



The Effect of Surgery and Anaesthesia in Clinical Parameters and Plasma Glucose Concentrations with LDH Enzyme in Donkey (*Equus asinus*)

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Abstract

This study was carried out to examine the effects of an aesthesia and surgery on clinical parameters such as heart rate, respiratory rate, rectal temperature and also the effects on plasma glucose concentrations and LDH enzyme on donkeys. Nine clinically healthy donkeys were used. The study consist of experiments trials, in the first trial (A) the animals received xylazine 2% (0.25 mg/kg) then after five minutes they received Diazepam 0.5% (0.25 mg/kg) and Propofol 1% (2mg/kg) intravenously. The second trial (B) was conducted by the injection of the above an aesthetic regime and immediately after recumbency surgical incision took place in the flank region through the skin and abdominal muscles. In both experiment A (an aesthesia) and B (surgery) heart rate, respiratory rate and rectal temperature were taken at 5, 10, 30 and 45 minutes after injection. Plasma glucose concentrations and plasma LDH enzyme were measured at 3, 6, 9, 24 and 48 hours after injection. Before administration of anaesthetic regime clinical parameters, glucose concentration and plasma LDH enzyme were measured (Controls). The sleeping time (an aesthetic phase) in experiment (A) was 18.11 ± 5.3 minutes compared to 20.89 ± 7.6 minutes in experiment (B). Recovery was smooth and of a good quality. In both experiments (A) and (B), heart rate significantly increased compared with the (Controls), respiratory rate significantly decreased ($P < 0.01$) when compared to baseline values, body temperature showed significant decrease ($P < 0.05$) at the 30, 45 minutes after injection. Plasma glucose concentrations revealed a significant different ($P < 0.01$, $P < 0.05$) at 3, 24 hours in both A and B. There was a significant difference in LDH after 24 hours from injection of propofol ($P < 0.05$). It is concluded that the an aesthetic regime alone (A) or followed by surgery (B) affected the heart rate, respiratory rate, rectal temperature, plasma glucose concentrations and plasma LDH enzyme. The study recommended to use this regime of an aesthesia in donkey's surgery.

Keywords: xylazine, propofol, diazepam, premedication, hypnosis.

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Introduction:

Donkeys are necessary in agricultural communities and farm work in many

areas of the world. They occupy rural areas; they were used in working life and transportation, due to their high

tolerance, disease resistance, less expensive to purchase and cheap in their health care expenses (Kreuchauf, 1984). They carry loads exceeding their body weight for long distances. Donkeys and mules have better tolerance for pain than horses (Ashley *et al.*, 2005). Many procedures could probably be performed in donkeys with sedation and appropriate use of local analgesia similar to that used in cattle (Matthews *et al.*, 2002).

Donkeys also appear to possess an increased metabolic capacity for certain drugs such as sulfamethoxazole, trimethoprim, flunixin meglumine, gentamicin, ketamine and xylazine (Peck *et al.*, 2002). Donkeys have a relative greater capacity in metabolism and elimination of drugs from their bodies. This fact is supported by the findings that total body clearance values for most of the drugs studied were greater for donkeys than for horses. For example, clearance of phenylbutazone is greater in donkeys than that in horses (Mealey *et al.*, 1997). Equine practitioners are often required to perform surgical procedures under field conditions. Few drugs have been authorized to be used in donkeys and they have a limited number of dosage regimens such as (Acepromazine, Detomidine, ketamine, phenylbutazone and Triclabendazole) (Lizarraga *et al.*, 2004). Total intravenous anesthesia is now a clinically accepted technique of veterinary anesthesia practice especially for short acting, non-cumulative anesthetic agents (Hughes and Nolan, 1999). Propofol is a popular anesthetic induction agent widely used in veterinary practice to produce a fast and smooth induction of anesthesia (Watkins *et al.*, 1987) and it is characterized by a lack of any cumulative effect and had a rapid recovery after administration either

by repeated bolus injection or by continuous infusion (Adetunji *et al.*, 2002). There was no available anaesthetic drug which can provide proper anesthesia alone now a day, therefore, combinations of sedatives and other anesthetics have been widely used in animal practice.

Xylazine HCL is pre-anaesthetic used in wide species of animals (Hall and Clarke, 1991). It was the first α_2 -adrenergic agonist that was used to induce sedation and analgesia in dogs and cats before using detomidine (Hall *et al.*, 2001). Central effect also was reported by Sanhoury *et al.*, (1989). It acts via central nervous system by activation of α - adrenergic system e.g the α_2 -adrenergic receptors (Thurmon *et al.*, 1996) intrathecal effects was also reported by Waterman (1989).

Diazepam (Valium) is the most broadly used of the benzodiazepines group; it induces sedation and hypnosis with less effects on the cardiopulmonary activities (Vicker *et al.*, 1984). In the present study a combination of xylazine and diazepam as pre-medication with propofol as a general intravenous anesthetic was used.

The aim of this study was to show the effect of the above mentioned anesthetic combination in clinical parameters (Heart rate, Respiratory rate, Rectal temperature), plasma glucose concentrations and plasma LDH enzyme activity, alone (A) or followed by surgical incision (B).

Materials and Methods:

Location of Study: The study was conducted at the Teaching Veterinary Hospital, University of Bahri, College of Veterinary Medicine.

Animals: Nine male mature donkeys (90-135 cm in height) ranging in age from 24 to 30 months and weighting

from 78 to 144 kg (average weight 110 kg) were used for the study. The donkeys were fairly tame. They were kept in paddocks a week before starting the experiments they had diet three times a day which composed of Alfalfa (*Medicago sativa*) and allowed free access to fresh water. The donkeys were examined clinically to prove freedom from diseases. The animals were fasted 12-15 hours before injection of anaesthesia. The experiments carried out indoors.

Physiological parameters:

Cardiopulmonary monitoring during anaesthesia: Base line data for heart rate, respiratory rate and temperature were determined in all donkeys standing in stocks before any medication was administered. They measured according to Geehan, 2014.

Heart rate: (beat / minute) was measured by auscultation of the cardiac area using stethoscope.

Respiratory rate: (breath / minute) was measured by observation of intercostal muscle movements.

Rectal temperature: (cellsius degrees °C) body temperature was measured by putting thermometer (Digital thermometer) in the rectum of the animal for two minutes. There after, temperature recorded from the digital thermometer.

Data Collection: Blood samples collection; 5 ml of blood were withdrawn from the jugular vein using sterile syringes. The blood was immediately transferred to heparinised containers (lithium heparin blood collection tube 4ml, Kang Jian), centerfuged at 5000xg for 5 minutes (Hettich Zentrifugen, Germany) and plasma decanted immediately after collection, plasma was pipetted into plastic tubes (Eppendorf tubes 1.5ml)

and stored at -20°C until analyzed. All blood samples were then transferred to haematology Lab. Teaching Veterinary Hospital, College of Veterinary Medicine, University of Bahri for plasma glucose concentrations measurement and LDH activity using commercial kits.

Experimental design: the experiments were carried out between 9:30 in the morning and lasted 48 hours after the beginning of each experiment, in each experiment anaesthesia was injected and blood samples were obtained via jugular vein. Before administration of the anaesthetic, cardiopulmonary parameters were measured, glucose concentrations and LDH activity were measured (Controls).

Anaesthetic Protocol: One anaesthetic regime was performed in this study but two experiments were conducted. In the first experiment animals were given xylazine 2% (0.25 mg/kg) then after five minutes they received Diazepam 0.5% (0.25 mg/kg) and Propofol 1% (2mg/kg) through jugular vein. In the second trial the animals were given the same anaesthetic regime followed by surgery on the flank.

Experiment A (Anaesthesia): Ten minutes prior to injection of xylazine, the donkeys were kept in a calm state, baseline samples were obtained and recorded for physiological parameters, blood profile and biochemical tests. The animals then received the sedatives in the jugular vein, once sedation took place, administration of 2 mg/kg Propofol 1% intravenously (IV) was carried out.

Clinical parameters and blood sampling: Immediately after recumbency donkeys positioned in right lateral recumbency to help monitor the heart rate and the respiratory rate.

clinical parameters were recorded 5, 10,30 and 45 minutes after administration of the general anaesthetic, and blood samples were collected from the jugular vein at 3, 6, 9, 24 and 48 hours after injection of an aesthetic drugs .

Experiment B (Surgery): The same above protocol was repeated in addition to flank surgery.

Surgical procedure: The left flank was prepared for surgery with clipping, shaving, disinfection and covered with thin layer of cotton saturated with absolute alcohol and also the surgical field was covered with sterile surgical towel which was fixed with towel clips. A 10 cm incision was made with scalpel and toothed thumb forceps through the skin, bleeding was controlled with artery forceps. Other incision was made through the external oblique abdominal muscle. The muscle was closed by simple continuous suture pattern with absorbable chromic cat gut. The skin was closed by simple interrupted pattern with silk. The wound was under some medical aftercare and protected from contamination by flie's eggs, dusts and micro-organisms.

Statistical Analysis: The findings were subjected to analysis of variance (ANOVA) followed by multiple comparison (LSD) and Chi-square Test using the method of SPSS.

Results

Clinical observations after anaesthesia and surgery: Administration of xylazine 2% (0.25 mg/kg) and Diazepam 0.5% (0.25 mg/kg) intravenously induced satisfied sedation in all animals subjected to the experiments. Sedation was characterized by reduced movement, ataxia, wide-base, lowered head, drooping of the ears and lower lips and penile protrusion. Injection of

propofol 1% (2 mg/kg) intravenously after 5 minutes from administration of xylazine and diazepam induced rapid onset of general anaesthesia. It took 10 to 15 seconds after injection of propofol to attain lateral recumbency, anaesthesia characterized by lateral recumbency, loss of consciousness and no limbs movement or head shaking after recumbency. All animals in experiment B (surgery) shows no signs of sensation at the site of surgery.

Quality of an aesthesia, muscle relaxation and recovery: The quality of an aesthesia was similar in all animals and the anaesthesia was smooth no excitement and excellent muscle relaxation in the two experiments. The sleeping time (anaesthetic phase) in experiment A (anaesthesia) was 18.11 ± 5.3 minutes and the sleeping time in experiment B (Surgery) was 20.89 ± 7.6 minutes. Recovery was smooth and of a quality good in both experiments A and B.

The effect of anaesthesia and surgery on Heart rate: Heart rate significantly increased ($P < 0.05$) compared with the baseline value (Control), in experiment A (Anaesthesia) the increase continued till the end of experiment. A sudden increase took place in experiment B (Surgery) at 5 minutes post dosing and started to decrease at 10 minutes post dosing to the end of the experiment, results depicted in Table (1).

The effect of anaesthesia and surgery on Respiratory rate: Table (2) shows that respiratory rate had a significant difference ($P < 0.01$) decreased from the baseline (Controls). In experiment A (anaesthesia) respiratory rate significantly decreased ($P < 0.01$). In Experiment B (Surgery) respiratory rate significantly decreased ($P < 0.01$) after 5 minutes after injection of propofol 1% 2mg/kg and

started to increase to the end of the experiment but not reached the control.

Table 1: The effect of anaesthesia (A) and surgery (B) on Heart rate (beats/min)

Treatment	After 5 min	After 10 min	After 30 min	After 45 min
Control	51.44±7.7 ^b	51.44±7.7 ^b	51.44±7.7 ^b	51.44±7.7 ^b
Anaesthesia	53.22±7.5 ^b	53.00±10.4 ^b	60.22±15.22 ^{a,b}	62.78±12.9 ^a
Surgery	65.56±9.1 ^a	63.67±10.9 ^a	64.22±12.1 ^a	63.56±9.9 ^a
Significance	0.002	0.028	0.092	0.036

a,b: means within the same columns followed by different superscripts are significantly (P<0.05) different.

Table 2: The effect of anaesthesia and surgery on Respiratory rate (breaths/min)

Treatment	After 5 min	After 10 min	After 30 min	After 45 min
Control	37.89±5.3 ^a	37.89±5.3 ^a	37.89±5.3 ^a	37.89±5.3 ^a
Anaesthesia	16.11±4.9 ^b	19.22±3.5 ^b	21.56±4.4 ^b	17.22±4.8 ^b
Surgery	15.44±3.1 ^b	20.89±4.9 ^b	24.33±5.7 ^b	22.89±7.6 ^b
Significance	0.00	0.00	0.00	0.00

a,b: means within the same columns followed by different superscripts are significantly (P<0.05) different

The effect of an aesthesia and surgery on Body Temperature: In both experiments A (Anaesthesia) and B (Surgery) body temperature showed no significant difference at 5, 10 minutes after injection of an aesthetic regime and

it was within the normal values compared with the controls. Significant decrease (P<0.05, P<0.01) at the 30, 45 minutes respectively after injection of an aesthetic regime.

Table 3: The effect of anaesthesia and surgery on Body Temperature in cellius degrees (°C)

Treatment	After 5 min	After 10 min	After 30 min	After 45 min
Control	37.18±0.6 ^{a,b}	37.18±0.6 ^a	37.18±0.6 ^a	37.18±0.6 ^a
Anaesthesia	37.02±1.1 ^b	36.70±0.9 ^b	36.13±1.0 ^b	36.19±0.8 ^b
Surgery	37.30±0.4 ^a	36.80±0.6 ^b	36.51±0.4 ^{a,b}	36.08±0.7 ^b
Significance	0.74	0.33	0.02	0.01

a,b: means within the same columns followed by different superscripts are significantly (P<0.05) different.

The effect of an aesthesia and surgery on plasma glucose: In experiment A (An aesthesia) and B (Surgery) plasma glucose concentrations revealed a

significant increase (P<0.01, P<0.05) at 3, 24 hours after injection of an aesthetic regime.

Table 4: The effect of anaesthesia and surgery on plasma Glucose concentration: (gm/disslitre)

Treatment	After 3 hours	After 6 hours	After 9 hours	After 24 hours	After 48 hours
Control	82.96±42.2 ^b	82.96±42.2 ^b	82.96±42.2 ^b	82.96±42.2 ^b	82.96±42.2 ^b
Anaesthesia	145.99±39.2 ^a	114.17±42.5 ^a	94.12±23.7 ^{a,b}	132.57±40.1 ^a	121.85±41.6 ^a
Surgery	107.64±24.2 ^b	94.09±30.8 ^{a,b}	103.69±18.8 ^a	112.43±378 ^{a,b}	102.15±24.0 ^b
significance	0.00	0.25	0.36	0.05	0.10

a,b: means within the same columns followed by different superscripts are significantly (P<0.05) different.

The effect of anaesthesia and surgery on plasma LDH: There was a significant

increase (P<0.05) in LDH activity in experiment A (Anaesthesia) and

experiment B (Surgery) compared with the baseline values (Controls) as in Table (5).

Table 5: The effect of anaesthesia and surgery on Plasma LDH

Treatment	After 3 hours	After 6 hours	After 9 hours	After 24 hours	After 48 hours
Control	516.92±121.9 ^b	516.92±121.9 ^b	516.92±121.9 ^b	516.92±121.9 ^b	516.92±121.9 ^b
Anaesthesia	581.27±525.0 ^b	702.33±458.0 ^{a,b}	635.54±421.8 ^b	472.11±261.6 ^b	779.57±264.7 ^a
Surgery	885.50±489.5 ^a	806.47±422.9 ^a	816.05±433.5 ^a	806.56±332.2 ^a	749.41±384.0 ^{a,b}
significance	0.16	0.26	0.22	0.02	0.11

a,b: means within the same column followed by different superscripts are significantly ($P < 0.05$) different

Discussion:

The onset of anaesthesia in the present study was rapid 10 to 15 seconds and recovery was free of untoward effects. These findings were in agreement with (Abd-Almaseeh, 2008),

All experimental animals showed similar quality of anaesthesia was smooth no excitement and excellent muscle relaxation, the duration of anaesthesia in experiment (A) was 18.11±5.3 minutes compared to 20.89±7.6 minutes in experiment (B), these findings were in accord with the previous studies in donkeys (Bettschart-Wolfensberger *et al.*, 2001)

Heart rate increased significantly which was also reported by (Naddaf *et al.* 2015), in donkeys. Increase in heart rate may be due to the bradycardia induced by xylazine as premedication to sedate the animals (Naddaf *et al.*, 2015). Mama, (1995) mentioned that tachycardia and hypertension were developed in horses anaesthetized with propofol alone. Injection of propofol may be associated with an increase in sympathetic tone as suggested by (Mama *et al.*, 1996), it reversed some cardiovascular properties such as bradycardia, sinoarterial and atrioventricular block which maybe attributed to xylazine as reported by (Faris *et al.*, 2003). Experiment B (Surgery) the tissue injury stimulate brain electrically and this stimulate defence reaction which characterized by tachycardia, increase in blood pressure ,

increase in sympathetic efferent activity and a relative vasodilatation to skeletal muscle with vasoconstriction in the splanchnic plexus these findings were cited by (Bernard Foex, 1999).

In this study the respiratory rate was significantly decreased from the baseline. These findings were similar to previous studies of propofol in donkeys (Coelho *et al.*, 2014). However, it differs from the results of (Quandt *et al.*, 1998) who reported that anaesthesia with propofol alone increased the respiratory rate in dogs. Propofol in most species associated with respiratory depression (Clarke *et al.*, 2014). Decrease of respiratory rate may be due to depression of central respiratory centre and ventilator response to arterial O₂ tension (Goodman *et al.*, 1987). And was explained by the fact that lateral or dorsal recumbent donkeys, the lower lung fields are compressed by the weight of the abdominal viscera pressing through the dome-shaped diaphragm, the problem is usually worst in larger donkeys lying in dorsal recumbency. Matthews *et al.*, (1999) suggested that the reduction in respiratory rate and increase of PaCO₂ may be associated with propofol given as induction of inhalation anaesthesia or may be due to positioning in dorsal recumbency.

In both experiments A (Anaesthesia) and B (Surgery) the rectal temperature in donkeys was significantly decreased, at 30 and 45 minutes from injection of

propofol, xylazine and diazepam. This is in agreement with previous studies in donkeys (Naddaf *et al.*, 2015). Although, the reduction in body temperature could be explained on the light of the fact that the body reacted to prevent itself from death due to hypothermia by rising of temperature.

Freeman and England, (2000) reported that general anaesthesia decrease body temperature by reducing metabolic rate and muscle activity, in addition the effects of α_2 adrenoceptor agonist caused depression of hypothalamic thermoregulatory center. Hypothermia may occur in animal under general anaesthesia when anaesthesia conducted in cold environment or administration of intravenous fluid (Hall *et al.*, 2001).

Plasma glucose concentration was significantly increased at 3, 24 hours post dosing, glucose concentration in plasma revealed an increase in experiment A (Anaesthesia) and experiment B (Surgery) and started to decrease gradually thereafter. After surgery blood glucose increased, the increase could be due to hepatic glycolysis as a result of cortisol and catecholamines which facilitated glucose production. Hyperglycaemia persists due to catabolic hormones stimulate glucose production and lack or reduction in insulin secretion (Desborough, 2000). Hyperglycaemia as a result of inhibition of insulin release from pancreatic beta cells due to α_2 adrenoceptor agonist injection (Hsu & Hummel, 1981). The increase in glucose concentration may be due to increase of glucose production from the liver reflecting increased sympatho - adrenal activity with less obvious effects on the pituitary – adrenal axis. Support for such a situation is provided by the observation that chlorpromazine induced hyperglycaemia

in mice by releasing adrenaline from the adrenal medulla (Nakadate *et al.*, 1980). Hyperglycaemia may also result from changes in rates of hepatic blood flow (Symonds, 1976).

There was a significant increase ($P < 0.05$) in LDH activity in experiment A (Anaesthesia) and experiment B (Surgery) at 24 hours post injection of anaesthetic regime. The results suggested that the increase might be due to the muscular damage which made by surgery. Serum activities can be used to evaluate effects of heart by xenobiotic (carcinogens, drugs, environmental pollutants, food additives, hydrocarbons and pesticides), LDH one of the markers enzymes which are distributed throughout the body and it contains isoenzymes that are recognized as markers for muscle and heart lesion (Anderson and Cockayne, 2003). Oluwatosin and Adaramoye *et al.*, (2013) in a study in rats reported that propofol at 2 and 4mg/kg significantly ($P < 0.05$) increase the activities of serum LDH. Hayden and Tyagi, (2002) also suggested that the increase in serum LDH of diabetic rats was due to cardiac muscular damage caused by (diabetes mellitus). Wiernsperger, (2003) stated that the activities of serum LDH could be used to measure the state of necrosis in cardiac tissues. In view of the observed increase in LDH activities in propofol-treated rats, the drug was found to elicit adverse effects on the cardiac tissue.

Conclusion and Recommendations:

The results concluded the combination of propofol 1% (2 mg/kg), xylazine 2% (0.25 mg/kg) and diazepam 0.5% (0.25 mg/kg) during both experiments is a safe regimen during both anaesthesia and recovery. Anaesthetic regime alone or followed by surgery had effects on

cardiopulmonary parameters, body temperature and plasma glucose concentrations and LDH activity. As the result this humble attempt was made to organize the study to provide an opportunity for each of these segments of the clinical practice to use the material as it applies to their needs.

The results recommended to use this regime of anaesthesia in donkey's surgery. Further studies are needed to monitor the level of the blood gases and remove the accumulated CO₂.

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تأثير التخدير والجراحة علي المؤشرات الإكلينيكية و تراكيز الجلوكوز و انزيم اللاكتات دي هيدروجينيز في البلازما في الد ر

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المستخلص

اجريت الدراسة لإختبار أثر التخدير و الجراحة علي المؤشرات الإكلينيكية مثل معدل ضربات القلب، معدل التنفس، درجة الحرارة و أيضا أثر التخدير و الجراحة علي تراكيز الجلوكوز و إنزيم اللاكتات دي هيدروجينيز في البلازما. أستخدمت في هذه الدراسة تسعة من در لائحة صحيا. إشملت الدراسة علي تجربتين. التجربة الأولى (أ) تم فيها حقن الحيوانات بعقار الزيليزي % 0.25 ملجرام/كيلوجرام) وبعد خمس دقائق تم حقن عقار الديازيبام 5. % 0.25 ملجرام/كيلوجرام) و عقار البروفول % 2 ملجرام/كيلوجرام) بالوريد. التجربة الثانية (ب) أجريت بحقن العقاقير أعلاه، في الحال بعد التخدير و حدوث الإستلقاء الجانبي تم فتح الخاصرة بالمبضع عبر الجلد وعضلات البطن. في كلتا التجربتين (أ) التخدير و (ب) الجراحة تم قياس معدل ضربات القلب، معدل التنفس، الحرارة عند 0. 10 45 دقيقة بعد الحقن. تراكيز الجلوكوز و إنزيم اللاكتات دي هيدروجينيز تم قياسها عند 6 9 24 48 ساعة بعد الحقن. تم أخذ المؤشرات الإكلينيكية و تراكيز الجلوكوز و إنزيم اللاكتات دي هيدروجينيز في البلازما وذلك قبل حقن البرتوكول التخديري (الكنترول). في التجربة (أ) كان الطور التخديري 5.3±18.11 دقيقة مقارنة ب 7.6±20.89 دقيقة في التجربة (ب). الإفاقة جيدة و غير مصحوبة بهيجان. في كلتا التجربتين (أ) و (ب) وجد هنالك زيادة معنوية في معدل ضربات القلب مقارنة بالقيم الطبيعية (الكنترول)، وجد أن هنالك إنخفاض معنويا في معدل التنفس مقارنة بالقيم الطبيعية، في كلتا التجربتين لوحظ إنخفاض معنويا في درجة الحرارة عند 10 45 دقيقة بعد حقن البرتوكول التخديري، في كلتا التجربتين وجد أن هنالك ارتفاع معنويا في نسبة الجلوكوز في البلازما عند 24 ساعة بعد حقن البرتوكول التخديري. وجد ان هنالك إرتفاع معنويا في تركيز إنزيم اللاكتات دي هيدروجينيز في البلازما في كلتا التجربتين. وجد أن البرتوكول التخديري (أ) لوحده أو متبوعا بالجراحة (ب) يؤثر علي ضربات القلب، معدل التنفس، درجة حرارة الجسم، نسبة الجلوكوز و انزيم اللاكتات دي هيدروجينيز في البلازما.