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

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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.

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).
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.

### Parameters Pre-induction | Post-induction
--- | ---
**WBC (X 10^3/µL)** | A | B | C | D | E | F | G
--- | --- | --- | --- | --- | --- | --- | ---
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 | 8.6±0.7 | 8±1 | 7±1.2 | 8±1.7
**Hemoglobin (g/dL)** | 18±1.2 | 18.5±1.4 | 19±1.4 | 19±1 | 18±1.7 | 16±1.9 | 16±3
**Hematocrit (%)** | 56±3 | 58±3.6 | 57.3±3 | 58±3.8 | 54±5.9 | 49±6.9 | 51±10
**MCV (fl)** | 68±1 | 67±0.6 | 68±1.2 | 68±0.9 | 68±1.5 | 67±1.7 | 67±1.7
**MCH (pg)** | 22±0.6 | 21±0.3 | 23±0.1 | 22±0.5 | 22±0.7 | 22±0.9 | 21±0.6 | 21±0.6
**MCHC (g/dL)** | 32±0.4 | 32±0.6 | 33±0.5 | 32±0.3 | 32±0.3 | 33±1.2 | 31±0.2 | 31±0.2
**Platelets X 10^3/µL** | 211±3 | 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 ≤ 0.05).

### Parameters Pre-induction | Post-induction
--- | ---
**AST (IU/L)** | A | B | C | D | E | F | G
--- | --- | --- | --- | --- | --- | --- | ---
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 | 73±3.5 | 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
**Glucose (mg/dL)** | 73±17 | 152±33 | 263±49 | 249±2 | 338±8 | 286±8 | 327±60
**Cholesterol (mg/dL)** | 239±7 | 238±39 | 235±7 | 410±6 | 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 | 8.8±0.4 | 11±0.5 | 11±0.4 | 9±0.9 | 10±0.4
**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±0.3
**Sodium (mmol/L)** | 1455±9 | 158±25 | 160±4.7 | 146±2 | 150±1.1 | 143±5 | 143±2.6
**Potassium (mmol/L)** | 4.6±0.1 | 4.7±0.3 | 4.3±0.3 | 4±0.1 | 4±0.5 | 4±0.3 | 3.6±0.4
**Chloride (mmol/L)** | 117±1.3 | 123±9 | 122±4 | 108±0.8 | 111±0.1 | 108±1.6 | 112±2.5
**BUN (mg/dL)** | 28±1.3 | 14±0.6 | 39±17 | 19±5 | 18±9 | 14±2 | 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 ≤ 0.05).
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 ≤ 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 ≤ 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 ≤ 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.
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 erratic 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 liver 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 hepatocellular 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


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