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Research Article

Short –term clinical and pathological alterations associated with a single intravenous injection of alloxan monohydrate in dogs

Zuhair Bani Ismail, Wael Hananeh, Musa Alshehabat, Mousa Daradka, Jafar Ali
Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, Jordan.

Abstract: This study aimed to investigate possible clinical and histological alterations associated with a single intravenous injection of alloxan monohydrate at 120 mg/kg to induce diabetes mellitus in dogs. A total of 6 (3 alloxan injected or DM group and 3 saline injected or control group) healthy, adult (2-3 years of age), male mixed breed dogs were used. Diabetes mellitus was confirmed in all DM group by performing an oral glucose tolerance test. Hematology parameters and serum biochemical analyses were performed before injection (Day -1), and at 1, 2, 5, 10, 21, and 28 days post injection. At the end of the study period (day 28), all dogs were humanely euthanized and complete necropsy was performed. All alloxan injected dogs developed diabetes and survived to the end of the study. Clinically, however, DM dogs suffered variable degrees of lethargy, partial anorexia and mild to moderate weight loss. In DM group, there was a significant increase in serum glucose and cholesterol levels. Histopathological alterations were observed in the hepatic parenchyma, bile ducts, pancreas and kidneys and were characterized by variable degrees of cytoplasmic vacuolation.

Key Words: diabetes mellitus, alloxan, dogs, clinico-pathology.

Corresponding Authors: Z. Bani Ismail, e-mail: zuhair72@just.edu.jo.

Introduction

Dogs are commonly used model for studying diabetes mellitus in human beings (Rees 2005; Chatzigeorgiou et al 2009; Valilou et al 2011). Furthermore, naturally occurring diabetes is very common in this animal species with all its clinical manifestations also similar to those reported in human patients (Valilou et al 2011). Experimentally, diabetes mellitus can be induced in animal models using dietary manipulations and injection of a diabetogernic drug such as alloxan or streptozocin or a combination of such methods (Szkudelski 2001; Akbarzadeh et al 2007; Abu Abeeleh et al 2009; Rohilla & Ali 2012). Diabetes mellitus is a common chronic endocrine disease that can cause a wide range of systemic abnormalities and clinical manifestations in both humans and animals such as hyperglycemia and glucoseuria, polydypsia, polyurea, polyphagea, weight abnormalities, microvascular and cardiovascular pathology, renopathy, retinopathy, heart and coronary problems, ketoacidosis and hypertension (Valilou et al 2011).

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is a derivative of pyrimidine (Rohilla & Ali 2012). It has been widely used as a diabetogenic agent in animal models (Rees 2005; Chatzigeorgiou et al 2009; Valilou et al 2011). It is a strong oxidizing agent that lead to a complete destruction of beta cells in the pancreas by releasing of oxygen free radicals (Valilou et al 2011). Alloxan is extremely cytotoxic to other tissues and body organs as well. Results of previous research in alloxan-induced diabetes in animal models were inconsistent regarding changes in the hematopoietic system (Watanabe et al 2004; Valilou et al 2007; Valilou & Lotfi 2010; Abu-Samak et al 2007; Azeez et al 2010). In addition, there is a paucity of information regarding changes in the biochemical profile in alloxan-induced diabetes in dogs. Hence the aim of this study was to document hematological, serum biochemical and histopathological changes associated with single intravenous injection of alloxan to induce diabetes mellitus in dogs.

Material and methods

All experimental procedures carried out in this project were reviewed and approved by the Jordan University of Science and Technology Institutional Animal Care and Use Committee (JUST-ACUC).

A total of 6, healthy male adult (2-3 years old) mixed breed dogs were used in this study. Dogs were housed individually in cages and allowed free access to fresh water. Dogs were fed twice daily regular dry pelleted dog feed (local supplier). All dogs were allowed outside exercise twice daily for 30 minutes. Before the start of the study, dogs were physically examined to make sure they were healthy. Whole blood was collected via jugular vein puncture and placed in plain and EDTA containing tubes

Table 1. Mean ± SD of hematology parameters in dogs administered alloxan intravenously to induce diabetes mellitus

Parameters	Pre-induction			Post-induction			
	Day -1	Day 1	Day 2	Day 5	Day 10	Day 21	Day 28
	A	B	C	D	E	F	G
WBC (X 10^3/µL)	8.8±2.4	9.8±2.9	8.9±1.1	9.7±1.2	11±1.7	11±1.4	9±0.3
RBCs (X 10^6/µL)	8±0.6	8.6±0.6	$8±0.6^{BD}$	$8.6±0.7^{CF}$	$8±1^{F}$	$7±1.2^{DE}$	8±1.7
Hemoglubin (g/dL)	18±1.2	18.53±1.4	19±1.4	19±1	18±1.7	16±1.9	16±3
Hematocrit (%)	56±3	$58±3.6^{C}$	$57±3^{B}$	$58±3.8^{F}$	$54±5.9^{F}$	$49±6.9^{DF}$	51±10
MCV (fL)	684±1	67±0.6	68±1.2	68±0.9	68±1.5	67±1.7	67±1.7
MCH (pg)	$22±0.6^{G}$	$21±0.3^{C}$	$23±0.1^{BG}$	$22±0.5^{G}$	22±0.7	22±0.9	$21±0.6^{CDE}$
MCHC (g/dL)	$32±0.4^{G}$	$32±0.6^{C}$	$33±0.5^{BG}$	$32±0.3^{E}$	$32±0.3^{D}$	33±1.2	$31±0.2^{AC}$
Platelets X 10^3/µL)	211±23	360±79	192±21	298±54	364±94	179±145	364±102

Superscript capital letters within rows indicate significant difference between different time points ($P \leq 0.05$).

Table 2. Mean ± SD of serum biochemical analysis in dogs administered alloxan intravenously to induce diabetes mellitus

Parameters	Pre-induction			Post-induction			
	Day -1	Day 1	Day 2	Day 5	Day 10	Day 21	Day 28
	A	B	C	D	E	F	G
AST (IU/L)	9±0.0	15±1.4	16±2	12±3	35±25	21±13	21±13
ALT (IU/L)	8±1.4	8±11	15±3.5	13±4	13±5.7	22±8	20±5.7
ALP (IU/L)	43±30	32±40	60±20	65±21	108±22	100±59	53±4
Total protein (g/L)	66±8.5	66±6	$65±2.8^{D}$	$73±3.5^{C}$	75±4	71±1.4	68±0.0
Fibrinogen (mg/dL)	233±57	300±0.0	300±0.0	233±58	333±115	333±58	$400±100^{D}$
Glucose (mg/dL)	$73±17^{DE}$	152±33	263±4.9	$249±2^{AE}$	$338±8^{A}$	286±8	327±60
Cholesterol (mg/dL)	$239±7^{D}$	238±39	$235±7^{D}$	$410±6^{AC}$	498±93	479±39	446±33
Triglycerides (mg/dL)	204±143	54±31	96±23	56±22	99±34	70±20	99±16
Calcium (mg/dL)	9.7±1.5	$8.7±0.2^{E}$	$8.8±0.4^{DE}$	$11±0.5^{BCF}$	$11±0.4^{BCFG}$	$9±0.9^{DE}$	$10±0.4^{E}$
Magnesium (mg/dL)	1.9±0.1	2.8±1.1	2.3±0.4	2±0.4	3±0.5	2±0.1	2.6±03
Sodium (mmol/L)	1455±9	158±25	$160±4.7^{F}$	$146±2^{G}$	150±1.1	$143±5^{C}$	$143±2.6^{D}$
Potassium (mmol/L)	4.6±0.1	$4.7±0.3^{C}$	$4.3±0.3^{B}$	4±0.1	$4±0.5^{G}$	4±0.3	$3.6±0.4^{E}$
Chloride (mmol/L)	$117±1.3^{D}$	123±9	122±4	$108±0.8^{A}$	111±0.1	108±1.6	112±2.5
BUN (mg/dL)	$28±1.3^{BF}$	$14±0.6^{A}$	39±17	19±5	18±9	$14±2^{A}$	27±9.6
Creatinine (mg/dL)	0.6±0.1	0.6±0.8	0.9±0.1	0.7±0.2	2.5±3	0.9±0. 6	1.3±0.8

Superscript capital letters within rows indicate significant difference between different time points ($P \leq 0.05$).

for serum biochemical and hematology analysis respectively. Any dog with abnormal findings was excluded from the study. To make sure dogs were not diabetic; an oral glucose tolerance test (OGTT) was performed in all dogs as described before (Watanabe et al 2004). Dogs with blood glucose over 180 mg/dl at 180 minutes post glucose administration were considered diabetic. Only non diabetic dogs were used in the study. Before alloxan injection, dogs were fasted over night. The next morning (Day -1), dogs (DM group; n=3) were administered a single intravenous injection of 120 mg/kg alloxan monohydrate (LKT Laboratories, Inc., MN, USA) while the other 3 dogs were administered normal saline intravenously and served as control. Blood glucose level was monitored every 2 hours after alloxan injection using Acura blood glucose meter (US Diagnostics, Inc., New York, USA) for the first 48 hours and twice daily for the rest of the study period (28 days). Dogs with 200mg/dl or more fasting blood glucose levels were considered diabetic. On as needed basis, hyperglycemic dogs were administered 10 to 20 units of Insulin (Mixtrad, Novo Nordisk, Denmark) subcutaneously. The OGTT was repeated again 1 week after alloxan injection to confirm diabetes status.

Whole blood was collected as described previously before induction of diabetes (Day -1) and at 1, 2, 5, 10, 21, and 28 days post injection. Hematology and serum biochemical analyses were determined using previously described methods (Thrall 2004). Parameters investigated were: total white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Serum was analyzed to determine total protein (TP), fibrinogen, glucose, cholesterol, triglycerides, blood urea nitrogen (BUN), creatinine, aspartate transaminase (AST), alanintransferase (ALT), alkaline phosphatase (ALP), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), and chloride (Cl). On day 28, all dogs were humanely euthanized using an overdose of an anesthetic solution containing pentobarbital sodium

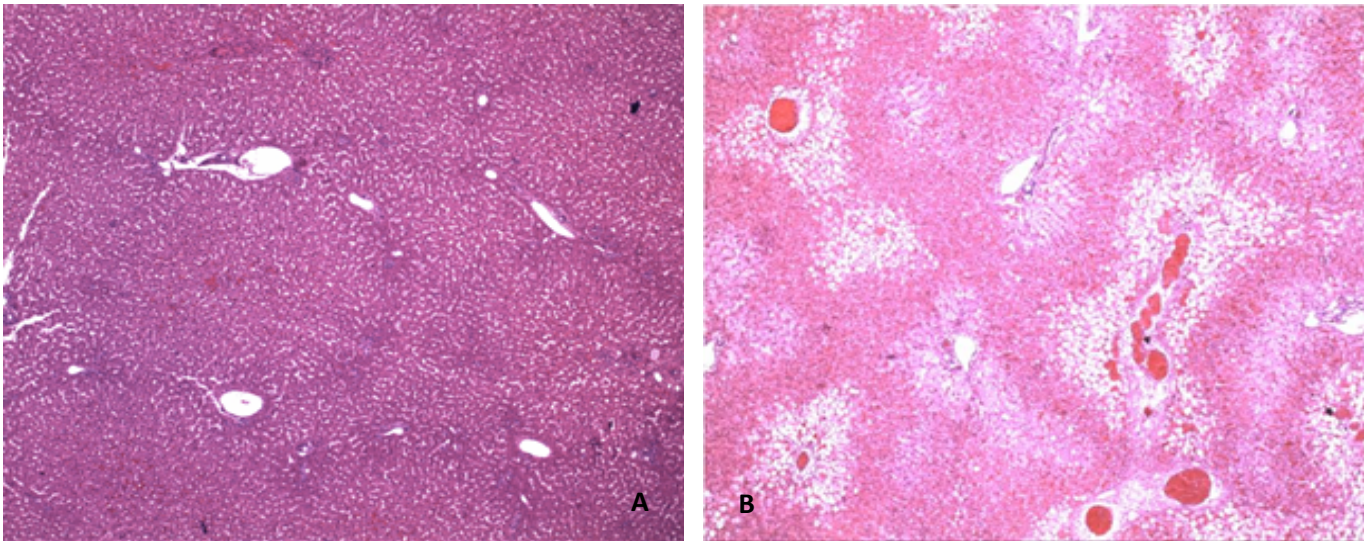

Fig. 1. HE stained sections of the liver from alloxan injected (B) and control (A) dogs. Notice diffuse cytoplasmic vacuolation and necrosis of the periportal hepatic parenchyma in alloxan injected dog. 4X.

and phenytoin sodium (Beuthanasia-D, Intervet, New Jersey, USA). Dogs were then subjected to complete necropsy procedure and tissue representative tissue samples were collected from liver, pancreas, kidney and adrenals. Tissue samples were placed immediately in 10% buffered formalin solution for 24 hours. The tissues were subjected to routine histopathological preparations. Glass slides were stained with Hematoxylin and Eosin (H & E) stain and examined under light microscopy.

Data was presented as mean ± standard deviation. Repeated measure analysis of each of the evaluated variables was performed using GLM repeated measure. Post-hoc pairwise comparisons were performed using Bonferroni test. Differences were considered statistically significant if the $P \leq 0.05$. The analysis was conducted using statistical software (SPSS, Version 19.0, SPSS Inc, Chicago, USA).

Results

All dogs survived to the end of the study. All alloxan injected dogs developed diabetes with a mean value for serum glucose significantly ($P \leq 0.05$) elevated to a level exceeding 200 mg/dl starting from 48 hours following induction of diabetes mellitus and remained high throughout the study period. Clinically, DM dogs suffered variable degrees of lethargy, partial anorexia and mild to moderate weight loss.

Results of the hematology analyses in dogs after a single intravenous injection of alloxan at a dose rate of 120 mg/kg are shown in Table 1. There were no statistically significant differences in any parameter when compared between values obtained before and after alloxan injection. In the serum biochemical analyses (Table 2), mean values for serum glucose were significantly ($P \leq 0.05$) elevated to a level exceeding 200 mg/dl starting from 48 hours following induction of diabetes mellitus and remained high throughout the study period. Data shows also a significant increase in cholesterol level beginning on day 5 of the study and remained high throughout the study. There were no other significant differences in any of the parameters studied.

There were no gross abnormalities that could be detected on necropsy except for mild weight loss and serous atrophy. Histologically, the hepatic parenchyma exhibited variable degrees of cytoplasmic vacuolation ranging from mild well rounded micro vacuoles to severe clearly vacuolated cytoplasm (Figure 1). The periportal hepatocytes were most severely affected. In these areas, the vacuolated hepatocytes exhibited variable degrees of degeneration and necrosis. The blood vessels were congested. The bile duct epithelia were also exhibiting moderate cytoplasmic vacuolation (Figure 2). In the untreated controls, the liver was within normal limits.

In comparison with normal pancreatic acini, pancreatic atrophy with loss of zymogen granules and mild cytoplasmic vacuolation of the pancreatic acini were seen in the alloxan treated dogs (Figure 3).

The kidneys in treated animals exhibited vacuolar changes concentrated in the proximal convoluted tubules. Multifocally, the tubular epithelia showed pale eosinophilic, vacuolated and lacy cytoplasm (glycogen accumulation) (Figure 4). A few numbers of these tubules exhibited necrotic epithelium. There were no histological lesions seen in the adrenal glands and skin of treated dogs.

Discussion

Several animal models have been used commonly to study diabetes mellitus in human beings (Rees 2005; Chatzigeorgiou et al 2009). Alloxan is considered one of the most common and effective chemicals used to induce diabetes in those animal models with clinical features similar to those observed in diabetic people (Watanabe et al 2004; Rohilla & Ali 2012; Valilou & Lotfi 2012). Alloxan is known to induce diabetes by rapid destruction of pancreatic beta cells (Lenzen 2008). Several toxic effects associated with alloxan injection have been reported previously in different animal models using lower doses than the one used in this study (Watanabe et al 2004; Abu-Samak et al 2007; Valilou et al 2007; Mir & Darzi 2009; Azeez al 2010; Valilou & Lotfi 2010; Valilou et al 2011; Rohilla & Ali 2012; Valilou and Lotfi 2012). Hence, this is the first study to be reported that describes the clinico-pathological and toxic effects associated with a single intravenous injection of a high dose (120 mg-kg) of alloxan in dogs.

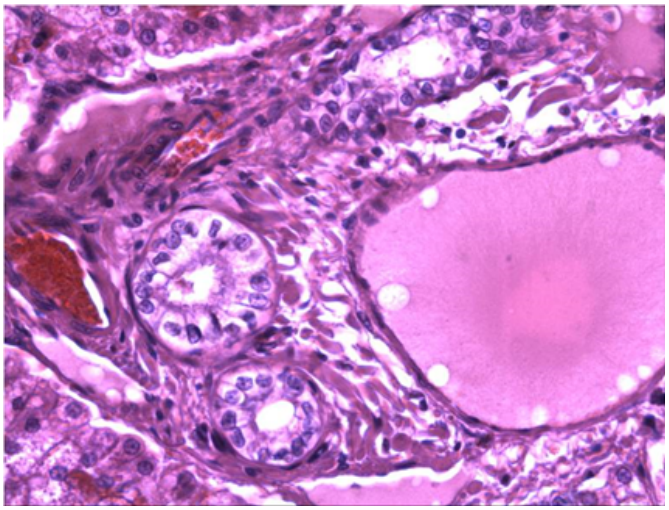

Fig. 2. Moderate cytoplasmic vacuolation of the epithelia lining the bile ducts in alloxan injected dog. HE 40X.

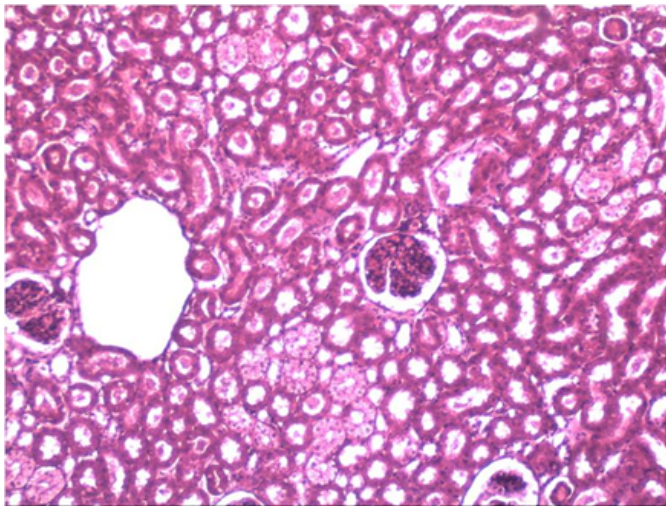

Fig. 4. Multifocal cytoplasmic vacuolation of the epithelia lining the proximal convoluted tubules in alloxan injected dog. HE 4X.

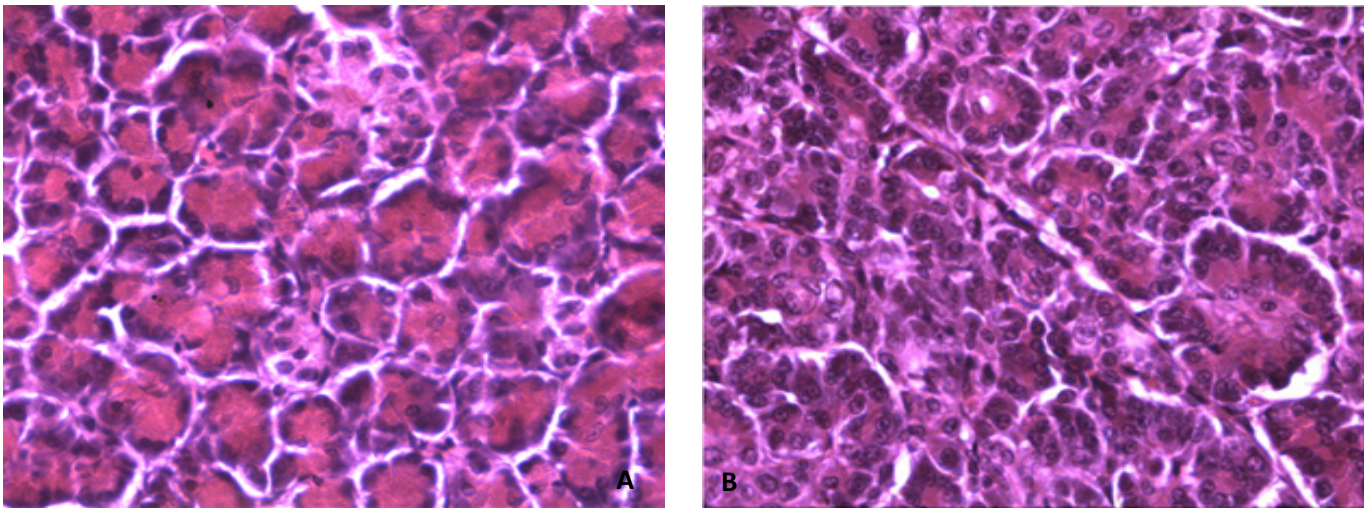

Fig. 3. HE stained sections of the pancreas from alloxan injected (B) and control (A) dogs. Notice diffuse atrophy of pancreatic acini with loss of zymogen granules in alloxan injected dog. 40X.

As expected all dogs in this study developed diabetes within 48 hours of alloxan injection. In addition, all dogs survived the initial hypoglycemic effect of alloxan and none of them needed extra care. It has been reported that alloxan induces a biphasic blood glucose response due erratical changes in the plasma insulin concentration associated with sequential ultrastructure changes in beta cells leading to necrosis (Lenzen 2008). Clinically, dogs in this study showed variable degrees of lethargy, partial anorexia and moderate to mild weight loss. These clinical effects are expected and are most likely due to the acute inflammatory reaction associated with beta cell death and due to extreme blood glucose variation in the immediate period after alloxan injection (Watanabe et al 2004; Valilou & Lotfi 2012). In the hematological analyses, data obtained in this study showed no significant differences in any of the studied parameters between DM and control groups. Inconsistent results were revealed when the literature concerning hematological changes associated with alloxan injection in different animal models were reviewed. Several studies had detected no abnormalities in the hemogram in alloxan injected animals while others had found a significant decrease in certain hematopoetic measures (Valilou et al 2011; Rohilla & Ali 2012; Valilou & Lotfi 2012). Moreover, similar conclusions could be drawn concerning changes in the serum biochemical analyses. In this study, the only abnormal significant change observed in DM dogs was an increase in serum total cholesterol concentration. These results were in agreement with previously reported data (Valilou & Lotfi 2012). Although not significant, results of this study showed a progressive increase in serum concentration of ALP in DM dogs while serum concentrations of other lever enzymes such as AST and ALT were not changed significantly. Others have reported significant elevations in serum activities of various liver enzymes AST, ALP and ALT in alloxan induced diabetes in animals (Valilou & Lotfi 2012). These changes were attributed to their release due to hepatocellualr damage, and their greater need for gluconeogenic substrate in diabetic conditions (Valilou & Lotfi 2012).

Similar to the results obtained here, it has been reported that alloxan injection was associated with major histological changes mainly involving the liver, pancreas and the kidneys (Watanabe et al 2004). The most notable changes were seen in the pancreatic acini which were atrophied with apparent loss of zymogen granules. Severe atrophy with lymphocytic infiltration of the islets of Langerhans in dogs administered a single injection of alloxan at a dose rate of 50 mg/kg was reported previously (Watanabe et al 2004). Similar changes were also observed in the acinar and ductal epithelia. Lesions observed in the liver and kidneys in DM dogs in this study were comparable to those

reported previously by others in dogs and rabbits (Watanabe et al 2004; Mir & Darzi 2009).

In conclusion, results of this study indicated that a single high dose (120 mg/kg) of alloxan monohydrate alone was associated with insignificant changes in the hematological and serum biochemical parameters in alloxan-induced diabetes mellitus dog model. The histopathological changes were characterized mainly by variable degrees of vacuolation in liver, kidney and pancreas in addition to pancreatic acini atrophy and degeneration.

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References

Abu Abeeleh M, Bani Ismail Z, Alzaben KR. Induction of Diabetes Mellitus in Rats Using Intraperitoneal Streptozotocin: A Comparison between 2 Strains of Rats. Europ J Sci Res 2009;3:398-402.

Abu-Samak M, Khuzaie R, Khataibeh M, and Mahmoud F. Melanocyte-stimulating hormone modulates blood viscosity in short-term alloxan-induced diabetic rats. J Biol Sci 2007;7:701-705.

Akbarzadeh A, Norouzian D, Mehrabi MR. Induction of diabetes by streptozotocin in rats. Indian J Clin Biochem 2007;22:60-64.

Azeez OI, Oyagbemi AA, Oyeyemi MO, et al. Ameliorative effects of Cnidoscolus aconitifolius on alloxan toxicity in Wistar rats. Afr Health Sci 2010;10:283-291.

Chatzigeorgiou A, Halapas A, Kalafatakis K, et al. The use of animal models in the study of diabetes mellitus. In Vivo 2009;23:245-58.

Lenzen S. The mechanisms of alloxanand streptozotocin-induced diabetes. Diabetol 2008;51:216-26.

Mir SH, Darzi MM. Histopathological abnormalities of prolonged alloxan-induced diabetes mellitus in rabbits. Int J Exp Path 2009; 90:66–73.

Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabet Med 2005;22:359-70.

Rohilla A, Ali S. Alloxan Induced Diabetes:Mechanisms and Effects. Int J Res Pharm Biom Sci 2012;819-823.

Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001;50:537-46.

Thrall MA. Veterinary hematology and clinical chemistry. Lippincott Williams & Wilkins, Pennsylvania. 2004.

Valilou MR, Lotfi AR. Changes in body weight and serum biochemical measures of German Shepherd dogs following alloxan induced diabetes mellitus. Asian J Biochem 2012;7:232-236.

Valilou, MR, Lotfi AR. Investigation of Ultra structural changes in testicles of German shepherd dogs following alloxan induced diabetes mellitus. Global Vet. 2010;4:86-89.

Valilou MR, Shayegh J, Eshratkhah B, et al. Hematopoietic measures in German shepherd dogs following Alloxan induced diabetes mellitus. Adv Environ Biol 2011;5:1177-1180.

Valilou MR, Sohrabi, HR, Mohammadnejad D, et al. Histopathological and ultra structural lesions study of kidney of Alloxan induced diabetes mellitus in German shepherd dogs. J Anim Vet Advan 2007;6:1012-1016.

Watanabe D, Nakara H, Akagi K. Oral glucose tolerance test and

determination of serum fructosamine level in Beagle dogs. J Toxicol Sci 2004;29:33-36.

Watanabe D, Nakara H, Yamaguchi Y. The pathological features of alloxan diabetes in Beagle dogs. J Toxicol Path 2004;17:187- 195.

Authors

•Zuhair Bani Ismail, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan, email: zuhair72@just.edu.jo

•Wael Hananeh, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan, email: whananeh@just.edu.jo

•Musa Alshehabat, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan, email: maalshehabat@just.edu.jo

•Mousa Daradka, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan, email: daradka@just.edu.jo

•Jafar Ali, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan, email: jthasanali12@vet.just.edu.jo

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