (p≤0.05) in the 15th day to be around the value of 72 hours.

Table 3 also show an increase in the activity of alkaline phosphatase (ALP) at 24 hours in the alloxan- treated group relative to control and zero hour, the increase in turns to be distinct at 48 hours reaching the peak level at 72 hours then ALP activity commenced to reduce in 7th and 15th days to stable around the value of 24 hours.

Creatine phosphokinase (CPK) activity increased significantly (p≤0.05) at 24 hours of the diabetic donkeys compared to control and zero time The significance increased at 48 hours then declined to approach the level of that shown at 24 hours while the end value observed after 15 days of treatment was significantly (p≤0.05) less than those of 72 hours and 7 days but still significant relative to control (Table 3).

Gamma glutamyl transferase (GGT) activity elevated significantly at 24 hours compared to control and zero time. At 48 hours, the value was statistically higher than that of 24 hours while the more significant ($p \leq 0.05$) elevation was recognized at 72 hours, 7 and 15 days (Table 3). Amylase activity fluctuates during the period of experiment however no distinct significance observed (Table 3).

Table 3: Influence of alloxan- induced diabetes mellitus on serum enzymes activity in donkeys.

Time / Groups	Zero	24 hours	48 hours	72 hours	7 days	15 days
ALT (U.L^{-1})						
Control	63.83 ±0.07 [d]	61.0 ±1.15 [d]	61.83 ±0.94 [d]	60.0 ±2.16 [d]	63.83 ±0.87 [d]	63.66 ±1.0 [d]
Diabetic	63.33 ±0.98 [d]	68.33 ±1.05 [c]	81.0 ±1.15 [a]	80.16 ±1.40 [a]	74.33 ±1.81 [b]	71.33 ±1.56 [bc]
AST (U.L^{-1})						
Control	36.0 ±1.21 [d]	35.83 ±0.74 [d]	35.83 ±0.65 [d]	36.33 ±1.08 [d]	36.66 ±0.95 [d]	36.50 ±1.64 [d]
Diabetic	36.33 ±0.76 [d]	36.83 ±0.98 [d]	44.33 ±0.98 [c]	60.66 ±2.36 [b]	66.50 ±1.60 [a]	59.33 ±1.45 [b]
ALP (U.L^{-1})						
Control	156.83 ±6.03 [d]	154.16 ±5.26 [d]	161.33 ±4.03 [d]	167.16 ±3.87 [d]	157.50 ±4.01 [d]	163.0 ±4.57 [d]
Diabetic	163.83 ±5.73 [d]	210.66 ±7.32 [c]	262.50 ±11.96 [b]	310.83 ±9.76 [a]	213.50 ±5.53 [c]	203.50 ±8.60 [c]
CPK (U.L^{-1})						
Control	126.50 ±3.07 [d]	124.50 ±2.83 [d]	131.66 ±1.74 [d]	130.16 ±2.15 [d]	126.50 ±1.83 [d]	129.16 ±2.08 [d]
Diabetic	129.83 ±0.89 [d]	199.66 ±7.92 [b]	222.0 ±5.70 [a]	192.83 ±6.11 [b]	196.0 ±4.93 [b]	162.83 ±10.34 [c]
GGT (U.L^{-1})						
Control	37.0 ±0.85 [d]	41.33 ±0.88 [d]	39.0 ±1.09 [d]	39.50 ±1.23 [d]	39.50 ±0.84 [d]	37.50 ±1.66 [d]
Diabetic	37.66 ±0.84 [d]	67.33 ±2.09 [c]	75.66 ±2.65 [b]	81.83 ±1.24 [a]	80.33 ±2.13 [a]	82.0 ±1.03 [a]
Amylase (U.L^{-1})						
Control	59.833 ±1.74 [ab]	62.833 ±0.79 [a]	62.333 ±1.11 [a]	61.833 ±1.53 [ab]	63.333 ±0.71 [a]	62.0 ±0.85 [ab]
Diabetic	57.666 ±1.30 [b]	62.666 ±1.56 [a]	60.833 ±2.60 [ab]	59.333 ±1.85 [ab]	62.166 ±0.79 [a]	61.166 ±1.40 [ab]

- Values are expressed as mean± SE
- Different letters in the columns or rows refers to a significant difference $p \leq 0.05$
- n= 7

Neither serum total bilirubin nor blood hemoglobin (Hb) concentrations were altered as a result of experimental diabetes in male donkey (Table 4). Sodium (Na^+) in serum concentration did not affected by the process of diabetes induction (Table 5) likewise the concentration of serum potassium (K^+) yet K^+ manifests a mildly fluctuation in comparison with control however there is no distinct significance ($p \leq 0.05$).

Table 4: Influence of alloxan- induced diabetes mellitus on serum total bilirubin and hemoglobin concentrations in donkeys.

Total bilirubin (mg.100 ml⁻¹)						
Time \ Groups	Zero	24 hours	48 hours	72 hours	7 days	15 days
Control	0.27 ±0.01 ᵃ⁻ᶜ	0.28 ±0.01 ᵃ⁻ᶜ	0.27 ±0.01 ᵃ⁻ᶜ	0.29 ±0.00 ᵃᵇ	0.30 ±0.00 ᵃ	0.30 ±0.00 ᵃ
Diabetic	0.26 ±0.01 ᵇᶜ	0.25 ±0.01 ᵇᶜ	0.25 ±0.01 ᵇᶜ	0.025 ±0.01 ᵇᶜ	0.27 ±0.01 ᵃ⁻ᶜ	0.27 ±0.01 ᵃ⁻ᶜ
Hb (g.100 ml⁻¹)						
Control	8.56 ±0.25 ᵃ	8.04 ±0.39 ᵃ	8.39 ±0.25 ᵃ	8.29 ±0.31 ᵃ	8.07 ±0.21 ᵃ	8.35 ±0.28 ᵃ
Diabetic	8.36 ±0.22 ᵃ	8.14 ±0.57 ᵃ	8.52 ±0.30 ᵃ	8.24 ±0.29 ᵃ	8.32 ±0.30 ᵃ	7.95 ±0.27 ᵃ

- Values are expressed as mean± SE
- Different letters in the columns or rows refers to a significant difference p≤0.05
- n= 7

Table 5: Influence of alloxan- induced diabetes mellitus on serum electrolytes concentration in donkeys.

Na⁺ (mmol.L⁻¹)						
Time \ Groups	Zero	24 hours	48 hours	72 hours	7 days	15 days
Control	103.0 ±2.69 ᵃ	101.0 ±1.96 ᵃ	102.16 ±2.25 ᵃ	101.16 ±4.10 ᵃ	98.16 ±2.62 ᵃ	97.33 ±1.56 ᵃ
Diabetic	97.50 ±3.08 ᵃ	102.50 ±2.81 ᵃ	104.66 ±3.91 ᵃ	96.50 ±3.87 ᵃ	98.83 ±1.62 ᵃ	98.83 ±1.49 ᵃ
K⁺ (mmol.L⁻¹)						
Control	5.42 ±0.16 ᵃᵇ	5.22 ±0.05 ᵃ⁻ᶜ	5.41 ±0.10 ᵃᵇ	5.35 ±0.10 ᵃ⁻ᶜ	5.45 ±0.12 ᵃ	5.18 ±0.04 ᵇᶜ
Diabetic	5.08 ±0.04 ᶜ	5.11 ±0.03 ᶜ	5.09 ±0.05 ᶜ	5.12 ±0.03 ᶜ	5.16 ±0.02 ᵇᶜ	5.11 ±0.08 ᶜ

- Values are expressed as mean± SE
- Different letters in the columns or rows refers to a significant difference p≤0.05
- n= 7

Calcium concentration decreased significantly ($p \leq 0.05$) after 24 hours compared to control and baseline value (Table 6). Inorganic phosphate concentration exhibited an obvious increase since 24 hours of treatment and so all over the period of experiment (Table 6).

Table 6: Influence of alloxan- induced diabetes mellitus on serum calcium and inorganic phosphate concentrations in donkeys.

Ca^{+2} (mg.100 ml^{-1})						
Time \ Groups	Zero	24 hours	48 hours	72 hours	7 days	15 days
Control	1.520 ±0.03 [a]	1.501 ±0.02 [a]	1.498 ±0.02 [a]	1.511 ±0.02 [a]	1.433 ±0.02 [ab]	1.514 ±0.03 [a]
Diabetic	1.491 ±0.03 [a]	1.368 ±0.03 [b-d]	1.311 ±0.02 [cd]	1.296 ±0.02 [cd]	1.293 ±0.00 [d]	1.296 ±0.03 [cd]
Inorganic phosphate (mg.100 ml^{-1})						
Control	1.28 ±0.01 [b]	1.28 ±0.02 [b]	1.29 ±0.01 [b]	1.27 ±0.01 [b]	1.26 ±0.01 [b]	1.31 ±0.04 [b]
Diabetic	1.30 ±0.02 [b]	2.24 ±0.03 [a]	2.24 ±0.03 [a]	2.24 ±0.02 [a]	2.24 ±0.03 [a]	2.25 ±0.02 [a]

- Values are expressed as mean± SE
- Different letters in the columns or rows refers to a significant difference $p \leq 0.05$
- n= 7

Serum creatinine concentration exhibited a significant ($p \leq 0.05$) elevation since 24 hours after injection compared to control, the value observed at 48 hours was significantly higher than that of 24 hours. The peak significant ($p \leq 0.05$) level of creatinine illustrated at 72 hours, 7 and 15 days (Table 7). Blood urea nitrogen BUN exhibited a significant elevation relative to the control at 48 and 72 hours whereas the level of BUN obtained in the 7th and 15th days was increased to be more significant ($p \leq 0.05$) than the values of both 48 and 72 hours (Table 7).

Table 7: Influence of alloxan- induced diabetes mellitus on some kidney function tests in donkeys.

Creatinine (mg.100 ml^{-1})						
Time ╲ Groups	Zero	24 hours	48 hours	72 hours	7 days	15 days
Control	0.91 ±0.00 ^{d}	0.91 ±0.00 ^{d}	0.90 ±0.02 ^{d}	0.92 ±0.01 ^{d}	0.91 ±0.01 ^{d}	0.90 ±0.00 ^{d}
Diabetic	0.93 ±0.01 ^{cd}	1.0 ±0.02 ^{c}	1.22 ±0.03 ^{b}	1.47 ±0.04 ^{a}	1.54 ±0.04 ^{a}	1.55 ±0.04 ^{a}
BUN (mg.100 ml^{-1})						
Control	12.66 ±0.21 ^{c}	12.50 ±0.22 ^{c}	12.50 ±0.22 ^{c}	11.83 ±0.30 ^{c}	12.16 ±0.30 ^{c}	11.83 ±0.30 ^{c}
Diabetic	11.66 ±0.42 ^{c}	12.83 ±0.54 ^{c}	17.33 ±0.33 ^{b}	17.31 ±.033 ^{b}	19.83 ±1.04 ^{a}	19.83 ±0.47 ^{a}

- Values are expressed as mean± SE
- Different letters in the columns or rows refers to a significant difference p≤0.05
- n= 7

As considered with serum glutathione (GSH) concentration, there was a sharp decrease just at the end of the experiment (15th day) as presented in Table (8) which is significantly (p≤0.05) less than the values of both baseline and counterpart value of control group (p≤0.05). Serum malondialdehyde (MDA) elevated (p≤0.05) at 48 and 72 hours compared with both control and zero hour, MDA values of both 7th and 15th days were greater (p≤0.05) than both there counterparts of control on one hand or 48 and 72 hours of diabetic group on the other hand (Table 8).

Table 8: Influence of alloxan- induced diabetes mellitus on the serum indices of oxidative stress in donkeys.

GSH ($\mu mol.L^{-1}$)					
Time \ Groups	Zero	24 hours	48 hours	72 hours	7 days	15 days
Control	2.05 ±0.11 [a]	2.06 ±0.11 [a]	2.08 ±0.09 [a]	2.06 ±0.07 [a]	2.06 ±0.11 [a]	2.15 ±0.10 [a]
Diabetic	2.09 ±0.09 [a]	2.06 ±0.10 [a]	2.06 ±0.10 [a]	2.07 ±0.09 [a]	2.06 ±0.17 [a]	1.49 ±0.16 [b]
MDA ($\mu mol.L^{-1}$)					
Control	0.58 ±0.02 [c]	0.53 ±0.02 [c]	0.53 ±0.02 [c]	0.54 ±0.00 [c]	0.54 ±0.02 [c]	0.53 ±0.02 [c]
Diabetic	0.60 ±0.04 [c]	0.56 ±0.04 [c]	0.74 ±0.03 [b]	0.83 ±0.04 [b]	0.94 ±0.02 [a]	0.98 ±0.02 [a]

- Values are expressed as mean± SE
- Different letters in the columns or rows refers to a significant difference p≤0.05
- n= 7

DISCUSSION

Hyperglycemia is the earliest logical result of alloxan injection in most species however human considered resistant to this effect, our present results are an assertion of many previous outcomes like Sikarwar and Patil (2010); Geetha *et al.* (2011); Rotimi *et al.* (2011) who reported that hyperglycemia is a common effect of insulin dependent diabetes mellitus IDDM. Hyperglycemia is the sequel of lack of circulating insulin so hepatic release of glucose is uninhibited (Yki-Jarvinen *et al*., 1984). The action of alloxan in the pancrease is preceded by it's rapid uptake by β- cells (Boquist *et al*., 1983) and liver (Tiedge *et al*., 1997). This action has been implicated in the alloxan diabetogenicity as well as the hypothesis of the role of reactive oxygen species (ROS) formation (Heikkila *et al*., 1976) as Udayakumar *et al.* (2009) presented that one of the diabetes manifestations is the enhancement of liver enzyme, glucose 6- phosphatase activity, the key enzyme for the process of gluconeogenesis. Lenzen *et al.* (1992) proposed an additional mechanism for alloxan- induced hyperglycemia which suggests that alloxan reacts with two sulfhydryl groups SH at the

sugar binding side of glucokinase which is essential for glycolysis and proper insulin secretion leading to the creation of disulfide bonds and inhibition of enzyme.

DNA of β- cells is well established as a target of the rising ROS so fragmentations will take place in β- cells exposure to alloxan (Sakurai and Ogiso, 1995). The damaged DNA stimulates poly ADP ribosylation which can partially regulates DNA reformation (LeDoux *et al*., 1988). Enzymatic and non enzymatic antioxidants also participate to some extent in the process of cell protection against alloxan- induced damage (Jórns *et al*., 1999), which may delay the sharp hyperglycemia. This hypothesis explains why did glucose elevation in this study did not detected until 24 hours of alloxan injection.

Means of glucose presented in figure 2 demonstrates that the onset of hyperglycemia commenced since 24 hours of alloxan injection however no detection of the early stage of hypoglycemia which must follow alloxan injection, this suggests that this stage might be detected in the donkeys between the 2nd and 6th hours after injection.

Hypercholesterolemia and hypertriglyceridemia is often arise from the state of diabetic hyperglycemia. This is similar to data of both Kheder, (2007); Geetha *et al*. (2011). The effect produced by insulin deficiency and it's most prominent feature, hypercholesterolemia attributed to the mobilization of fats from peripheral adipose tissues due to underutilization of glucose (Nimenibo-Uadia, 2003). The lack of insulin inhibitory action of lipase and related elevation of counter regulatory hormones lead to activation of lypolytic enzymes in adipose tissues, acyl Co-A: Cholesterol acyl transferase, the enzyme engaging a role in the intestinal absorption of cholesterol which is up regulated during insulin deficiency which can be elucidates another aspect of the hypercholesterolemia (Hori *et al*., 2004). Furthermore, lipoprotein lipase may be inhibited as a consequence of insulin deficiency and hypertriglyceridemia will be common resulted from diminished rate of triglycerides mobilization into glycerol and free fatty acids (Nelson and Cox, 2005).

Regarding serum proteins concentration, present study demonstrated a condition of hyperproteinemia and hyperalbuminemia resulted from experimental diabetes in the donkeys counterwise to the most studies Udayakumar *et al*. (2009); Mansour *et al*. (2002) who subjected lab. animals to alloxan. These studies interpreted diabetic

hypoproteinemia as a result of negative nitrogen balance, enhanced proteolysis and lowered protein synthesis in the case of lack of insulin (Pathak and Dhawan, 1998). The reports of Peavy *et al.* (1978) described the metabolic effect of hypoproteinemia and the sequence hypoalbuminemia as an outcome of decreased level of albumin mRNA in the liver of diabetic animals while Pushkina *et al.* (1987) reported that there is no alteration in serum proteins during experimental diabetes. However the observations of present study resembled that of Kheder, (2007) who detected a significant (p≤0.05) hyperproteinemia following the induction of diabetes. The findings might be explained as a result of diabetic polyurea and consequence dehydration and hemococentration (Khan and Hershey 2001).

Liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are elevated as a result of alloxan-induced diabetes mellitus which agree with Udayakumar *et al.* (2009); Hamden *et al.* (2009a). Transaminases are directly related to changes in the metabolic activities in which the enzymes are involved. Both ALT and AST activities might increased during insulin deficiency because of the availability of amino acids in blood as well as the implication of increased gluconeogenesis and ketogenesis (Gokce and Haznedaroglu, 2008), also alloxan- induced liver damage might be partially responsible for elevated transaminases activity, the findings based on decreased activity of both ALT and AST in the liver of diabetic rats in contrast elevation in plasma (Mansour *et al.*, 2002).

Gamma glutamyl transferase GGT and alkaline phosphatase ALP activities are also elevated in the present study likewise Udayakumar *et al.* (2009); Hamden *et al.* (2009b). Both GGT and ALP acts as indicators of liver function hence the disturbance of their plasma activity indicates abnormal liver function which attributes to alloxan- induced lipid peroxides (LPO) mediated tissue damage in pancrease, liver, kidney and heart (Prince *et al.*, 1997). So increased activity of these two enzymes in this trail may be a result of leaking out from tissues into the circulation through alteration membrane's architecture. The event enhances liver enzymes activity in plasma (Prince and Menon, 2000).

An elevated creatine phosphokinase CPK activity in the present study is in agreement with the findings of Kain *et al.* (2010) and it can be elucidated as a score of cardiac complications and damage represented as heart infarction (Howard-Alpe *et al.*, 2006). Amylase activity did not reflect a clear cut significant values however previous

studies demonstrates a decline in amylase activity as a result of diabetes in lab. animals (Otsuki and Williams, 1982; Pierzynowski and Barej, 1984) which may reflect a species variations in donkeys compared to others.

The level of total bilirubin insignificantly decreased. This observations did not harmonized with both Hamden *et al*., (2009a); Mansour *et al.* (2002) and who reported a condition of hyperbilirubinemia in alloxan- induced diabetic rats. On the other hand, Takayanagi *et al.* (2011) demonstrated that diabetes might induce hypobilirubinemia as an of inverse correlation between diabetic hyperglycemia and level of plasma bilirubin a fact of equine feature in bilirubin excretion. Hemoglobin (Hb) concentration in the present study also kept on the same basal level with no diabetic- induced alteration in the blood of treated donkeys.

Insignificant changes in serum sodium (Na^+) concentration observed from alloxan- injected donkeys was evident whereas a few previous studies differed about this aspect when Ikpi *et al.* (2009) referred to a status of decreased plasma concentration of sodium as a result of alloxan- induced diabetes in rats. However our present observations stayed in agreement with Soto *et al*. (2001). Potassium (K^+) also considered insignificant changed similarly in diabetic donkeys when compared with baseline value although a significant hypokalemia observed in both 48 hours and 7 days after alloxan injection in respect to control only. A fact which was partially agreed with Dhawan *et al*. (1999) in diabetic rats. On the contrary, Kpi *et al*. (2009) who did not report any alteration in potassium level.

The reduced serum calcium (Ca^{+2}) confirms the findings of Duarte *et al.* (2005), a result which can be explicated as a result of diabetic lipidemia and it's significant impact to impair Ca^{+2} efflux from the cell (Witczak and Sturek, 2004). Elevated extracellular glucose concentration triggers the opening of Ca^{+2} channels and consequence influx of Ca^{+2} into the cells which serves as critical trigger for insulin exocytosis process (Laughlin *et al*., 1989). The disturbances in the intracellular Ca^{+2} homeostasis considered an important step in the diabetogenic effect of alloxan which demonstrated as alloxan- induced elevation in cytosolic free Ca^{+2} in β- cells (Park *et al*., 1995). The change may also arises from exaggerated mobilization from intracellular medium and it's limited elimination from the cytoplasm. Ca^{+2} influx may result from alloxan ability to depolarize β- cells (Dean and Matthews,

1972). Depolarization of cell membrane enhances the opening of voltage- dpendent Ca^{+2} channels and increases Ca^{+2} entry into the cells (Lenzen *et al*., 1992).

Alloxan- induced restriction of Ca^{+2} removal from the cells due to alloxan- induced inhibition of liver plasma membrane Ca^{+2}- ATPase was reported (Seckin *et al*., 1993). So the result of these events is hypocalcemia which confirms our data. Park *et al*., (1995) interpreted the effect of alloxan on cell Ca^{+2} as a consequence of elevated hydrogen peroxide H_2O_2 accompany alloxan administration.

Hyperphosphatemia affirms the observations of Mahmud *et al*. (2011) versus to Bicer *et al*. (2011) who recorded the reverse result in diabetic rats. Also Duarte *et al*. (2005) did not recorded a significant alteration in the serum inorganic phosphate resulted from alloxan treatment. In vitro, cell incubation with alloxan yields an activation of ALP activity in rat kidney and small intestine (Parsadanian *et al*., 1989) Also alloxan- induced hyperphosphatemia suggests that diabetic status and resulted ketoacidosis prohibits the uptake of glucose and inorganic phosphate by the insulin- sensitive cells (Ditzel and Lervang, 2010).

It has been well established that the elevation of both creatinine and blood urea nitrogen BUN is a common result of diabetes mellitus which is in agreement with Hamden *et al*. (2009b); Tavafi *et al*. (2011). Early stages of diabetic nephropathy characterized by glomerular hyperfiltration which increases glomerular filtration rate (GFR) and it is contributes to the progression of nephropathy (Sochett *et al*., 2006). Also there was a down- regulation of nitric oxide synthase binding sites in addition to a reduction in NADPH- diaphorase activity in the diabetic renal cortex, medulla and glomeruli (Mumtaz *et al*., 2004). Winiarska *et al.* (2011) documented that diabetes- induced elevated NADPH oxidase activity catalyses superoxide radical formation which implicated to be the source of ROS in kidney. Thus the development of diabetic nephropathy is prevailing. Evan *et al.* (1984) proved the prejudicial role of alloxan on kidney of lab animals through a histopathological study which revealed a focal glomerular basement membrane thickening as well as glomerular capillary endothelial abnormalities and visceral foot process fusion and reduction in the size of endothelial fenestae. Furthermore, alloxan- induced activation in sphingosine kinase- sphingosine 1- phosphate signaling pathway might be involved in the pathogenesis of diabetic nephropathy and renal injury (Lan *et al*., 2010).

The state of hyperglycemia discussed above beside to the increased ROS production resulted from alloxan injection are implicated in motivating the formation of ROS creating an oxidative atmosphere in the cells through impairment the endogenous antioxidant system (West, 2000). Reduced glutathione GSH protects the cellular system against the detrimental effect of LPO (Nicotera and Orrenius, 1986) and also serves as a free radical scavenger, co- substrate for glutathione peroxidase GSH_{px} as well as GSH forms conjugates in xenobiotic reactions (Gregus *et al*., 1996). So the elevated serum GSH observed in this study might be explored as a result of alloxan and it's reduced product dialuric acid when they reacts with oxygen yields superoxide radical O^-_2 and hydrogen peroxide H_2O_2 and in the presence of O^-_2. The formation of hydroxyl radical $OH^{\cdot}$ is predicted leading to peroxidation of numerous biomolecules including proteins (Halliwell and Gutteridge, 1985). Damaged protein is a common reason for the decreased activity of GSH-S transferase, GSH reductase and glucose 6- phosphate dehydrogenase (Letelier *et al*., 2005), the enzymes responsible for GSH biosynthesis and reactivation.

Kochar and Umathe, (2009) recorded a decrease in GSH beside increase in malondialdehyde MDA as well as a decrease in the activity of antioxidant enzymes including superoxide dismutase SOD and catalase CAT. These findings confirms the observations of Fisher and Humburger, (1980) in pancreatic islets, erythrocytes (Yadav *et al*., 1996), kidney (Kêdziora-Kornatowska *et al*., 2002), liver and testis (El-Missiry and El Gindy, 2000) which attributed to inactivation of mitochondrial and cytosolic antioxidant enzymes due to decreased their protein expression levels in the diabetic conditions (Sindhu *et al*., 2004).

Thiobarbituric acid reacting substance (MDA) which represents one of the lipid peroxidation products was elevated as a result of alloxan injection, a result can be explained on the base of previous in vitro study observed that occurrence of glucose enolization results in reduction of molecular oxygen and production of oxygen free radicals and α-ketoaldehydes which ultimately cause peroxidative breakdown of phospholipids and accumulation of MDA (Kochar and Umathe, 2009).

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