Effects of Diazepam and Xylazine on Changes of Blood Oxygen and Glucose Levels in Mice

ABD EL-NASSER AHMED MOHAMMED1,2, ADEL AL-HOZAB1, TAREK ALSHAHEEN1

1Department of Animal and Fish Production, College of Agriculture and Food Sciences, King Faisal University, Saudi Arabia; 2Department of Animal Production, Faculty of Agriculture, Assiut University, Egypt, 71526.

Abstract | The present study aimed to explore the changes of body temperature, heart rate, blood partial pressure of oxygen (PO2) and glucose levels upon diazepam and/or xylazine administration in mice. Sixty male albino mice (33.89 ± 0.41 g body weight; BW) were distributed randomly into five groups given intraperitoneal (IP) injection: control group given physiological saline; diazepam group (D) given 13.3 mg/kg BW; xylazine group (X) given 26.6 mg/kg BW; DX group given both 13.3 mg/kg BW diazepam and 26.6 mg/kg BW xylazine; DXVas group given DX dose for vasectomy surgical operation. The values of body temperature, PO2, heart rate and blood glucose were recorded of groups at 0, 20 min, 40 min, 1h, 2h, 3h, 4h, 8h of diazepam and/or xylazine injection. The results indicated a significant (P < 0.05) transient negative side effect of diazepam and/or xylazine dosages on body temperature, PO2, heart rate and blood glucose, which were the highest at 1-2 h post-drug administration. The transient negative side effects decreased gradually thereafter at 2h, 3h, 4h and 8h of injection. The transient negative side effects were more significantly pronounced in xylazine, DX and DXVas groups compared to diazepam and control groups and extended to 8h of injection. In conclusion, the given dosage of diazepam and/or xylazine resulted in transient negative side effects in body temperature, PO2, heart rate and blood glucose, which returned approximately close to normal levels at 8h of injection. The DX anesthesia dose is sufficient and safe for performing minor and major surgeries in mice.

Keywords | Diazepam, Xylazine, Analgesia, Anesthesia, Oxygen, Glucose

INTRODUCTION

Diazepam is a medication of the benzodiazepine family. Diazepam produces in human a calming effect and is commonly used to treat a range of conditions as anxiety, muscle spasms, and trouble sleeping (Calcaterra et al., 2014). Xylazine is a clonidine analogue used for sedation, analgesia and anesthesia in animals (Mohammed et al., 2011; Mohammed et al., 2012) and the overdose of xylazine is usually fatal in humans (Greene and Thurmon, 1988).

Mice and rats were used in scientific research for different studies (Mohammed et al., 2008; Mohammed, 2009; Mohammed et al., 2010; Mohammed and Al-Suwaiegh, 2016; Mohammed, 2017; Mohammed, 2018). Mice and rats used in scientific research must be sedated or anaesthetized for surgical operation (Mohammed and Al-Hozab 2016; Mohammed, 2018). The proper use of analgesics and/or anesthetics in research animals is imperative for an ethical and scientific perspective. The use of analgesics and/or anesthetics for samples’ collection and minor/major surgeries is more frequent for general scientific applications. Some of these drugs can cause serious reversible/irreversible disruption to pulmonary and cardiovascular organs (Flecknell et al., 1983; Peeters et al., 1988; Borkowski et al., 1990). Diazepam and xylazine were used for induction of sedation in research animals but the drugs caused reversible/irreversible side effects (Ghurashi et al., 2009; Mohammed et al., 2012; Karcz and Papadakos, 2013). Analgesics diaze-
Pam and xylazine drugs were reported to cause respiratory, cardiovascular and hematological complications (Mohammed et al., 2012; Yadav et al., 2008). Changes of blood parameters were found upon diazepam or xylazine injection (Fani et al., 2004; Mohammed et al., 2012).

Maintenance of body temperature and blood glucose during anesthesia is the most important metabolic reactions in addition to blood oxygen level (Ljungqvist et al., 2012; Mohammed et al., 2012; Behdad et al., 2014). Hypothermia in addition to hyperglycemia is expected in the post-anesthesia period due to inflammatory mediators and stress hormones such as cortisol and epinephrine. In addition, respiratory complications such as hypoxemia (hemoglobin oxygen saturation <90%) is expected due to hyperventilation (Karcz et al., 2013) in the post-anesthesia period as well. Turina et al. (2006) reported that short-term hyperglycemia causes immunosuppression and significant increase of infectious conditions. During surgery, acute hyperglycemia worsens prognosis even in patients had normal glucose level (Bochicchio et al., 2005; Puskas et al., 2007). Muir and Mason (1993) found that diazepam and/or xylazine drugs have a dose-dependent effect in mice. In rodent research, the effects of analgesic and anesthetic dose of diazepam and xylazine drugs were evaluated on the glucose, hemoglobin, and urea levels in rats at 120 min post-anesthesia (Mohammed et al., 2012). Therefore, the aim of the present study was to investigate the time-dependent analgesic and anesthetic dose of diazepam and xylazine drugs were evaluated on the glucose, hemoglobin, and urea levels in rats.

Site of Study and Animal Management
The study was conducted during the period from January to February 2018 of Animal and Fish Production department. Sixty male albino mice of 33.89 ± 0.41 g body weight (BW) and six months of age were used for the study. The animals were bred in the animal lab of the department of Animal and Fish Production. Mice were fed commercial pellet diet (Arasco, KSA), which composed of 21.0% protein, 2.9% fat and 3.3% fiber, 1% mixture of vitamins and minerals, and 3300 kcal/kg energy. Animals had free access to food and water. Mice were kept controlled under 12 h light and 12 h dark cycle starting at 7 a.m. The controlled temperature and relative humidity during the experiment were 25.5±2.8°C and 50±10%, respectively.

Injection of Diazepam and Xylazine Dosages
The sixty male albino mice (33.89 ± 0.41 g BW) were distributed over five groups. Fasting males for six hours were given intraperitoneally (IP) the dosages of injection. The control group injected with 0.2 ml physiological saline. The diazepam group injected with 13.3 mg/kg BW of diazepam (Neuril 2.5 mg/ml; Memphis Co. Egypt). Xylazine group injected with 26.6 mg/kg BW of xylazine (Xylject 10 mg/ml; Adwia Co. Egypt). Diazepam and xylazine group (DX) injected with both 13.3 mg/kg BW of diazepam and 26.6 mg/kg BW of xylazine. Diazepam and xylazine group used for vasectomy surgical operation (DXVs). The dosages of diazepam and xylazine used in this study were chosen according to the drugs’ safety margin of our previous studies (Mohammed et al., 2012; Mohammed, 2012).

Surgical Operation of Vasectomy
Vasectomy surgical operation of males was carried out according to the method of Bermejo-Alvarez et al. (2014) using our general anesthesia dose (diazepam 13.3 mg/kg and xylazine 26.6 mg/kg) (Mohammed, 2018). Briefly, incision 1 cm of skin and muscle above the penis was done upon general anesthesia. Cauterization of the vas deferens in two points at once through flaming forceps followed by muscle and skin suture.

Body Temperature Monitoring
Body temperatures were recorded using Digital LCD IR Infrared Thermometer Body Surface Temperature (Cofoe Portable Digital Termometre Infrared Thermometer Gun Non-contact IR LCD). The measurement time is less than 2 seconds, measurement range error is 0.1 – 0.3 degree Celsius and the measurement distance is 1-3 cm.

Pulse Oximeter and Heart Rate Monitoring
The pulse oximeter and heart rate monitor was used (CMS60D-VET Handheld Veterinary Pulse Oximeter) to measure partial pressure of oxygen (PO2) and heart rate. The male mice were restrained in one hand and proper sensor put on the chest. The partial pressure of oxygen (PO2) and heart rate were recorded since no shaving or hair removal is required.

Blood Glucose Monitoring
Blood glucose monitoring recorded of male mice using blood glucose meter (iCare advanced Medical) (King 2012). Blood glucose levels were monitored at 0, 20 min, 40 min, 1h, 2h, 3h, 4h and 8h of injection. The tip of tail was punctured and the drop of blood put on strips for measuring blood levels.

Statistical Analysis
Statistical analysis was done according to general linear model (GLM) of SAS program (2008). Differences be-
between control and diazepam, xylazine, DX and DXVs treated groups were evaluated in body temperature, PO2, heart rate and glucose level by one-way ANOVA. Duncan Multiple Range Test (Steel and Torrie, 1980) was used to test the effect of treatments. The data were presented as mean ± S.E.M. Level of significance was set at P<0.05. Statistical model as follow:

\[
Y_{ij} = \mu + T_i + E_{ij}
\]

Where: \(Y_{ij}\) = the experimental observation \(ij\), \(\mu\) =the overall mean, \(T_i\) = the effect due to treatment \(i\), \(E_{ij}\) = the experimental error.

**RESULTS**

**Body Temperature**

Body temperature of male mice is shown in (Table 1) at 0, 20 min, 40 min, 1h, 2h, 3h, 4h and 8h over injection of diazepam (D; 13.3 mg/kg), xylazine (X; 26.6 mg/kg), DX (DX; D 13.3 mg/kg & X; 26.6 mg/kg) and both DX used for vasectomy surgical operation (DXVs). Mean of body temperature before drugs’ injection did not have significant differences between the five groups, but starting from 20 minutes of diazepam and/or xylazine dosage injection, body temperature values were significantly lower than control one. The amount of body temperature at 20, 40, 1h and 2h of diazepam and/or xylazine decreased significantly (P<0.05) compared to control group. The significant decrease (P<0.05) of body temperature was more pronounced in DX and DXVs groups followed by xylazine and diazepam groups, respectively. The lowest amount of body temperature recorded at 2h of drugs’ injection in all groups. Thereafter, the values of body temperature started to increase in diazepam and/or xylazine groups. The significant return (P<0.05) of body temperature thereafter was more pronounced in diazepam followed by xylazine, DX and DXVs groups, respectively, but it was still lower significantly than control group.

**Partial Pressure of Oxygen in Blood**

Partial pressure of oxygen (PO2) reflects the amount of oxygen gas dissolved in the blood. Amount of oxygen in the blood measured of male mice at 0, 20 min, 40 min, 1h, 2h, 3h, 4h and 8h over injection of diazepam (D; 13.3 mg/kg), xylazine (X; 26.6 mg/kg), both DX (D 13.3 mg/kg & X 26.6 mg/kg), and both DX used for vasectomy surgical operation (DXVs) is shown (Table 2). Mean of PO2 before drugs’ injection did not have significant differences between the five groups, but starting from 20 minutes of diazepam and/or xylazine dosage injection, PO2 values were significantly decreased than control group. The amount of oxygen in the blood at 20 and 40 min of diazepam and/or xylazine decreased significantly (P<0.05) compared to control group. The lowest amount of oxygen in the blood recorded at 1h of injection in all injected groups. The amount of oxygen in the blood started to increase in diazepam and/or xylazine groups thereafter. The increase of PO2 was more pronounced in diazepam group where it was not differed than control group at 3h, 4h and 8h of injection.

**Heart Rate**

Pulse rate of male mice at 0, 20 min, 40 min, 1h, 2h, 3h, 4h and 8h over injection of diazepam (D; 13.3 mg/kg), xylazine (X; 26.6 mg/kg), both DX (D 13.3 mg/kg & X 26.6 mg/kg), and both DX used for vasectomy surgical operation (DXVs) is shown (Table 3). Mean of heart rate before drugs’ injection did not have significant differences between the five groups, but starting from 20 minutes of diazepam and/or xylazine dosage injection, heart rate values of diazepam and/or xylazine groups was significantly lower than control group. Heart rate at 20 and 40 min of diazepam and/or xylazine groups decreased significantly (P<0.05) compared to control group. The decrease of pulse rate was significantly more pronounced in xylazine, DX and DXVs groups compared to diazepam and control ones. The lowest pulse rate recorded at 1h of diazepam and/or xylazine injection. The pulse rate started to increase in diazepam and/or xylazine groups thereafter. The increase of pulse rate was more pronounced in diazepam group where it was not differed significantly than control group at 3h, 4h and 8h of injection. The values of heart rate at 3h, 4h and 8h of xylazine, DX and DXVs as groups was still significantly lower than those of diazepam and control ones.

**Blood Glucose Level**

Blood glucose levels of mice at 0, 20 min, 40 min, 1h, 2h, 3h, 4h and 8h over injection of diazepam (D; 13.3 mg/kg), xylazine (X; 26.6 mg/kg), both DX (D 13.3 mg/kg & X 26.6 mg/kg), and both DX used for vasectomy surgical operation (DXVs) is shown (Table 4). Blood glucose level increases significantly among groups due to diazepam and/or xylazine injection over time of injection. Mean of blood glucose levels before drugs’ injection did not have significant differences between the five groups, but starting from 20 minutes of diazepam and/or xylazine dosage injection, blood glucose values of diazepam and/or xylazine groups was significantly higher than control group. Blood glucose levels at 20 and 40 min of diazepam and/or xylazine increased significantly (P<0.05) compared to control group. The highest glucose level recorded at 1h of diazepam and/or xylazine injection. The increase of blood glucose levels was significantly more pronounced in xylazine, DX and DXVs as groups compared to diazepam and control ones. The highest blood glucose levels recorded at 1h of diazepam and/or xylazine injection. The blood glucose levels started to decrease in diazepam and/or xylazine groups.
### Table 1: Effect of diazepam (13.3 mg/kg) and/or xylazine (26.6 mg/kg) on body temperature (°C) in mice

| Time (min) | Control | Diazepam (D) | Xylazine (X) | DX | DXVas |
|------------|---------|--------------|--------------|----|-------|
| 0          | 37.22 ± 0.061a | 37.21 ± 0.06a | 37.10 ± 0.10a | 37.23 ± 0.12a | 37.28 ± 0.12a |
| 20         | 37.17 ± 0.07a  | 35.26 ± 0.01b | 34.19 ± 0.02c | 33.10 ± 0.02d | 33.0 ± 0.10d  |
| 40         | 37.15 ± 0.07a  | 34.29 ± 0.02b | 32.52 ± 0.06c | 32.16 ± 0.04d | 32.07 ± 0.07d |
| 1          | 37.20 ± 0.06a  | 33.34 ± 0.02b | 32.24 ± 0.05c | 31.48 ± 0.02d | 31.36 ± 0.13d |
| 2          | 37.22 ± 0.07a  | 33.11 ± 0.03b | 32.06 ± 0.07c | 31.23 ± 0.07d | 31.1 ± 0.14d  |
| 3          | 37.21 ± 0.07a  | 34.57 ± 0.12b | 33.65 ± 0.20c | 31.53 ± 0.10d | 31.44 ± 0.17d |
| 4          | 37.16 ± 0.06a  | 35.08 ± 0.13b | 34.63 ± 0.23c | 31.80 ± 0.05d | 31.66 ± 0.17d |

*Values with different superscripts between groups of the same row differed significantly at P < 0.05.*

### Table 2: Effect of diazepam (13.3 mg/kg) and/or xylazine (26.6 mg/kg) on partial pressure of oxygen (PO2) in mice

| Time (min) | Control | Diazepam (D) | Xylazine (X) | DX | DXVas |
|------------|---------|--------------|--------------|----|-------|
| 0          | 90.45 ± 2.13a | 91.18 ± 2.11a | 90.54 ± 2.17a | 89.36 ± 2.21a | 89.00 ± 2.13a |
| 20         | 93.72 ± 1.67a | 84.09 ± 2.10b | 87.25 ± 1.44b | 84.81 ± 1.61b | 85.18 ± 1.58b  |
| 40         | 95.54 ± 1.35a | 78.63 ± 1.79c | 86.00 ± 1.62b | 83.18 ± 1.82b | 82.63 ± 1.88b  |
| 1          | 87.63 ± 2.37a | 78.09 ± 1.80b | 79.90 ± 3.53c | 75.81 ± 1.78b | 74.81 ± 1.92b  |
| 2          | 91.45 ± 1.82a | 79.90 ± 1.01c | 85.36 ± 2.05b | 76.00 ± 2.98c | 76.45 ± 3.32c  |
| 3          | 90.18 ± 1.62a | 79.09 ± 2.71b | 86.72 ± 0.66a | 79.18 ± 3.71b | 78.27 ± 3.64b  |
| 4          | 91.27 ± 1.82a | 80.72 ± 1.82b | 89.18 ± 1.85a | 81.45 ± 2.23b | 79.54 ± 2.97b  |
| 8          | 90.36 ± 1.46ab | 86.36 ± 2.40bc | 90.90 ± 0.93a | 85.18 ± 0.52c | 83.81 ± 1.46c  |

*Values with different superscripts between groups of the same row differed significantly at P < 0.05.*

### Table 3: Effect of diazepam (13.3 mg/kg) and/or xylazine (26.6 mg/kg) on pulse rate in mice

| Time (min) | Control | Diazepam (D) | Xylazine (X) | DX | DXVas |
|------------|---------|--------------|--------------|----|-------|
| 0          | 178.18 ± 6.39a | 186.00 ± 5.32a | 185.09 ± 3.86a | 186.54 ± 5.09a | 185.45 ± 5.07a |
| 20         | 188.00 ± 4.53a | 155.18 ± 11.03b | 120.72 ± 5.98c | 119.09 ± 8.39c | 118.27 ± 8.05c |
| 40         | 187.63 ± 10.55a | 146.09 ± 9.94b | 119.18 ± 5.83c | 118.36 ± 7.27c | 117.45 ± 6.99c |
| 1          | 176.45 ± 8.5a  | 129.45 ± 6.48b | 113.54 ± 5.61c | 116.36 ± 7.39c | 115.09 ± 7.22c |
| 2          | 197.27 ± 8.40a | 162.45 ± 6.15b | 114.09 ± 5.33c | 124.27 ± 9.10c | 124.90 ± 8.73c |
| 3          | 192.27 ± 6.76a | 163.36 ± 14.49b | 114.54 ± 8.41b | 132.36 ± 13.69b | 131.45 ± 13.2b |
| 4          | 191.09 ± 6.62a | 180.09 ± 8.37a | 145.36 ± 2.47b | 138.45 ± 9.53c | 138.72 ± 9.16b |
| 8          | 189.09 ± 6.45a | 186.18 ± 4.40a | 160.18 ± 5.71b | 156.45 ± 6.16b | 154.63 ± 6.14b |

*Values with different superscripts between groups of the same row differed significantly at P < 0.05.*

### Table 4: Effect of diazepam (13.3 mg/kg) and/or xylazine (26.6 mg/kg) on blood glucose levels in mice

| Time (min) | Control | Diazepam (D) | Xylazine (X) | DX | DXVas |
|------------|---------|--------------|--------------|----|-------|
| 0          | 115.63 ± 5.94a | 118.63 ± 4.05a | 122.36 ± 3.03a | 123.27 ± 3.72a | 122.54 ± 3.68a |
| 20         | 123.27 ± 1.47c | 168.54 ± 6.48b | 238.54 ± 5.42a | 239.00 ± 10.0a | 237.18 ± 10.2a |
| 40         | 121.00 ± 2.72c | 163.36 ± 14.49b | 261.54 ± 24.21a | 259.27 ± 4.31a | 258.36 ± 3.97a |
| 1          | 136.09 ± 4.29c | 176.27 ± 10.08b | 263.25 ± 6.86a | 277.54 ± 19.76a | 275.72 ± 18.97a |
| 2          | 114.72 ± 3.80b | 141.09 ± 5.80b | 226.54 ± 32.90a | 253.18 ± 30.30a | 255.0 ± 28.80a |
| 3          | 111.18 ± 3.40c | 128.00 ± 6.61b | 187.81 ± 9.99b | 227.90 ± 16.77a | 228.81 ± 16.32a |
| 4          | 107.54 ± 2.74b | 127.18 ± 0.85b | 172.54 ± 6.84a | 176.63 ± 19.83a | 177.54 ± 19.44a |
| 8          | 102.00 ± 2.54c | 126.54 ± 4.98b | 154.00 ± 1.69a | 152.54 ± 5.71a | 150.72 ± 5.40a |

*Values with different superscripts between groups of the same row differed significantly at P < 0.05.*
thereafter. The decrease of blood glucose levels was more pronounced in diazepam group where it was not differed significantly than control group at 3h and 4h of injection. The values of blood glucose at 3h, 4h and 8h of xylazine, DX and DXVas as groups were still significantly higher than those of diazepam and control ones.

**DISCUSSION**

Results of the current study demonstrated the effects of diazepam (D; 13.3 mg/kg), xylazine (X; 26.6 mg/kg), both diazepam and xylazine (DX; D 13.3 mg/kg & X; 26.6 mg/kg), and both diazepam and xylazine used for vasectomy surgical operation (DXVas) at 0, 20 min, 40 min, 1h, 2h, 3h, 4h and 8h of injection on body temperature, PO2, heart rate and blood oxygen level (Tables 1-4). Diazepam and/or xylazine drugs were used for sedation, analgesia and anesthesia in rabbit, mice and rats (Mohammed et al., 2011; Ljungqvist et al., 2012; Mohammed et al., 2012).

Because of their transient negative side effects (Mohammed et al., 2012), the current study recorded the values of body temperature, PO2, pulse rate and blood glucose at the previous aforementioned times to explore the time of recovery over diazepam and/or xylazine injection relevant to surgical practice.

The significant reduction of body temperature in this study over diazepam (13.3 mg/kg) and/or xylazine (26.6 mg/kg) presented in Table 1. The decrease was more pronounced in both X, DX and DXVas groups as previously indicated over 6.2 mg/kg diazepam and/or 13.3 mg/kg xylazine in rats at 2h after injection (Mohammed et al., 2012) as in other species [cattle (Yadav et al., 2008); male Mongolian gerbils (Sarnowska et al., 2009); rabbits (Mohammed et al., 2011)]. The changes of thermoregulatory control upon diazepam and/or xylazine administration might be due to the decrease of vital functions of cardiovascular and respiratory systems.

The significant reduction of blood oxygen and heart rate in this study due to diazepam (13.3 mg/kg) and/or xylazine (26.6 mg/kg) presented in tables (2-3). Diazepam caused minimal cardiovascular effects compared to xylazine. The appropriate use of analgesic and anesthetic drugs clearly influences pulmonary outcomes. The decrease of myocardial contractility and cardiac output is body of evidence of some studies (Kul et al., 2000; Ismail et al., 2010) due to xylazine injection. Decreased heart rate could be attributed to sinus carotid barore ceptorreflex in response to an initial hypertension due to vasoconstriction caused by peripheral postsynaptic adreno-receptors (Garner et al., 1971). Decreased heart rate due to xylazine administration was found in pregnant goats (Sakamoto et al., 1996), pregnant cows (Hodgson et al., 2002), dogs (Ilbäckand Stalhandske, 2003), and heifers (Araujo and Ginther, 2009).

Blood glucose levels increased significantly among groups due to diazepam and/or xylazine injection over time of injection. Our previous study in rats using 6.2 mg/kg diazepam and/or 13.3 mg/kg xylazine (Mohammed et al., 2012) presented the insignificant increase of plasma glucose level at 2h of injection. Other studies in other species (cattle, buffaloes, camels and dogs) indicated hyperglycemia upon drugs’ administration (Symonds, 1976; Custer et al., 1977; Dwivedi et al., 2004; Fani et al., 2004). Custer et al. (1977) found that the value of glucose was approximately twice in xylazine-induced restrain camels compared to the value of manually restrained camels. Hyperglycemia of anesthesia drugs might be due to the stress-induced gluconeogenesis and the probable suppression of insulin.

**CONCLUSION**

It could be concluded that administration of 13.3 mg/kg BW of diazepam and/or 26.6 mg/kg BW xylazine resulted in transient negative side effects in body temperature, heart rate and blood oxygen and glucose levels, which returned approximately close to normal levels at 8h of injection in mice.

**CONFLICTS OF INTEREST**

No conflict of interest of this article to declare.

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All authors contributed equally.

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