Abstract:

The current study conducted in the period from August 2016 to January 2017 to evaluate the effect of the general anaesthetic propofol with premedication xylazine and diazepam in cardiopulmonary parameters (heart rate, respiratory rate, rectal temperature), Haematological parameters (RBCs, WBCs, Hb and PCV), biochemical parameters (Glucose, Total protein, Albumin, ALT, AST and LDH enzyme activity) and some reflexes (anal reflex, tail reflex, spinal reflex, tongue reflex, pedal reflex, salivation and lacrimation). Also to evaluate the effect of surgery with the mentioned regimen in the mentioned parameters. The study consist of two experiment conducted in nine donkeys (*Equuas asinus*), the first experiment (A) performed by administered propofol 1% (2 mg/kg), xylazine 2% (0.25 mg/kg) and diazepam 0.5% (0.25 mg/kg). the second experiment (B) performed by administered the above mentioned anaesthetic regime then surgical incision in the flank through the skin and abdominal muscle took place. In both experiments cardiopulmonary parameters, haematological parameters and reflexes were taken at 5, 10, 30 and 45 minutes after injection of anaesthetic drugs, blood samples were taken to separate plasma to measure biochemical parameters at 3, 6, 9, 24 and 48 hours after injection of anaesthetic drugs. The sleeping time (anaesthetic phase) in

experiment A(anaesthesia) was 18.11±5.3 and the sleeping time in experiment B(Surgery) was 20.89±7.6 . recovery was smooth and of a quality good in both experiments A and B. Heart rate significantly increased compared with the baseline values (Controls) , gradually in experiment A (Anaethesia) continued till the end of experiment. And a sudden increase took place in experiment B (Surgery) and started to decrease gradually to the end of the experiment. respiratory rate had a highly significant different ($P < 0.01$) it had been dramatically decreased from the baseline values (Controls). In experiment A (anaethesia) respiratory rate decreased , and started to rise till 30 minutes after injection of propofol (21.56±4.4) breath/min. Then decreased again. In Experiment B (Surgery) respiratory rate decreased after 5 minutes after injection of propofol 1% 2mg/kg and started to increase to the end of the experiment. In experiment A (Anaethesia) and B (Surgery) Body temperature showed no significant difference after 5 minutes, 10 minutes of injection of propofol it was within the normal values compared with the controls. And significantly decreased ($P<0.05$, $P<0.01$) at the 30, 45 minutes after injection of propofol. Haematological parameters (RBCs, WBCs, Hb and PCV) revealed no significant difference but a very small variation was noticed when compared with the normal values. Glucose concentration revealed a significant different ($P<0.01$, $P<0.05$) at 3 , 24 hours after injection of propofol, plasma glucose

concentration revealed an increasing pattern at 3 , 24 hours after injection of propofol intravenously in both experiment A (Anaethesia) and experiment B (Surgery) compared with the baseline values (Controls) and started to decrease gradually there after. Total protein revealed no significant difference but there was a small decrease in experiment A (Anaethesia) and experiment B (Surgery) compared with the baseline values (Controls). There was a significant decrease ($P<0.01$, $P<0.05$) in plasma albumin in both experiment A (Anaethesia) and B (Surgery) compared with the baseline values (Controls). No significant different in plasma AST. It had been increased dramatically three hours post propofol injection then started to decrease to the normal values gradually thereafter in both experiment A (Anaethesia) and B (Surgery). ALT revealed no significant different, In experiment A (Anaethesia) ALT had small increase at three hours post propofol injection and started to decrease to the end of the experiment, in experiment B (Surgery) ALT increased more than experiment A (Anaesthesia) when compared with baseline value (Control) and started to decrease gradually thereafter. There was no significant difference in LDH in experiment A (Anaethesia) and experiment B (Surgery) compared with the baseline values (Controls) , But a small increase in LDH enzyme activity in both experiment took place at 3, 6, 9, 24 in surgery and 48 hours after injection of propofol 1% 2mg/kg intravenously, and there was a significant increase after

24 hours from injection of propofol ($P<0.05$). It is concluded that the anaesthetic regime alone (A) or followed by surgery (B) affected the heart rate, respiratory rate, rectal temperature, plasma glucose concentrations, albumin, total protein, AST, ALT and plasma LDH enzyme activity, haematological parameters were not affected . The study recommended to use this regime of anaesthesia in donkey's surgery.

المستخلص

اجريت الدراسة بالمستشفى البيطري التعليمي بجامعة بحري كلية الطب البيطري في الفترة من أغسطس 2016 وحتى أبريل 2017 وذلك لتقييم أثر التخدير والجراحة علي المؤشرات الإكلينيكية مثل معدل ضربات القلب، معدل التنفس، الحرارة والمؤشرات الدموية (RBCs, WBCs, Hb and PCV) وبعض المؤشرات الكيموحيوية (تراكيز الجلكوز في الدم, البروتين, الألبيومين, الالنين امينوترانسفيريز, الاسبارتيت امينوترانسفيريز وإنزيم الاكتات دي هيدروجينيز). أستخدمت في هذه الدراسة تسعة حمير لائقة صحيا. إشتملت الدراسة علي تجربتين. التجربة الأولي(أ) تم فيها حقن الحيوانات بعقار الزيلزين2% (0.25 ملجرام/كيلوجرام) وبعد خمس دقائق تم حقن عقار الديازيبام 0.5% (0.25 ملجرام/كيلوجرام) وعقار البروفول 1% (2 ملجرام/كيلوجرام) بالوريد. التجربة الثانية (ب) أجريت بحقن العقاقير أعلاه، في الحال بعد التخدير وحدوث الإستلقاء الجانبي تم فتح الخاصرة بالمبضع عبر الجلد وعضلات البطن. في كلتا التجربتين تم قياس المؤشرات الاكلينيكية والمؤشرات الدموية والمنعكسات عند 5, 45,30,10 دقيقةبعد الحقن. تم أخذ عينات دم وفصل البلازما لقياس المؤشرات الكيموحيوية عند 6,3 ,9 ,24 ,48 ساعة بعد الحقن. في التجربة (أ) كان الطور التخديري 18.11±5.3 دقيقة مقارنة ب 20.89±7.6 دقيقة في التجربة (ب). الإفاقة جيدة وغير مصحوبة بهيجان. وجد هنالك زيادة معنوية في معدل ضربات القلب مقارنة بالقيم الطبيعية (الكنترول)، وجدت الزيادة تدريجية في التجربة (أ) إستمرت حتى نهاية التجربة , وجدت زيادة مفاجئة في معدل ضربات القلب في التجربة (ب) ثم بدأت في التناقص تدريجيا حتى نهاية التجربة. وجد أن هنالك إنخفاضا معنويا في معدل التنفس مقارنة بالقيم الطبيعية، في التجربة (أ) وجد انخفاض في معدل التنفس حتى 30 دقيقة (21.56 ±4.4) نفس/ الدقيقة, في التجربة (ب) وجد إنخفاض في معدل التنفس عند 5 دقائق بعد حقن البرتوكول التخديري ثم بدأ في الارتفاع تدريجيا حتى نهاية التجربة.في كلتا التجربتين (أ) و (ب) لوحظ عدم

وجود إختلاف معنوي في درجات حرارة الجسم عند 5, 10 دقائق من حقن البرتوكول التخديري كانت ضمن القيم الطبيعية , وجد هنالك انخفاضا معنويا($P<0.01< P<0.05$) في درجات الحرارة عند 30, 45 دقيقة بعد حقن البرتوكول التخديري.لوحظ عدم وجود اختلاف معنوي في المؤشرات الدموية مع وجود اختلاف صغير إذا ما قورنت بالقيم الطبيعية. وجدت زيادة معنوية ($P< 0.01, P< 0.05$) في تركيزالجلكوز في البلازما عند 3, 24 ساعة بعد حقن البرتوكول التخديري ثم بدأ ينخفض تدريجيا . لوحظ عدم وجود إختلاف معنوي في مستوى بروتينات البلازما في كلتا التجربتين لكن هنالك نقصان طفيف إذا ما قورن بالقيم الطبيعية. وجد هنالك انخفاضا معنويا في مستوى الألبيومين في البلازما ($P < 0.01, P< 0.05$) في كلتا التجربتين إذا ماقورن بالقيم الطبيعية . في كلتا التجربتين لوحظ عدم وجود إختلاف معنوي في مستوى ALT, AST في البلازما لكن هنالك زيادة ضئيلة عند 3ساعات من حقن البرتوكول التخديري ثم بدأ في الانخفاض تدريجيا ليصل الى القيمة الطبيعية. في كلتا التجربتين لوحظ وجود زيادة معنوية في مستوى انزيم LDH عند 24 ساعة بعد حقن البرتوكول التخديري .في كلتا التجربتين وجد هنالك تأثير في المنعكسات مابين جيد وضعيف وغياب للمنعكسات.وجد أن الجراحة والتخدير لها تأثير على المؤشرات الاكلينيكية والمؤشرات الكيموحيوية والمنعكسات وليس لها تأثير على المؤشرات الدموية . الدراسة توصي باستخدام البرتوكول التخديري في جراحة الحمير.

Chapter One

1.1 Introduction:

Donkeys are necessary in agricultural communities and farm work in many areas of the world. They occupy rural areas, generally they were used in working life and transportation due to its high tolerance disease resistance less expensive to purchase and cheap in their health care expenses (Kreuchauf . 1984). The huge number of donkeys were found in developing countries where most of them have less medical following up and veterinary care . The donkey population was estimated to be 7.5 millions (MARF, 2009). There were located in north east region of Africa, where there originated the Nubian wild ass (Beja-Pereira *et al.*, 2004) . It was then domesticated and used for multiple purposes. Donkeys were widely distributed throughout Northern, Eastern, Western and Central Sudan. Owners depend on them in most of their activities. These animals are used for transport of wood, water, hay, tools needed for field work, pull carts are also ridden by their owners, used for cultivating broad beans and wheat. They may carry loads exceeding their body weight for long distances. It is a beast of burden which is tough enough to survive with such a workload in the harsh environments. The donkeys are immensely tolerant creatures, living intimately with humans and always working, quietly carrying their loads with endurance and patiency (Powell, 2004). The first warning a practitioner must remind himself that donkeys and

mules don’t look like horses they don’t act exactly like horses (they have been given the reputation of being stubborn). Perhaps in different ways they are more like cattle, they are not showing distress and pain and they are easier to be restrained with hobbles or ropes, if securely applied. 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). Although donkeys should not be worked until they are three years old when they are physically mature, in Mexico and developing countries, they were often forced to work earlier than that (De Aluja & Lopez, 1991).

Donkeys are desert-adapted animals, they can survive where horses cannot. Reasons which were attributed to their hardiness and survival include: desert-adaptations to water shortages, the ability to rehydrate quickly when water is presented, willingness to eat unpalatable feeds which is harsh for horses, and perhaps, differences in susceptibility to diseases highly fatal to horses, they are more patient, tolerable and show more strength and endurance, however, they lack the desire of competing in race in that aspect they differ from horses.

Donkeys are differentiated by size and range from miniature (<85-90 cm)through standard (90-35cm) to Mammoth (>135cm) (Naddaf,*et al.*, 2015). Many physiological and pharmacological feature of donkeys (Equus asinus) are different from those of horses(Equus caballus). For example the donkeys were

desert- adapted; therefor, fluid-balance mechanism are different than in the horse. It was also found that the donkeys have a greater capacity to metabolize certain drugs, they will often require higher doses of drugs or more frequent doses to maintain drugs effectiveness. In some certain anaesthetic (Guaifenesin) donkeys were showed sensitivity to depression , administration of guaifenesin produce respiratory depression in donkeys more faster than it does in horses (Matthews *et al*., 2002) . Donkeys also appear to posses an increased metabolic capacity for certain drugs such as sulfamethoxazole, trimethoprim, flunixin meglumine, gentamicin, ketamine and xylazine (Peck. *et al* 2002, Coakley.*et al*. 1999, Welfare .,*et al* 1996, Matthews .*et al* 1994, 1997,2002) which could be attributed to differences in cytochrome P450 isoenzyms. 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 (Cheng *et al*. 1996; Mealey *et al*. 1997). Other differences between donkeys and horses that the plasma volume is maintained in dehydrated donkeys even when the loss is 20% of normal body water, while horses are by far less resistant to this challenge. 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 dosages regimens such as (Acepromazine, Detomedine, ketamine, clenbuterol, Griseofulvin, Ivermectin, Mebendazole, Permethrin, phenylbutazone and Triclabendazole) (Lizarraga *et al*. 2004). Total intravenous anesthesia is now a clinically accepted technique of veterinary anesthesia 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 was characterized by a virtual 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 were limited studies on TIVA in donkeys, but propofol has been used in donkeys premedicated with xylazine (Matthews *et al*. 2002; Abd-Almaseeh 2008). Amin & Mohammed (2012) found that, Propofol and Ketamine injected after detomedine was satisfactory. Acepromazine has been used satisfactorily for tranquilization of donkeys and mules (Matthews & van Loon 2013). 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. In the last 15 years the greatest interest and emphasis for equine field anesthesia have been on various combinations of an alpha2-adrenergic agonist sedative/analgesic with a phencyclidine.

Xylazine HCL is pre-anaesthetic used in wide species of animals (Hall and Clarke.,1991). It was the first α2 - adrenergic agonist that was used to induce sedation and analgesia in dogs and cats before using detomidine (Hall *et al.,* 2001) Central effect was also reported by Sanhouri *et al* (1989). It act via central nervous system by activation of α - adrenergic system e.g the α2 - adrenergic receptors (Thurmon *et al*., 1996) intrathecal effects was also reported by Waterman (1989). To achieve adequate sedation in mules dose of xylazine should be increased 50% (Matthews et al. 1997). Donkeys need higher doses of α2-adrenoceptor agonist to produce adequate sedation and analgesia (El-Maghraby and Atta 1997; Joubert *et al*. 1999). Alpha 2-adrenoceptor agonists are capable of producing atrio-ventricular and sino-atrial blockade in donkeys as in horses (El-Maghraby and Atta 1997; Joubert *et al*. 1999). Subsequently, xylazine has been used clinically to induce ejaculation in stallion with neurologic or lameness problems that prevent normal copulation (Johnston, *et al*., 1998).

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). It can be administered intravenously, intramuscularly, orally and rectally via suppository enema (Forney., 2002), it has receptors in peripheral organs and receptors in central nervous system (Pierre *et al*., 2002). In the present

study a combination of xylazine and diazepam as pre-medication with propofol as a general intravenous anaesthetic.

1.2 General Objectives:

The aims of the present study were: To determine the onset time of anaesthesia , duration of anaesthesia and recovery time after a single intravenous infusion.

1.3 Specific Objectives:

1- To study reflexes, clinical and biochemical parameters during and after anaesthesia induced with propofol with xylazine and diazepam.

2- To study blood profile (haematological conditions) during and after surgery and anaesthesia.

3- Provide relief from pain and desensitization during surgery.

4- To evaluate anaesthetic quality produced by propofol (2mg kg^{-1} , I.V), xylazine (1 mg kg^{-1}, I.V) and diazepam (0.02 mg kg^{-1} , I.V) alone and during surgery.

Chapter Two

Literature review

2.1 Background:

In the early 1970s many studies were performed to establish new generation of injectable anaesthesia, the studies revealed that some phenol derivatives had hypnotic properties. So they can be used in surgery, these studies resulted in the development of a new molecule 2,6-di-isopropylphenol (propofol) the first trail performed by Kay and Stephenson (1980).

Anaesthesia and surgery in horses still carries an unacceptably high risk of death. The death rate may range from 0– 63% (Mee *et al*., 1998) to 1–5% (Young and Taylor, 1993, Johnston *et al*., 1995). This compares unfavourably with the anaesthetic-related death rate of 0·15% in small animals (Clarke and Hall, 1990) and 0·01% in man (Duberman and Bendixen, 1986).

Propofol had became popular and widely used as intravenous anaesthetic in both induction and maintenance general anaesthesia in the last 10-15 years. Recovery in animal which injected with propofol is smooth and fast without excitement whether it was injected as a bolus or infusion (Hall and Chambers 1987).

Propofol decreases arterial blood pressure and cardiac output in a dose-dependent manner, probably because of reduction in preload by direct venodilation (Goodchild *et al.*, 1989 , Bentley *et al*., 1989).

2.2 Propofol intravenous anaesthetic:-

Tow,6-di-isopropyl phenol (alkyl phenol), nonbarbiturate and relatively noncumulative intravenous anaesthetic agent with rapid onset and smooth

recovery and has neutral PH, and it provided as an oil in water emulsion (Abd-Almaseeh., 2008), Induction and recovery were smooth, unexcited with good stability of blood profile (watkins *et al*.,1987).

2.2.1 Composition of propofol:

The present formulation of propofol consist of 1% of propofol in intralipid, a parental nutritional agent consist of 10% soybean oil, 2.25% glycerol and 1.2% purified egg phosphate. Propofol appears as slightly milky viscous substance of pH 7 and stable at room temperature, not sensitive to light. If a diluted solution of propofol is required, it is compatible with 5% dextrose in water (Lumb and Jones., 1996). As it capable of supporting bacterial growth, a septic precaution should be maintained in removing propofol from ampules or vials (Andrews *et al* ., 1997).

2.2.2 Commercial and Chemical names:

Propofol has many commercial names : diprivan, rapinovet and propofol . it's chemical name is 2,6-di-isopropyl phenol.

2.2.3 Formula and Structure:

Propofol consist of twelve carbon atom, eighteen hydrogen atom and one oxygen atom ($C_{12}H_{18}O$). And it's structure as below.

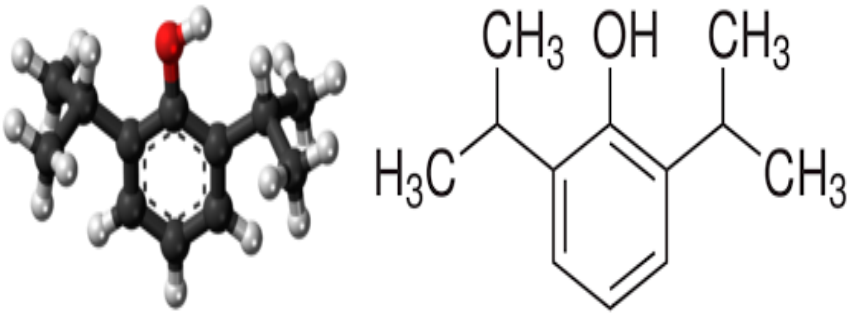

Figure (1) Chemical structure of propofol

2.2.4 Propofol in Total Intravenous Anaesthesia:

In recent years, total IV anesthesia (TIVA) protocols became widely used in humans. In horses, a variety of TIVA techniques have been used for short procedures in the field, but for longer procedures, maintenance of anesthesia by use of TIVA has been limited to combinations of muscle relaxants, α2-adrenoceptor agonists, or both, with ketamine. Although cardiopulmonary function during their use is acceptable (Greene, *et al*. 1986,.Taylor, *et al*., 1995). These techniques were limited in duration by accumulation of the ketamine metabolite, norketamine, causing poor recoveries with signs of apparent ketamine overdose (Taylor, *et al*., 1995, Nolan, *et al*. 1996) Propofol has the ideal pharmacokinetic profile for infusion, but it provides poor surgical analgesia, and in humans and other species it is used in combination with sedatives, analgesics, or both for TIVA. Propofol has been used for TIVA in horses (Nalon, et al., 1996,Nolan, *et al* ., 1985, Mama.*et al*., 1998) but adequate anesthesia to enable surgical procedures with relatively low doses of propofol and with acceptable cardiopulmonary function was only achieved when propofol was combined with ketamine infusion (Nalon, *et al*., 1996,Flaherty, *et al*., 1997) which again limited its use for prolonged anesthesia.

The effect of propofol was seen clearly when injected intravenously but oral rout didn't show any effect because of it's rapid metabolism, the intramuscular rout did not produce a state of anaesthesia.

2.2.5 Total Intravenous Anaesthesia in Equine:

Both inhalant and injectable anaesthetics are used in mammals (Ghurashi *et al*., 2016). Inhalant anaesthetics are easier to administer and allow more control of the depth of anaesthesia, but it requires expensive and sophisticated equipment for

application, Injectable anaesthetics are often preferred because they can be used without special equipment (Ghurashi *et al*., 2016). A variety of methods can be used for induction of anaesthesia depending on availability of drugs, size and condition of the patient donkey and familiarity of anaesthesia staff and the animal with different protocols (Matthews and van Dijk, 2004).

Total intravenous anaesthesia is now a clinically accepted technique of veterinary anaesthesia especially for short acting non-cumulative anaesthetic agents (Hughes and Nalon, 1999). The most popular technique used to maintain intravenous anesthesia in horses is often referred to as triple drip (TD). This refers to a mixture of a dissociative anesthetic (ketamine), an adrenergic a2- receptor agonist (xylazine, romifidine or detomidine), and a centrally acting muscle relaxant (guaifenesin) (Staffieri & Driessen 2007). This solution is infused at predetermined rates or to effect (Taylor et al. 1995; El-Ghoul *et al*. 2004). The TD can be employed for induction in horses (Taylor *et al*. 2008) and to maintain anesthesia, as a sole technique or in association with inhalational anesthetics (Yamashita *et al*. 2002, Staffieri & Driessen 2007). This technique produces excellent muscle relaxation and mild to moderate analgesia. Although hypoxemia can occur, severe cardiorespiratory depression is rarely seen in short procedures (Young *et al*. 1993; Taylor et al. 1995). However, the application of this technique in horses is restricted to procedures up to 60–90 minutes, since the potential accumulation of drugs and metabolites might lead to unsatisfactory prolonged recoveries and toxicity (Taylor *et al*. 1995).

2.2.6 Propofol in Equine practice:

Propofol was reported as an induction agent in premedicated horses or miniature donkeys with alpha 2 adrenoceptor agonist to provide a satisfactory anaesthesia with excellent induction, muscle relaxation and smooth recovery (Matthews and

van-Dijk, 2004). Administration of propofol by the continuous infusion rate for maintenance of anaesthesia resulted in stable cardiopulmonary effects in donkeys (Naddaf *et al*., 2015). Although propofol has been used for TIVA in horses, adequate anesthesia enabling surgical procedures and acceptable cardiovascular function was only achieved when propofol was administered with an infusion of ketamine, medetomidine, or both (Young *et al*., 1993,Mama *et al*., 1998, Flaherty *et al*., 1997).

2.3 Xylazine

Xylazine is on of α2 adrenoceptor agonists. Sedation is achieved five minutes after injection and lasts 30 to 60minutes and is dose dependent. Xylazine is a powerful tranquilizer which was commonly used as premedication to sedate large animals prior surgery, it used primarily for sedation , anaesthesia, analgesia and muscle relaxation (Kollias *et al*., 1993; Cuvilliez *et al*., 1995). Xylazine is strong analgesic agent, particularly in colic (England & Clarke, 1996) and in colic cases the analgesia is associated with the marked reduction in gastrointestinal tract motility. Sedated horses with xylazine were very sensitive to touch or any external stimulants and the apparently well sedated horse may, if disturbed, respond with a very sudden and quick kick. The cardiopulmonary effects of xylazine in horses have been well investigated (Muir *et al*., 1979a; England & Clarke, 1996). At doses of up to 1.1mg/kg xylazine did not cause severe respiratory depression although there may be a small rise in $PaCO_2$ and a slight decrease in PaO_2. However, upper airway obstruction may occur. Heavy coated horses may sweat as sedation is waning off this is most commonly seen if atmospheric temperatures are high. Other side effects were those typical of α2 adrenoceptor agonists and include hyperglycaemia (Thurmon *et al*., 1982) and diuresis. When used as a premedicant, xylazine greatly reduces the amount of both injectable and volatile anaesthetic

agents subsequently required. In the 30 years since its launch xylazine has become the ‘gold standard’ for equine sedation and premedication, particularly in North America. In Europe until the advent of generic forms and alternative agents forced prices to reduce to a realistic level, its use was limited by much higher pricing.

2.3.1 Commercial and Chemical names:-

Xylazine HCL, Rompun HCL. The chemical name of Xylazine is 2(2,6-dimethyl phenylammine)-4-H-5,6-dihydro-1,3-thiazine (Prys-Robert., 1991).

2.3.2 Formula and Structure:-

Xylazine consist of eleven carbon atom, sixteen hydrogen atom, two nitrogen atom and one sulphar atom ($C_{12}H_{16}N_2S$).

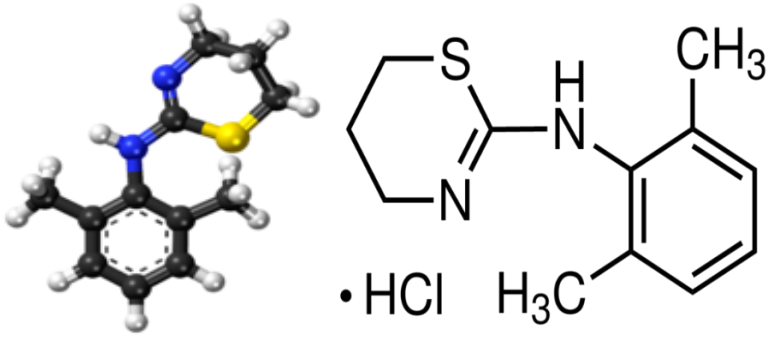

Figure (2): Chemical structure of xylazine HCL

2.4 Diazepam:-

Diazepam is one of benzodiazepines group, is used for sedation of animals. Diazepam is a white to yellow , tasteless, the drug is insoluble in water except for one member of the group named midazolam and solution for injections contain solvents such as propylene glycol, ethanol and sodium benzoic acid (Hall *et al*., 2001, Taylor and Clarke., 2007). The anxiolytic properties of diazepam are not obvious in horses and the drug should not be used on its own as it gives rise to

ataxia, sometimes associated with panic, possibly through its muscle relaxing properties (Muir *et al*., 1982). Horses when they were given diazepam they showed some clinical signs such as: muscle fasiculations, weakness, and ataxia at sedative doses. Over doses (greater than 0.2 mg/kg) may induce recumbancy as a result of its muscle relaxant properties and general CNS depressant effects, severe respiratory depression or cardiovascular complications (Susan and Donald, 2003). When administrated alone, its overdoses caused: coma, decreased reflexes, hypotension, respiratory depression and cardiac arrest, drowsiness, mental confusion, impaired motor functions (impaired reflexes, impaired coordination, impaired balance), and dizziness (Bendarzewska-Nawrocka *et al*., 1980). Diazepam increases the central depressive effects of alcohol, other hypnotics/sedatives (e.g. barbiturates), narcotics, and other muscle relaxants. The euphoriant effects of opioids may be increased, leading to increased risk of psychological dependence (Holt, 1998). The benzodiazepine agents can be used in foals which produce recumbency following i.v. 0.1–0.25mg/kg of diazepam or midazolam (a water soluble benzodiazepine).

2.4.1 Commercial and Chemical Name:-

Valium and Diastast, It is a 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-2H-1, 4-benzodiazepin-2-one (Marrs, 2004).

2.4.2 Formula and Structure:-

Diazepam consist of sixteen carbon atom, thirteen hydrogen atom, two nitrogen atom and one oxygen atom ($C_{16}H_{13}CIN_2O$).

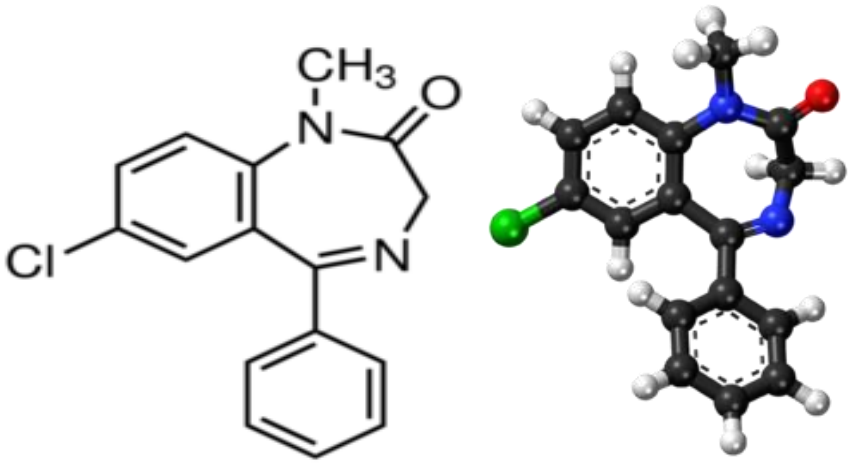

Figure (3): Chemical structure of diazepam

2.5 Combination of Propofol, Xylazine and Diazepam:-

Propofol anaesthesia with combination with diazepam is reported in camel to cause quick, smooth, excitement free recovery (Fahmy *et al*., 1995). Diazepam envolving anaesthetic combination with xylazin and propofol posses properitis desireable for an ideal general anaesthetic as reported by (Singh, 2003). Combination of xylazine and propofol is used to induce surgical anaesthesia in dog, horses and camels (Cullen and Reynoldson., 1993; Robertson *et al.*, 1992; Mama *et al*., 1996; Al-Mubarak., 2008) respectively. Duration of anaesthesia and recovery time is dependent on the premedication dose of xylazine in propofol anaesthetized dog (Kim and Jang., 1999). It also reported that premedication with xylazine, papaveretum, diazepam, pethidin, atropine and scopolamine did not affect recovery time of propofol in which animal (Weaver and Raptopoulos, 1990).

2.6 Effect on clinical parameters:

2.6.1 Effect on respiratory rate:

propofol anaesthesia in animals causes respiratory depression expressed by a decrease in tidal volume and respiratory rate, occurrence of apnoea. And it was

reported to be dependent on dose, speed of injection, premedication and the presence of hyperventilation and hypoxia (Langley and Heel, 1988; Smith *et al*., 1994).

The respiratory values (minute volume and tidal volume) decrease significantly after onset till 15 min and gradually resume normal values thereafter (Bayan *et al*., 2002). It was very well established that when xylazine injected it produce bradycardia and respiratory depression (Sanger*et al*., 1968).

2.6.2 Effect on heart rate:

Propofol alone (Kim *et al*., 1999) or in combination showed a significant decrease in heart rate.

Propofol alone produces higher heart rate than Xylazine-Ketamin (Brussel *et al*., 1989). When xylazine was used as sedative in animals it produces immediate decreases in heart rate, respiratory rate (Campbell *et al*., 1979; Kumar and Thurmon., 1979; Sanhouri *et al* 1989). In horses xylazine produce significantly decreased heart rate, increased incidence of atrioventricular block, and decrease cardiac output (Wanger *et al*., 1991).

2.6.3 Effect on Body Temperature:

Body temperature significantly decrease after propofol injection (Kim and Jang, 1999).

2.6.4 Effect on haematological parameters:

haematological parameters are reported to be within the physiological Limits during propofol anaesthesia in camel (Brzeski *et al*., 1994). A decrease in haematocrit, haemoglobin and Red Blood Cells (RBCs) and White Blood Cells

(WBCs) count values and an increase in white blood cells (WBCs) count values after surgery performed (Gill *et al*., 1996).

2.6.4.1 Effect on Packed Cell Volume (PCV):

PCV is reported to fall during the first 10 min of anaesthesia in ruminant (Handel et al., 1991). A significant decrease in packed cell volume (PCV) during diazepam-propofol anaesthesia in goats was also recorded (Kalawala *et al*., 1991). PCV appears unaltered when xylazine premedication was used in combination with low and high doses of propofol in equines (Mama *et al*., 1998).

No significant change in Total Leukocyte Count (TLC). Total Erythrocyte Count (TEC) and PCV values is observed when propofol is used for seven consecutive days in dogs. (Kwon *et al*., 1999). Similarly, no significant changes in TLC, TEC, Mean Corpouscular Volume (MCV), Mean Haemoglobin Concentration (MHC) and Mean Corposcular Haemoglobin Concentration (MCHC) values were noticed when Xylazine- propofol combination were used for general anaesthesia in dogs (Kim and Jang ., 1999).

2.6.4.2 Effect on Red Blood Cells (RBCs) and White Blood Cells (WBCs):-

No significant changes in either total red blood cells and total leukocyte count was recorded after propofol and premedication injection (Kwon *et al* 1999).

2.6.4.3 Effect on Blood Indices:

No significant changes in blood indices like mean corpouscular volum (MCV), mean haemoglobin concentration (MHC), mean corpouscular haemoglobin concentration (MCHC) values observed after propofol injection (Kwon *et al* 1999).

2.6.5 Effect on Biochemical parameters:- Total Plasma Protein (TPP) and Albumin (ALB) increased significantly after the injection diazepam- propofol-

ketamin in goats (Kalawala *et al*., 1991, 1993). But Alanin aminotransferase (ALT) and Aspartate aminotransferase (AST) were decreased, and they were in normal range in dogs anaesthetized with propofol (Kwon *et al*., 1999).

2.6.6 Diagnostic characteristic of biochemical parameters:-

2.6.6.1 Diagnostic characteristics of plasma glucose:-

Glucose is the major source of energy in the body .insulin, produced by islet cells in the pancreas, facilitates glucose entry into the tissue cells. A deficiency of insulin or decease of its effectiveness increases blood glucose.

Elevated serum or plasma glucose concentration was found in diabetes mellitus (insulin dependent, non-insulin dependent) and in other conditions and syndromes.

Hypoglycemia can occur in response to fasting, or it may be due to drugs, poisons, inborn errors of metabolism or previous gastrectomy.

2.6.6.2 Diagnostic characteristics of plasma Total protein:-

Most of the plasma proteins are synthetized by the liver. The major exception to this is the immunoglobulins which are produced by plasma cells found in the spleen, lymph nodes and bone marrow.

The two general causes of alterations of serum total protein are either a change in the volume of plasma water or a change in concentration of one or more of the serum proteins or both.

Hyperproteinemia can be caused by dehydration (inadequate water intake, severe vomiting, diarrhea, diabetic acidosis) or as a result of an increase in the concentration of specific proteins (immunoglobulins in chronic infections, multiple myeloma).

Hypoproteinemia caused by hemodilution (salt retention syndromes, massive intravenous infusions), impaired synthesis (sever malnutrition, chronic liver disease such as hepatitis or parasitic diseases of the liver such as fascioliasis or schistosomiasis, intestinal malabsorptive disease), or by excessive protein loss due to chronic kidney disease or severe burns.

2.6.6.3 Diagnostic characteristics of plasma Albumin:-

Albumin is the most abundant protein in plasma. It has three main function: it contributes towards maintaining the colloid oncotic pressure of plasma , it acts as non-specific transport vehicle for many nonpolar compounds and it is a source of endogenous amino acids.

Hyperalbuminemia is of little diagnostic significance except in dehydration.

Hypoalbuinemia is found as a result of several factors: reduced synthesis caused by liver disease; reduced absorption of amino acids due to malabsorption syndromes or malnutrition; increased catabolism as a result of inflammation or tissue damage; altered distribution between intravascular and extravascular space due increased capillary permeability, over hydration or ascites; abnormal losses caused by renal disease (nephrotic syndrome, diabetes mellitus, chronic glomerulonephritis, system lupus erythematosus), gastrointestinal tract disease (ulcerative colitis, Crohn’s disease) or skin damage (exfoliative dermatitis, extensive burns); congenital absence or albuminemia.

Albumin plasma concentration, although important for management and follow-up, have very little value in diagnosis.

2.6.6.4 Diagnostic characteristics of plasma AST:-

The aminotransferase catalyze the formation of glutamic acid from 2-oxyglutarate by transfer of amino groups. AST is found in highest concentration in the liver and heart muscle but it is also abundant in skeletal muscle, kidney and pancreas.

The serum concentration of AST is elevated in hepatitis and other form of hepatic disease associated with necrosis: infectious mononucleosis, cholestasis, cirrhosis, metastatic carcinoma of the liver, delirium tremers, after administration of various drugs (Friedman 2001) and parasitic diseases.

Serum AST is also elevated after myocardial infarction, in skeletal muscle disease (as progressive muscular dystrophy), in acute pancreatitis or hemolytic disease (Friedman 2001).

2.6.6.5 Diagnostic characteristics of plasma ALT:-

ALT is normally present in various tissue but its higher concentration found in liver and kidney.

The serum concentration of ALT is elevated in hepatitis and other form of hepatic disease associated with necrosis: infectious mononucleosis, cholestasis, cirrhosis, metastatic carcinoma of the liver, delirium tremers, and after administration of various drugs, such as opiates, salycilates or ampicillin (Friedman 2001) and parasitic diseases.

Serum ALT concentration can also be elevated in skeletal or cardiac muscle disease (Friedman 2001 ;Burtis *et al*., 2005).

2.6.6.6 Diagnostic characteristics of plasma LDH:-

Lactate dehydrogenase is present in all cells of the body but its higher concentrations are found in liver, heart, kidney, skeletal muscle and erythrocytes.

Total LDH concentration in serum or plasma is increased in patients with liver disease, renal disease, myocardial infarction, many malignant diseases, progressive muscular dystrophy and almost any cause of hemolysis (Friedman 2001 ;Burtis *et al*., 2005).

Chapter Three

Materials and Methods:

3.1 Location of Study:

The study was conducted at the Teaching Veterinary Hospital, University of Bahri, College of Veterinary Medicine in the period from August 2016 to January 2017.

3.2 Animals:

Nine male mature donkeys (90-135 cm in high at the withers) ranging in age from 24 to 30 months and weight to be 78-144 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 experiment 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 any diseases. The animals were fasted 12-15 hours before injection of anaesthesia. The experiments carried out indoors.

3.3 Control tools:

Labor , Ropes

3.4 Drugs:

Propofol Lipuro 1% (10 mg/ml). B.Braun Melsungen AG, Germany.

Xylazine HCL(xylaject 2%). ADWIA Co. S.A.E, Egypt.

Diazepam (Valium 10 mg)

3.5 Instrumentation:

3.5.1 Injection Set:

Disposable Syringe 20ml and 10ml, Intravenous catheters.

3.5.2 Monitoring Tools

Stethoscope , Digital Thermometer, Stop watch

3.5.3 Surgical Set

Scalpel plates size (15 and 22), Scalpel Handle size (3 and 4), Needle holders, Artery forceps, Surgical Scissors, Toothed and Non toothed thumb forceps, Sterile Surgical gloves, Latex powder Gloves, Chromic cat gut, Non absorbable silk, Towel clips, Surgical Towels, Sterile gauze, Cotton, Absolute alcohol, Clipping machine, Shaving blades and shaving machine.

3.6 Clinical Parameters During the Experimental works:

3.6.1 Reflexes:

Anal and tail Reflex, Spinal Reflex, Tongue and Jaw Relaxation Reflex, Pedal Reflex, Salivation and Lacrimation.

3.6.2 Anaesthetic Parameters:

Onset Time , sleeping time and recovery time.

3.6.3 Clinical Parameters:

Respiratory Rate, Heart Rate and Rectal Temperature.

3.6.4 Biochemical Tests:

Glucose Concentration, ALT, AST, Total protein, Albumin and LDH.

3.6.5 Blood Profile (Haematological Parameters):

RBCs, TWBCs, PCV and HB

3.7 Experimental Design:

The experiment was 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 (Geehan*et al*., 2014).

3.8 Anaesthetic Protocol:

One anaesthetic regime was performed in this study but two experiments were conducted. In the first experiment animals were given Propofol with Xylazine and Diazepam. In the second trial the animals were given Propofol with Xylazine and Diazepam, after induction of anaesthesia, Surgery was then carried out.

3.8.1 Experiment A (Anaesthesia):-

Ten minutes prior to premedication, with the donkeys outwardly in a calm state, baseline measurement were obtained and recorded for physiological parameters, blood profile and biochemical tests. The animals then received Diazepam 0.5% (0.25 mg kg^{-1}) and xylazine 2% (0.25 mg kg^{-1}) which were given in the jugular vein. Five minutes after premedication , once sedation took place anaesthesia was given by the administration of 2 mg kg^{-1} Propofol 1% intravenously (IV). Immediately after recumbency donkeys positioned in right lateral recumbency to help us monitor the heart rate and the respiratory rate. Then haematological parameters and selected reflexes were recorded 5, 10, 30 and 45 minutes after administration of the general anaesthetic.

3.8.2 Experiment B (Surgery):-

Ten minutes prior to premedication, with the donkeys lied in a calm state. Baseline measurement were obtained and recorded for physiological parameters, blood profile and biochemical tests. The animals received Diazepam 0.5% (0.25 mg kg-1) and xylazine 2% (0.25 mg kg-1) which were given in the jugular vein. five minutes after premedication , once sedation took place.Anaesthesia with 2 mg kg^{-1} Propofol 1% administered intravenously(IV). Immediately after recumbency donkeys positioned in right lateral recumbency, surgical incision was made

through the skin and abdominal muscle. Then haematological parameters and selected reflexes were recorded 5, 10,30 and 45 minutes after administration of the general anaesthetic.

3.8.2.1 Surgical procedure:-

The left flank was prepared for surgery with clipping,shaving, disinfectant 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. By using needle holder the muscle was closed by simple continuous suture pattern with chromic cat gut. The skin was closed by simple interrupted pattern with non absorbable silk. The wound was then followed up and protected from contamination by flie's eggs, dusts and micro-organisms.

3.9 Anaesthetic Parameters:

3.9.1 Onset time:-start of anaesthesia effect.

3.9.2 Recovery time:-

Is the time where the animal started to wake up till regaining the standing position.

3.9.3 Quality of Anaesthesia Onset:-

Quality of onset was judged in each donkey using a subjective scoring system from 0 to 4, as follows

0 (poor) : Ataxia and paddling; danger to the donkey and handler.

1 (fair) : Purposeful paddling with or with out attempt to regain feet.

2 (satisfactory) : Ataxia with or without paddling before falling to the ground.

3 (good) : donkey took 1 or 2 steps with no paddling before falling to the Ground

4 (excellent) : donkey sank smoothly to the ground

3.9.4 Anaesthetic Phase (sleeping time

Was considered as the period during which the animal showed signs of unconscious, no reflexes, response negatively to painful stimuli (Tammisto *et al*.; 1981).

3.9.5 Quality of muscles relaxation:

The quality of muscle relaxation scored as following

1- Excellent: characterized by complete relaxation.
2- Good: characterized by adequate relaxation of muscle that permits surgery

3- Moderate: with partial relaxation of neck, head and limbs muscles.

4- Poor: characterized by rigidity of neck, head and limbs muscles.

3.9.6 Recovery phase:

The animal was considered to be recovered from anaesthesia when it is capable of supporting itself in standing position and walk for ten steps without falling down.

3.9.6 Recovery time:

From the cessation of anaesthesia to first movement, gaining sitting on a sternal position and standing (Tomohito *et al*.; 2012).

3.9.8 Quality of recovery:

A score 0 to 5 described by Tomohito *et al* (2012) was used for assessment of quality of recovery from anaesthesia, as following

0 (unable to stand): donkey could not stand for > 2 hrs after multiple attempts To stand; excitement was evident; injury or high risk of Injury.

1 (poor): multiple attempts to stand; excitement was evident ; high Risk of injury.

2 (fair): multiple attempts to stand; substernal ataxia.

3 (satisfactory): Stood after 1 to 3 attempts; prolonged ataxia but no Excitement.

4 (good) : stood after 1 or 2 attempts; mild, short- term ataxia.

5 (excellent): stood after the first attempt; no ataxia.

3.9.9 Reflexes of anaesthesia and Surgery:

All anaesthetic reflexes were recorded at different periods during anaesthesia and surgery and then measured

3.9.9.1Tail reflex:

Detected when the tail is lifted upwardly, then if tension arise or the animal showed some kind of resistance the reflex is present (Sanhouri, *et al.;* 1989).

3.9.9.2 Anal reflex

Anal reflex was assessed by inducing tension of anal sphincter with two fingers. Positive response was considered when the movement of the anal sphincter was noticed (Geehan *et al.;* 2014).

3.9.9.3 Pedal reflex:

Pedal reflex was assessed by pinprick on the coronary band of the digit. If the animal moved its leg or leg muscle, the reflex was considered positive (Williams and Wyatt.; 2007).

3.9.9.4 Spinal reflex:

Detected by pinbrick in the skin with sharp object when animal move the skin reflex is present (Sanhuri *et al*.; 1989). In this work spinal reflex was tested with sharp object in the skin of lumbo-sacral region.

3.9.9.5 Tongue reflex:

Was assessed by pulling the tongue outside the mouth, when the animal capable to pull its tongue into the mouth, the reflex was considered positive (Nuha; 2004).

3.9.9.6 Jaw relaxation reflex:

Persistence of open mouth due to induced jaw retraction was considered to be positive jaw relaxation reflex. The reflex was considered regained when the animal was reluctant to open its mouth (Geehan.; 2014).

3.10 Cardiopulmonary monitoring during anaesthesia:

Base line heart rate and respiratory rate were determined in all donkeys standing in stocks before any medication was administered.

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

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

3.10.3 Body temperature: 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.

3.11 Data Collection:

Blood samples collection; five ml of blood were withdrawn from the jugular vein using sterile syringes. The blood was immediately transferred to EDITA container to examine the blood profile , other blood samples 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 ^{o}Cuntil analyzed. All blood samples were then transferred to haematology Lab. Teaching Veterinary Hospital, College

of Veterinary Medicine, University of Bahri for hematological examinations and Biochemical examinations. Blood profile (WBCs, RBCs, Haemoglobin concentration and PCV), Biochemical and Physiological parameters (Heart Rate, Rectal Temperature and Respiratory rate) were evaluated before anaesthesia and surgery took place (baseline, one sample), 5, 10, 30 and 45 minutes after protocol of anaesthesia administration. And when the donkeys stood. Biochemical parameters (Glucose concentration in Plasma, Total protein, Albumin, Alanine Aminotransferase ALT, Aspartate Aminotransferase AST, and Lactate Dehydrogenase LDH) were evaluated at baseline (one sample), 3, 6, 9, 24 and 48 hours after anaesthesia onset and after surgery.

3.12 Haematological values:

3.12.1 Packed Cell Volume (PCV%):

Tubes with EDTA mixed well with blood , capillary tubes were filled with blood until ¾ of its length, then sealed at the end. The sealed end is placed at the outer angle of microhaematocrit centrifuge. The tubes were centrifuged at 12000 (rpm)for 5 minutes then removed and placed on a reader. Values were recorded as percentage of packed red cells to the total blood volume.

3.12.2 Haemoglobin Concentration Hb (g/dl).

In this test used fresh blood, drabkin solution,microtiter pipetts and chlorimeter apparatus bichrome number (07500 Cambridge, UK). 20µl of blood were drown , the outside of the tip was whipped with a piece of cotton and then inserted in 5ml of drabkin solution. The mixture left for 10 minutes to allow full color development. The colorimeter switched on and allowed to reach umbient temperature , then adjusted to zero using plain drabkin solution as blank. The samples were then read and their optical densities (O.D)were recorded. The Hb concentration calculated using the following formula;

$$\frac{\text{O.D of sample}}{\text{O.D of standard}} \times \text{concentration}$$

3.12.3 Red Blood Cells (RBCs):

3.12.3.1 Materials:

Blood, haemocytometer, RBCs reagent, microtiter pipette, cover slip and microscope. The working dilution was prepared in eppindorff tubes in suitable volumes to reach a final dilution of 1:200. The 1:200 was gently put in haemocytometer to fill the chambers using microtiter pipette.

After leaving the haemocytometer for 2-3 minutes to settle the RBCs counting is made in 5 of 25 small squares using 40x objective lens and then multiplied by the

haemocytometer correction factor to give the number of the RBCs per µl (x 10^6), the number of cell counted x 5 x dilution.

Number of cells count x 50 x200 = --------/ µl.

3.12.4 White Blood Cells (WBCs):

3.12.4.1Materials:

Fresh blood, WBCs reagent, microtiter pipette, haemocytometer, cover slip and microscope.

The working dilution prepared in eppendorff tubes in suitable volumes to reach the final dilution of 1:20 .the 1:20 was gently put in haemocytometer to fill the chambers using microtiter pipette .

After leaving the haemocytometer for 2-3 minutes to settle the WBCs counting was made by using the microscope in the four squares which found in the corner, each of them divided into16 squares by using 40x objectives eyepiece and then multiplied the result by the haemocytometer correction factor to give the number of WBCs per µl (x10^3).

Number of cells count x 50= ---------/ µl

3.13 Plasma Glucose and Enzymes Assay

Plasma which collected in lithium heparin tubes were used to assay the concentrations of plasma glucose, Total Protein (TP), Albumin (ALB), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Lactate dehydrogenase (LDH) analyzed by Digital spectrophotometer and all results were recorded.

3.14 Plasma Glucose concentration:-

Glucose in samples originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry

$Glucose + {}^{1}/_{2}\ O_2 + H_2O \qquad Gluconate + H_2O_2$

$2H_2O_2 + 4\text{- Aminoantipyrine} + Phenol \qquad Quinoneimine + 4H_2O_2$

3.14.1 Plasma glucose Requirements and Instrumentation:-

Plasma, glucose reagent (A), glucose standard (S), test tubes, pipette and spectrophotometry.

3.14.2 Plasma glucose procedure:-

glucose reagent and glucose standard were brought to room temperature $25^{O}C$. labelled test tubes (blank, standard and sample) were pipetted with 10µL of

glucose standard (S), 10µL of sample and 1.0 ml of glucose reagent (A) which added to all tubes.

The tubes were mixed and incubated for 10 minutes at room temperature $25^{O}C$.

The absorbance (A) of the standard and the sample were measured at 500 nm against the blank.

The glucose concentration in the sample was calculated using the following general formula:

$$\frac{A\ sample}{A\ standard} \times C\ standard = C\ sample$$

3.15 Total protein:-

Protein with the sample reacts with copper (II) ion in alkaline medium forming a coloured complex that can be measured by spectrophotometry.

3.15.1 Total protein Requirements and Instrumentation:-

Plasma, protein reagent (A), protein standard (S), test tubes, pipette and spectrophotometry.

3.15.2 Total protein procedure:-

protein reagent and protein standard were brought to room temperature 25^{O}C. labelled test tubes (blank, standard and sample) were pipetted with 20µL of protein standard (S), 20µL of sample and 1.0 ml of protein reagent (A) which added to all tubes.

The tubes were mixed and incubated for 10 minutes at room temperature 25^{O}C.

The absorbance (A) of the standard and the sample were measured at 545 nm against the blank.

The total protein in the sample was calculated using the following general formula:

$$\frac{A\ sample}{A\ standard} \times C\ standard = C\ sample$$

3.16 Plasma Albumin:-

Albumin in the sample reacts with bromocresol green in acid medium forming a coloured complex that can be measured by spectrophotometry.

3.16.1 Plasma Albumin Requirements and Instrumentation:-

Plasma, albumin reagent (A), albumin standard (S), test tubes, pipette and spectrophotometry.

3.16.2 Plasma Albumin procedure:-

albumin reagent and albumin standard were brought to room temperature 25^{O}C.

labeled test tubes (blank, standard and sample) were pipetted with 10µL of albumin standard (S), 10µL of sample and 1.0 ml of albumin reagent (A) which added to all tubes.

The tubes were mixed and incubated for 10 minutes at room temperature 25^{O}C.

The absorbance (A) of the standard and the sample were measured at 630 nm against the blank.

The albumin concentration in the sample was calculated using the following general formula:

$$\frac{A\ sample}{A\ standard} \times C\ standard = C\ sample$$

3.17 Aspartate aminotransferase:-

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutrate forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction.

Aspartate + 2-Oxoglutarate _____Oxalacetate + Glutamate

Oxalacetate + NADH + H^+———— Malate + NAD^+

3.17.1 AST Requirements and Instrumentation:-

Plasma, eppindorff tubes, pipett, working reagent, cuvette with 1 cm light path and spectrophotometer.

3.17.2 AST procedure:-

Working reagent and the instruments were brought to reaction temperature 37^{o}C .

1.0 ml of working reagent and 50 µl of sample were pipetted into a cuvette.

Working reagent and sample were mixed and then inserted into spectrophotometer.

Absorbance of each sample was recorded in 180 seconds.

The difference between consecutive absorbance and the average absorbance difference per minute (ΔA/min) were calculated with the general formula:

$\Delta A/min \times (Vt \times 10^6) \div (\varepsilon \times 1 \times VS) = U/L$

3.18 Alanine aminotransferase:-

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The

catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase (LDH) coupled reaction.

Alanine + 2-Oxoglutarate ——————Pyruvate + Glutamate

Pyruvate + NADH + H^+——————Lactate + NAD+

3.18.1 ALT Requirements and Instrumentation:-

Plasma, eppindorff tubes, pipett, working reagent, cuvette with 1 cm light path and spectrophotometer.

3.18.2 ALT procedure:-

Working reagent and the instruments were brought to reaction temperature 37^{o}C .

1.0 ml of working reagent and 50 µl of sample were pipetted into a cuvette.

Working reagent and sample were mixed and then inserted into spectrophotometer.

Absorbance of each sample was recorded in 180 seconds.

The difference between consecutive absorbance and the average absorbance difference per minute (ΔA/min) were calculated with the general formula:

Δ A/min × (Vt × 106) ÷ (ε × 1× VS) = U/L

3.19 Lactate dehydrogenase:-

Lactate dehydrogenase (LD or LDH) catalyzes the reduction of pyruvate by NADH, to form lactate and NAD^+ . the catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm.

Pyruvate + NADH + H+ ————Lactate + NAD^+

3.19.1LDH Requirements and Instrumentation:-

Plasma, eppindorff tubes, pipett, working reagent, cuvette with 1 cm light path and spectrophotometer.

3.19.2 LDH procedure:-

Working reagent and the instruments were brought to reaction temperature 37^{o}C .

1.0 ml of working reagent and 20 µl of sample were pipetted into a cuvette.

Working reagent and sample were mixed and then inserted into spectrophotometer.

Absorbance of each sample was recorded in 180 seconds.

The difference between consecutive absorbances and the average absorbance difference per minute (ΔA/min) were calculated with the general formula:

Δ A/min × (Vt × 106) ÷ (ε × 1× VS) = U/L

3.20 Statistical Analysis:-

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

Chapter Four

Results

4.1 Clinical observation after anaethesia and surgery:

Administration xylazine 2% (0.25 mg kg^{-1}) andDiazepam 0.5% (0.25 mg kg^{-1}) 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 anaethesia . 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. Surgery performed respectively, all animals in experiment B (surgery) shows no signs of sensation at the site of surgery.

4.2 Quality of anaethesia, muscle relaxation and recovery:

The quality of anaethesia was similar in all animals and the duration of anaethesia 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.

4.3 The effect of anaethesia and surgery on Physiological parameters:

4.3.1 The effect of anaethesia and surgery on Heart rate:

Heart rate significantly increased compared with the baseline values (Controls) , gradually in experiment A (Anaethesia) continued till the end of experiment. And

a sudden increase took place in experiment B (Surgery) and started to decrease gradually to the end of the experiment, result depicted in table (1).

Table (1): The effect of anaethesia (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}
Anaethesia	53.22 ± 7.5^{b}	53.00 ± 10.4^{b}	$60.22\pm15.22^{a,b}$	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

4.3.2 The effect of anaethesia and surgery on Respiratory rate:

Table (2) shows that respiratory rate had a highly significant different ($P < 0.01$) it had been dramatically decreased from the baseline values (Controls). In experiment A (anaethesia) respiratory rate decreased , and started to rise till 30 minutes after injection of propofol (21.56±4.4) breath/min. Then decreased again. In Experiment B (Surgery) respiratory rate decreased after 5 minutes after injection of propofol 1% 2mg/kg and started to increase to the end of the experiment.

Table (2): The effect of anaethesia and surgery on Respiratory rate (breath/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]
Anaethesia	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

4.3.3 The effect of anaethesia and surgery on Body Temperature:

In experiment A (Anaethesia) and B (Surgery) Body temperature showed no significant difference after 5 minutes, 10 minutes of injection of propofol it was within the normal values compared with the controls. And significantly decreased (P<0.05, P<0.01) at the 30, 45 minutes after injection of propofol .

Table (3): The effect of anaethesia and surgery on Body Temperature in cellius degrees(C^O)

Treatment	After 5 min	After 10 min	After 30 min	After 45 min
Control	37.18±0.6	37.18±0.6	37.18±0.6[a]	37.18±0.6[a]
Anaethesia	37.02±1.1	36.70±0.9	36.13±1.0[b]	36.19±0.8[b]
Surgery	37.30±0.4	36.80±0.6	36.51±0.4[a,b]	36.08±0.7[b]
significance	0.735	0.331	0.023	0.006

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

4.4 The effect of anaethesia and surgery on blood profile:

4.4.1 The effect of anaethesia and surgery on RBCs

No significant difference in RBCs but there were a small decrease in RBCs in experiment A (Anaethesia) and experiment B (Surgery) when compared with base line values (Controls).

Table (4):The effect of anaethesia and surgery onRBCs (No× 10^6 µl)

Treatment	After 5 min	After 10 min	After 30 min	After 45 min
Control	9.75±1.5	9.75±1.5	9.75±1.5	9.75±1.5
Anaethesia	9.22±2.7	8.46±2.3	8.78±2.3	8.65±2.0
Surgery	9.85±1.5	7.94±1.9	9.18±1.2	9.25±0.8
significance	0.771	0.142	0.496	0.331

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

4.4.2 The effect of anaethesia and surgery on WBCs

Table (5) shows no significant different in WBCs but a very smallincrease in WBCs in Experiment A (Anaethesia) and experiment B (Surgery) compared with baseline values (Controls).

Table (5): The effect of anaethesia and surgery on WBCs

Treatment	After 5 min	After 10 min	After 30 min	After 45 min
Control	6.20±2.4	6.20±2.4	6.20±2.4	6.20±2.4
Anaethesia	7.69±2.9	7.02±2.1	7.82±2.5	7.33±2.9
Surgery	7.40±2.3	6.41±1.2	7.02±1.6	7.38±1.8
significance	0.442	0.666	0.314	0.515

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

4.4.3 the effect of anaethesia and surgery on Hb:

No significant difference in Haemoglobin concentration (Hb) in experiment A (Anaethesia) and experiment B (Surgery) compared with the baseline values (Controls).

Table (6): The effect of anaethesia and surgery on Hb

Treatment	After 5 min	After 10 min	After 30 min	After 45 min
Control	11.78±1.9	11.78±1.9	11.78±1.9	11.78±1.9
Anaethesia	12.01±1.6	10.98±1.5	11.77±1.4	12.41±1.5
Surgery	12.43±2.1	12.39±2.4	12.04±2.0	11.89±1.9
significance	0.751	0.333	0.933	0.725

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

4.4.4 the effect of anaethesia and surgery on PCV:

As described in table (7) no significant different in PCV in experiment A (Anaethesia) and experiment B (Surgery) compared with the baseline values (Controls).

Table (7): The effect of anaethesia and surgery on PCV

Treatment	After 5 min	After 10 min	After 30 min	After 45 min
Control	29.44±5.4	29.44±5.4	29.44±5.4	29.44±5.4
Anaethesia	29.00±4.0	28.33±31	29.67±4.0	29.78±3.2
Surgery	30.33±3.6	29.11±4.5	27.78±3.4	28.11±3.5
significance	0.808	0.862	0.607	0.668

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

4.5 The effect of anaethesia and surgery on biochemical parameters:

4.5.1 The effect of anaethesia and surgery on plasma glucose

There were a significant different ($P<0.01$, $P<0.05$) at 3 , 24 hours after injection of propofol, plasma glucose concentration revealed an increasing pattern at 3 , 24 hours after injection of propofol intravenously in experiment A (Anaethesia) and at 3 , 24 hours after injection of propofol intravenously experiment B (Surgery)

compared with the baseline values (Controls) and started to decrease gradually there after(see table 8).

Table (8): The effect of anaethesia 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	82.96±42.2	82.96±42.2[b]	82.96±42.2[b]
Anaethesia	145.99±39.2[a]	114.17±42.5	94.12±23.7	132.57±40.1[a]	121.85±41.6[a]
Surgery	107.64±24.2[b]	94.09±30.8	103.69±18.8	112.43±378[a,b]	102.15±24.0[b]
significance	0.004	0.245	0.356	0.047	0.104

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

4.5.2 The effect of anaethesia and surgery on plasma total protein:

Total protein revealed no significant difference but there was a small decrease in experiment A (Anaethesia) and experiment B (Surgery) compared with the baseline values (Controls).

Table (9): The effect of anaethesia and surgery on plasma Total protein

Treatment	After 3 hours	After 6 hours	After 9 hours	After 24 hours	After 48 hours
Control	92.33±46.0	92.33±46.0	92.33±46.0	92.33±46.0	92.33±46.0
Anaethesia	60.98±29.1	83.00±64.2	92.68±52.4	59.96±40.6	69.28±42.8
Surgery	81.57±45.3	73.29±41.2	82.57±61.4	84.10±22.3	83.84±45.8
significance	0.274	0.738	0.903	0.188	0.553

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

4.5.3 The effect of anaethesia and surgery on plasma Albumin

Table (10) showed significant decrease ($P<0.01$, $P<0.05$) in plasma albumin in both experiment A (Anaethesia) and B (Surgery) compared with the baseline values (Controls).

Table (10): The effect of anaethesia and surgery on plasma Albumin

Treatment	After 3 hours	After 6 hours	After 9 hours	After 24 hours	After 48 hours
Control	5.61 ± 2.2^{a}	5.61 ± 2.2^{a}	5.61 ± 2.2^{a}	5.61 ± 2.2^{a}	5.61 ± 2.2^{a}
Anaethesia	$4.66\pm2.3^{a,b}$	3.26 ± 2.7^{b}	$3.76\pm3.2^{a,b}$	3.50 ± 2.3^{b}	3.61 ± 2.3^{b}
Surgery	2.95 ± 1.7^{b}	1.72 ± 0.9^{b}	2.47 ± 2.0^{b}	1.94 ± 1.3^{b}	2.09 ± 1.0^{b}
significance	0.036	0.002	0.044	0.003	0.003

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

4.5.4 The effect of anaethesia and surgery on plasma AST:

No significant different in plasma AST. It had been increased dramatically three hours post propofol injection then started to decrease to the normal values gradually thereafter in both experiment A (Anaethesia) and B (Surgery).

Table (11): The effect of anaethesia and surgery on plasma AST

Treatment	After 3 hours	After 6 hours	After 9 hours	After 24 hours	After 48 hours
Control	206.85± 118.6	206.85±118.6	206.85±118.6	206.85±118.6	206.85±118.6
Anaethesia	230.42± 146.8	219.64±132.4	269.82±134.9	221.92±104.7	183.93±127.9
Surgery	323.36± 151.1	211.65± 79.6	210.82±40.4	248.80±122.7	232.40± 64.0
significance	0.195	0.971	0.386	0.740	0.637

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

4.5.5 The effect of anaethesia and surgery on plasma ALT:

Table (11) revealed no significant different, In experiment A (Anaethesia) ALT had small increase at three hours post propofol injection and started to decrease to the end of the experiment, in experiment B (Surgery) ALT increased more than experiment A (Anaesthesia) when compared with baseline value (Control) and started to decrease gradually thereafter.

Table (11): The effect of anaethesia and surgery on Plasma ALT

Treatment	After 3 hours	After 6 hours	After 9 hours	After 24 hours	After 48 hours
Control	12.53±10.9	12.53±10.9	12.53±10.9	12.53±10.9	12.53±10.9
Anaethesia	16.85±12.8	9.66±6.2	9.00±5.5	11.79±7.9	13.42±4.5
Surgery	17.68±9.4	15.80±8.7	11.95±6.8	16.79±13.6	20.89±13.1
significance	0.581	0.351	0.617	0.591	0.181

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

4.5.6 The effect of anaethesia and surgery on plasma LDH:

There was no significant difference in LDH in experiment A (Anaethesia) and experiment B (Surgery) compared with the baseline values (Controls) as in table (12). But a small increase in LDH in both experiment took place at 3, 6, 9, 24 in surgery and 48 hours after injection of propofol 1% 2mg/kg intravenously, and there was a significant difference after 24 hours from injection of propofol ($P<0.05$).

Table (12): The effect of anaethesia 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	516.92±121.9	516.92±121.9	516.92±121.9[b]	516.92±121.9
Anaethesia	581.27±525.0	702.33±458.00	635.54±421.8	472.11±261.6[b]	779.57±264.7
Surgery	885.50±489.5	806.47±422.9	816.05±433.5	806.56±332.2[a]	749.41±384.0
significance	0.161	0.257	0.221	0.020	0.112

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

4.6 The effect of anaethesia and surgery on body reflexes:

4.6.1 The effect of anaethesia and surgery on anal reflex

Anal reflex showed significant different at 5 minutes after injection of propofol ($P<0.01$), as in table (13) revealed that in experiment A (Anaesthesia), (5) donkeys showed good anal reflex, (1) donkey showed poor anal reflex and (3) donkeys showed no anal reflex, and also in experiment B (Surgery) 1 donkey

showed good anal reflex, (4)donkeys showed poor anal reflex and (4) donkeys showed no anal reflex.

Table (13): The association between treatment and Anal reflex at (A: 5 min, B: 10 min, C: 30 min, D: 45 min)

A: X^2 = 15.3 , significance (0.004)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	5(55.6)	1 (11.1)
Poor reflex	0 (0.00)	1 (11.1)	4 (44.4)
No reflex	0 (0.00)	3 (33.3)	4 (44.4)

At 10 minutes after injection of anaesthetic regimen there were no significant different in anal reflex, in experiment A (Anaesthesia), (7) donkeys showed good anal reflex, (1) donkey showed poor anal reflex and (1) donkey showed no anal reflex, in experiment B (Surgery), (6) donkeys showed good anal reflex, (3) donkeys showed poor anal reflex and no donkeys showed no anal reflex. Results depicted in table (14).

Table (14): The association between treatment and Anal reflex

B: X^2 = 6.1 , significance (0.189)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	7 (77.8)	6 (66.7)
Poor reflex	0 (0.00)	1 (11.1)	3 (33.3)
No reflex	0 (0.00)	1 (11.1)	0 (0.00)

No significant different in anal reflex at 30 minutes after administration of anaesthetic regimen, in experiment A (Anaesthesia), (7) donkeys showed good anal reflex and (2) donkeys showed poor anal reflex. In experiment B (Surgery), (8) donkeys showed good anal reflex and (1) donkey showed poor anal reflex. No donkey in both experiments showed no anal reflex (see table 15)

Table (15): The association between treatment and Anal reflex

C: X^2 =2.3 , significance (0.325)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	7 (77.8)	8 (88.9)
Poor reflex	0 (0.00)	2 (22.2)	1 (11.1)

No significant different in anal reflex at 45 minutes after administration of anaesthetic regimen, in experiment A (Anaesthesia), (7) donkeys showed good anal reflex and (2) donkeys showed poor anal reflex. In experiment B (Surgery), (8) donkeys showed good anal reflex and (1) donkey showed poor anal reflex. No donkey in both experiments showed no anal reflex (see table 16)

Table (16): The association between treatment and Anal reflex

D: X^2 = 2.3 , significance (0.325)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	7 (77.8)	8 (88.9)
Poor reflex	0 (0.00)	2 (22.2)	1 (11.1)

4.6.2 The association between treatment and Tail reflex:

Tables (17) revealed there was a significant different ($P<0.01$) in tail reflex at 5 minutes after injection of anaesthetic regimen. In experiment A (Anaethesia), (1) donkey showed good tail reflex, (1) donkey showed poor tail reflex and (7) donkeys showed no tail reflex. In experiment B (Surgery), no donkey showed good tail reflex, (1) donkey showed poor tail reflex and (8) donkeys showed no tail reflex.

Table (17): The association between treatment and Tail reflex at (A: 5 min, B: 10 min, C: 30 min, D: 45 min)

A: $X^2 = 23.2$, significance (0.00)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	1 (11.1)	0 (0.0)
Poor reflex	0 (0.0)	1 (11.1)	1 (11.1)
No reflex	0 (0.0)	7 (77.8)	8 (88.9)

Tables (18) revealed there was a significant different ($P<0.05$) in tail reflex at 10 minutes after injection of anaesthetic regimen. In experiment A (Anaethesia), (3) donkey showed good tail reflex, (5) donkey showed poor tail reflex and (1) donkeys showed no tail reflex. In experiment B (Surgery), (2) donkeys showed

good tail reflex, (5) donkeys showed poor tail reflex and (2) donkeys showed no tail reflex.

Table (18): The association between treatment and Tail reflex

B: $X^2 = 13.1$, significance (0.011)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	3 (33.3)	2 (22.2)
Poor reflex	0 (0.0)	5 (55.6)	5 (55.6)
No reflex	0 (0.0)	1 (11.1)	2 (22.2)

There were a significant different ($P<0.01$) at 30 minutes after administration of anaesthetic regimen. In experiment A (Anaesthesia), (7) donkeys showed good tail reflex, (2) donkeys showed poor tail reflex and no donkey showed no tail reflex. . In experiment B (Surgery), (3) donkeys showed good tail reflex, (6) donkeys showed poor tail reflex and no donkey showed no tail reflex. Results depicted in table (19).

Table (19): The association between treatment and Tail reflex

C: $X^2 = 9.9$, significance (0.007)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	7 (77.8)	3 (33.3)
Poor reflex	0 (0.0)	2 (22.2)	6 (66.7)

Table (20) revealed that there were a significant different (P<0.01) at 45 minutes after administration of anaesthetic regimen. In experiment A (Anaesthesia), (9) donkeys showed good tail reflex, no donkey showed poor tail reflex and no donkey showed no tail reflex. . In experiment B (Surgery), (5) donkeys showed good tail reflex, (4) donkeys showed poor tail reflex and no donkey showed no tail reflex.

Table (20): The association between treatment and Tail reflex

D: X^2 =9.4 , significance (0.009)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	9 (100)	5 (55.6)
Poor reflex	0 (0.0)	0 (0.0)	4 (44.4)

4.6.3 The association between treatment and Pedal reflex:

In this study pedal reflex revealed a significant different (P<0.01) at 5 minutes after injection of anaesthetic drugs. In experiment A (Anaesthesia), no donkey showed good pedal reflex, (5) donkeys showed poor pedal reflex and (4) donkeys showed no pedal reflex. In experiment B (Surgery) , no donkey showed good pedal reflex, no donkey showed poor pedal reflex and (9) donkeys showed no pedal reflex (see table 21).

Table (21): The association between treatment and Pedal reflex at (A: 5 min, B: 10 min, C: 30 min, D: 45 min)

A: X^2 = 37.4 , significance (0.00)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9(100)	0 (0.0)	0 (0.0)
Poor reflex	0 (0.0)	5 (55.6)	0 (0.0)
No reflex	0 (0.0)	4 (44.4)	9 (100)

Pedal reflex revealed significant different (P<0.01) at 10 minutes after injection of anaesthetic drugs, as in table (22) revealed that in experiment A (Anaesthesia), there were (5) donkeys showed good anal reflex, (2) donkeys showed poor anal reflex and (2) donkeys showed no anal reflex, and also in experiment B (Surgery),(1) donkey showed good anal reflex, (5)donkeys showed poor anal reflex and (3) donkeys showed no anal reflex.

Table (22): The association between treatment and Pedal reflex

B: X^2 = 14.6 , significance (0.006)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	5 (55.6)	1 (11.1)
Poor reflex	0 (0.0)	2 (22.2)	5 (55.6)
No reflex	0 (0.0)	2 (22.2)	3 (33.3)

No significant different at 30 minutes after injection of anaesthetic drugs. In experiment A (Anaesthesia), there were (8) donkeys showed good pedal reflex, (1)

donkey showed poor pedal reflex and no donkey showed no pedal reflex, and also in experiment B (Surgery), (6) donkey showed good pedal reflex, (3)donkeys showed poor pedal reflex and no donkey showed no pedal reflex. Results depicted in table (23).

Table (23): The association between treatment and Pedal reflex

C: $X^2 = 4.1$, significance (0.128)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	8 (88.9)	6 (66.7)
Poor reflex	0 (0.0)	1 (11.1)	3 (33.3)

No significant different at 45 minutes after injection of anaesthetic drugs. In experiment A (Anaesthesia), there were (8) donkeys showed good pedal reflex, (1) donkey showed poor pedal reflex and no donkey showed no pedal reflex, and also in experiment B (Surgery), (6) donkey showed good pedal reflex, (3) donkeys showed poor pedal reflex and no donkey showed no pedal reflex (see table 24). It started to attain normality coinciding with the awakening of the animal with fully attained pedal reflex coinciding with complete recovery from anaethesia and surgery.

Table (24): The association between treatment and Pedal reflex

D: $X^2 = 4.1$, significance (0.128)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	8 (88.9)	6 (66.7)
Poor reflex	0 (0.0)	1 (11.1)	3 (33.3)

4.6.4 The association between treatment and Tongue reflex:

There was a significant different (P<0.01) in tongue reflex at 5minutes after injection of propofol. In both experiments A (anaethesia) and B (Surgery) no donkey showed good tongue reflex, no donkey showed poor tongue reflex and (9) donkeys showed no tongue reflex. Tongue observed clearly protruding out site of mouth in both experiments slightly all the time of experiments and started to attain normal position inside the mouth.

Table (25): The association between treatment and Tongue reflex at

(A: 5 min, B: 10 min, C: 30 min, D: 45 min)

A: X^2 = 27.0 , significance (0.000)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	0 (0.0)	0 (0.0)
No reflex	0 (0.0)	9 (100)	9 (100)

Tongue reflex revealed significant different (P<0.01) at 10 minutes after injection of anaesthetic drugs, as in table (26) revealed that in experiment A (Anaesthesia), there were (4) donkeys showed good tongue reflex, (1) donkey showed poor tongue reflex and (4) donkeys showed no tongue reflex, and also in experiment B (Surgery), (1) donkey showed good tongue reflex, (2)donkeys showed poor tongue reflex and (6) donkeys showed no tongue reflex.

Table (26): The association between treatment and Tongue reflex

B: X^2 =14.6 , significance (0.006)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	4 (44.4)	1 (11.1)
Poor reflex	0 (0.0)	1 (11.1)	2 (22.2)
No reflex	0 (0.0)	4 (44.4)	6 (66.7)

No significant different at 30 minutes after injection of anaesthetic drugs. In experiment A (Anaesthesia), there were (9) donkeys showed good tongue reflex and no donkey showed poor tongue reflex, and also in experiment B (Surgery), (7) donkey showed good tongue reflex and (2) donkeys showed poor tongue.No donkey in both experiments showed no tongue reflex. Results depicted in table (27).

Table (27): The association between treatment and tongue reflex

C: X^2 = 4.3 , significance (0.115)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	9 (100)	7 (77.8)
Poor reflex	0 (0.0)	0 (0.0)	2 (22.2)

No significant different in tongue reflex at 45 minutes after administration of anaesthetic regimen, in experiment A (Anaesthesia), (9) donkeys showed good tongue reflex andno donkey showed poor tongue reflex. In experiment B

(Surgery), (8) donkeys showed good tongue reflex and (1) donkey showed poor tongue reflex. No donkey in both experiments showed no tongue reflex (see table 28)

Table (28): The association between treatment and Tongue reflex

D: $X^2 = 2.1$, significance (0.354)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	9 (100)	8 (88.9)
Poor reflex	0 (0.0)	0 (0.0)	1 (11.1)

4.6.5 The association between treatment and Lacrimation, Salivation:

Lacrimation and salivation in experiment A (Anaethesia) and experiment B (Surgery) had seen in some donkeys but not all. There were no significant different at 5 minutes after injection of anaesthetic drugs. In experiment A(Anaesthesia), (2) donkeys showed good reflex, (4) donkeys showed poor reflex and (3) donkeys showed no reflex, also in experiment B (Surgery), (1) donkey showed good reflex, (3) donkeys showed poor reflex and (5) donkeys showed no reflex, as in table (29).

Table (29): The association between treatment and Lacrimation, Salivation at (A: 5 min, B: 10 min, C: 30 min, D: 45 min)

A: $X^2 = 9.0$, significance (0.061)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	0 (0.0)	2 (22.2)	1 (11.1)
Poor reflex	0 (0.0)	4 (44.4)	3 (33.3)
No reflex	9 (100)	3 (33.3)	5 (55.6)

Lacrimation and salivation revealed significant different (P<0.01) at 10 minutes after injection of anaesthetic drugs, as in table (30) revealed that in experiment A (Anaesthesia), there were (3) donkeys showed good reflex, (5) donkeys showed poor reflex and (1) donkey showed no reflex, and also in experiment B (Surgery), (1) donkey showed good reflex, (1)donkey showed poor reflex and (7) donkeys showed no reflex.

Table (30): The association between treatment and Lacrimation, salivation

B: $X^2 = 16.6$, significance (0.002)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	0 (0.0)	3 (33.3)	1 (11.1)
Poor reflex	0 (0.0)	5 (55.6)	1 (11.1)
No reflex	9 (100)	1 (11.1)	7 (77.8)

No significant different in lacrimation and salivation at 30 minutes after administration of anaesthetic regimen, in experiment A (Anaesthesia), (2) donkeys showed good reflex, (2) donkeys showed poor reflex and (5) donkeys showed no reflex. In experiment B (Surgery), no donkey showed good reflex, no donkey showed poor reflex and (9) donkeys showed no reflex. Results depicted in table (31).

Table (31): The association between treatment and Lacrimation ,Salivation

C: X^2 = 9.4 , significance (0.052)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	0 (0.0)	2 (22.2)	0 (0.0)
Poor reflex	0 (0.0)	2 (22.2)	0 (0.0)
No reflex	9 (100)	5 (55.6)	9 (100)

No significant different in lacrimation and salivation at 45 minutes after administration of anaesthetic regimen, in experiment A (Anaesthesia), (2) donkeys showed good reflex and (7) donkeys showed no reflex. In experiment B (Surgery), no donkey showed good reflex and (9) donkeys showed no reflex. No donkey showed poor reflex in both experiment A (Anaesthesia) and B (Surgery) as shown in table (32).

Table (32): The association between treatment and Lacrimation ,Salivation

D: X^2 = 4.3 , significance (0.115)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	0 (0.0)	2 (22.2)	0 (0.0)
No reflex	9 (100)	7 (77.8)	9 (100)

4.6.6 The association between treatment and Spinal reflex:

In this study spinal reflex in both experiment A(Anaesthesia) and B(Surgery) showed significant different ($P<0.01$) at 5 minutes after administration of anaesthetic regimen. Experiment A (Anaesthesia) revealed (1) donkey showed

poor spinal reflex and (8) donkeys showed no spinal reflex. In experiment B (Surgery) no donkey showed poor spinal reflex and (9) donkeys showed no spinal reflex. In both experiments no donkey showed good spinal reflex as shown in table (33).

Table (33): The association between treatment and Spinal reflex at (A: 5 min, B: 10 min, C: 30 min, D: 45 min)

A: X^2 = 28.6 , significance (0.000)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (0.0)	0 (0.0)	0 (0.0)
Poor reflex	0 (0.0)	1 (11.1)	0 (0.0)
No reflex	0 (0.0)	8 (88.9)	9 (100)

Spinal reflex in both experiment A(Anaesthesia) and B(Surgery) showed significant different ($P<0.01$) at 10 minutes after injection of anaesthetic drugs. Experiment A (Anaesthesia) revealed (4) donkeys showed poor spinal reflex and (5) donkeys showed no spinal reflex. In experiment B (Surgery) no donkey showed poor spinal reflex and (9) donkeys showed no spinal reflex. In both experiments no donkey showed good spinal reflex as shown in table (34).

Table (34): The association between treatment and Spinal reflex

B: X^2 =34.7 , significance (0.000)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	0 (0.0)	0 (0.0)
Poor reflex	0 (0.0)	4 (44.4)	0 (0.0)
No reflex	0 (0.0)	5 (55.6)	9 (100)

There were a significant different (P<0.01) in spinal reflex at 30 minutes after administration of anaesthetic regimen. In experiment A (Anaesthesia), (2) donkeys showed good spinal reflex, (4) donkeys showed poor spinal reflex and (3) donkeys showed no spinal reflex. . In experiment B (Surgery), no donkey showed good spinal reflex, (2) donkeys showed poor spinal reflex and (7) donkeys showed no spinal reflex. Results depicted in table (35).

Table (35): The association between treatment and Spinal reflex

C: X^2 =23.6 , significance (0.000)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	2 (22.2)	0 (0.0)
Poor reflex	0 (0.0)	4 (44.4)	2 (22.2)
No reflex	0 (0.0)	3 (33.3)	7 (77.8)

A significant different (P<0.05) in spinal reflex at 45 minutes after injection of anaesthetic drugs. In experiment A (Anaesthesia), (4) donkeys showed good spinal reflex, (2) donkeys showed poor spinal reflex and (3) donkeys showed no spinal reflex. . In experiment B (Surgery), (2) donkeys showed good spinal reflex, (3) donkeys showed poor spinal reflex and (4) donkeys showed no spinal reflex. Results depicted in table (36).

Table (36): The association between treatment and Spinal reflex

D: X^2 =11.7 , significance (0.020)			
Treatment	Control No. (%)	Anaethesia No. (%)	Surgery No. (%)
Good reflex	9 (100)	4 (44.4)	2 (22.2)
Poor reflex	0 (0.0)	2 (22.2)	3 (33.3)
No reflex	0 (0.0)	3 (33.3)	4 (44.4)

Chapter Five

Discussion

Propofol studies were very limited in donkeys (Naddaf *et al*. 2015). This study performed in donkeys pertained to cardiopulmonary effects (heart rate, respiratory rate) , body temperature, blood profile (RBCs, WBCs, Hb and PCV) and Biochemical parameters (Glucose, Total protein, Albumin, ALT, AST and LDH) during anaesthesia induced with propofol 1% (2 mg/kg), xylazine 2% (0.25 mg/kg) and diazepam 0.5% (0.25 mg kg-1). A lot of considerable effort was made to stabilize the environment of the work such as strangers, or noise.

propofol 1% (2 mg/kg), xylazine 2% (0.25 mg/kg) and diazepam 0.5% (0.25 mg kg-1) were chosen for these study because they are inexpensive and were very commonly used in practice.

Propofol induced short duration of anaesthesia in donkeys sedated with xylazine (Matthews *et al*. 2002 and Ab-Almaseeh. 2008). (Naddaf *et al*. 2015) used combination of propofol with acepromazin, (Amin & Mohammed 2012) used combination of propofol and ketamine , (Howaida Abu-Ahmed. 2014) used combination of propofol with detomidine. Propofol alone as total intravenous

anaesthetic produce excellent anaesthesia for carotid artery translocation (Umar *et al.*, 2006) and performed abdominal surgery in horses (Matthews *et al*., 1999).

Although donkeys are very similar to horses, but they are not were the same (Abd-Almaseeh. 2008) , (Howaida M.Abu-Ahmed. 2014) reported that donkeys and horses have different fluid balance. (Moloiy, 1970; Matthews *et al*., 1997) reported that pharmacokinetics of drugs and behavioral difference between donkeys and horses make difficulties to extrapolate the results of horses anaesthesia in donkeys anaesthesia. Donkeys and mules have better tolerance for pain than horses (Taylor & Matthews 2002; Ashley *et al*. 2005).

Ketamine and guaifenesin have metabolized faster in donkeys if compared with horses (Matthews *et al*., 1994 , 1997), donkeys may require higher doses of certain anaesthetic drugs and sometimes lower doses of others (Matthews & van loon 2013).

Propofol in this study at a dose rate of 2mg/kg was chosen according to the based data published literature by Abd-Almaseeh (2008) , Howaida Abu-Ahmed (2014) and Naddaf *et al* (2015).

Our results showed that the dose rate (0.25mg/kg) of xylazine which was used in horses were enough to induce satisfactory sedation in donkeys, results in

experiment A (Anaesthesia) and B (Surgery) confirmed the clinical signs of sedation typically reported in horses by (Yamashit *et al*., 2002).

In this study was observed after injection of propofol (2 mg/ kg) that onset of anaesthesia was rapid 10 to 15 seconds and recovery was clear of any untword effects. All these are due to the highly lipid solubility properties of propofol (Langley and Keel. 1988) and rapid uptake by organs rich with blood vessels (brain, heart, liver and kidney) but is very quickly redistributed to muscle and fat and is subsequently metabolized and rapidly transformed by liver to be excreted from the body compared with thiopental (Abd-Almaseeh. 2008, Mckelvey and Hollingshead, 2003; Muir *et al*., 2007; Bettschrt-Wolfensberger *et al*., 2005.

All animals shown the similar quality of anaethesia,also in aspect of the study reported that the duration of anaethesia was smooth no excitement and excellent muscle relaxation in the two experiments, these in agreement with the previous studies in donkeys which were reported by Nolan, *et al*.,1996, Mamma., *et al* 1998, Bettschart-Wolfensberger .,*et al* (2001).

Heart rate increased significantly and this result agreed with (Naddaf *et al*. 2015, HowaidaAbu-Ahmed. 2014, Jassim Khalaf *et al*., 2012, Abd-Almaseeh. 2008, Matthews *et al*. 2002) in donkeys, also agreed with horses studies with Umar *et al*., (2006). Ender *et al*., (2002). Ohta *et al*., (2004) in hourse, Nalon *et al*. 1996,

Flaherty *et al*. (1997) in ponies and Adetunji *et al*., (2002) in dogs. This 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 and Yamashita, 2009) reported that tachycardia and hypertension were developed in horses anaesthetized with propofol alone. Injection of propofol may be connected with an increasing in sympathetic tone (Mama *et al*., 1996), it reversed some cardiovascular properties such as bradycardia, sinoarterial and atrioventricular block which are produced by xylazine (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 sympathetic efferent activity and a relative vasodilatation to skeletal muscle with vasoconstriction in the splanchnic plexus (Coote. *et al*., 1979, Hilton. *et al*., 1983, Bernard Foex, 1999) .

In this study the respiratory rate decreased from the baseline, these findings were similar to those previous studies of propofol induction in donkeys (Cassia Molinaro *et al* . 2014. Flaherty *et al.* 1997; Matthews *et al*. 1999. Muir *et al* 2007. Abd-Almaseeh 2008; Nolan and Hall. 1985; Mama *et al*., 1996). And disagrees with (Cullen and Reynoldson., 1997; Quandt *et al.*, 1988) who reported that anaetheasia with propofol alone increase the respiratory rate in dogs. Propofol in most species associated with respiratory depression (Tranquili *et al*. 2007; Clarke

et al. 2014). Decrease of respiratory rate may be due to depression of central respiratory centre and ventilator response to arterial O_2tension (Goodman *et al* ; 1987). This occurs in laterally or dorsally recumbent horses because the lower lung fields are compressed by the weight of abdominal viscera pressing through the dome-shaped diaphragm,The problem is usually worst in larger horses lying in dorsal recumbency. Matthews *et al*; (1999) suggested that the reduction in respiratory rate and increase of $PaCO_2$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) revealed that rectal temperature in donkeys was significantly decreased from the base line values 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 reduction in body temperature pointed that the body reacted to prevent itself from death due to hypothermia by rising of temperature.

England and Clark, (1996); Freeman and England, (2000) reported that general anaesthesia decrease body temperature by reducing metabolic rate and muscle activity, in addition the effect of $\alpha_2$adrenoceptor agonist caused depression of hypothalamic thermoregulatory center. Other researchers reported that ketamine and other disassociative anaesthetic caused decrease in body temperature by

releasing monoamines which centrally inhibit endogenous norepinephrine (Afshar, *et al* ., 2005 ;Wyatt, *et al* ., 1989). Hypothermia may occur in animal under general anaesthesia when anaesthesia conducted in cool environment or administration of intravenous fluid (Hall, *et al*., 2001).

Haematological parameters (RBCs, WBCs, Hb, PCV) in this study revealed no significant different in both experiments A(Anaesthesia) and B(Surgery) compared with the baseline values (Controls). But a small decrease in RBCs took place and this may be due to the bleeding during surgery, and very a small increase in WBCs. This indicated that there was no effect of the drugs used on the contraction of the spleen, liver etc. these suggested by (Thakur *et al*. 2011) in their studies in ponies .

Anaethesia and Surgery were considered as stress factors. Stress lead to disturb animals welfare through many bioactive pathways such as clinical, physiological, biochemical, behavioral and others (Kannan *et al*., 2000; Ali *et al*., 2001), stress may has negative consequences on the reproductive potential of animals (Dobson *et al*., 2001; Sevi *et al*., 2001).

There were a significant different ($P<0.01$, $P<0.05$) in glucose concentration at 3 , 24 hours after injection of propofol, glucose concentration in plasma revealed an increase in experiment A (Anaethesia) and experiment B (Surgery) compared with

baseline values (Controls) and started to decrease gradually thereafter. After surgery blood glucose increased, increasing 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).

Hsu & Hummel (1981) reported that hyperglycaemia as result of inhibition of insulin release from pancreatic beta cells due to $\alpha_2$adrenoceptor agonist injection.

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).

Levels of total protein in plasma revealed no significant different but there was a decrease in total protein in experiment A (Anaethesia) and experiment B (Surgery) compared with baseline values (Controls). Desborough, (2000) reported that an increase of cortisol concentration stimulate protein catabolism and release amino acids which could be produced by catabolism of protein in skeletal muscle after

surgery or trauma, some visceral muscle protein is also catabolized to release amino acids, These amino acids may further be catabolized to produce energy or consumed by the liver to form new protein, the liver may converts amino acids into other substrates (glucose, fatty acid or ketone bodies).

These study revealed a significant decrease ($P<0.01$, $P<0.05$) in plasma albumin in both experiment A (Anaethesia) and B (Surgery) compared with baseline value (Control).

Findings of plasma alanine aminotransferase (ALT) revealed no significant different on both surgery and anaesthesia. In experiment A (Anaethesia) ALT increased three hours post anaesthesia injection and started to decrease, (Kwon *et al*., 1999) reported that no significant difference after injection propofol in dogs, and in this aspect our study agrees with them.There was suggestion by (Omid *et al*., 2012) that values of ALT increased in camels premedicated with xylazine after injection of xylazine.in experiment B (Surgery) ALT increased more than experiment A (Anaesthesia) when compared with baseline value (Control) and started to decrease gradually. ALT values were unchanged and still stable in all time of experiment, these findings were reported by (Brazeski *et al*., 1994) more or less our study agrees with these findings except from the non significant fluctuations in ALT seen in our study. (Fani *et al*., 2008) published that ALT values increased post epidural injection of xylazine in dogs. (Jassim Khalaf *et al*.,

2012) reported that ALT test is used to detect liver injury or liver disease which produce high level of ALT indicate hepatitis caused by (Virus, bacterial, fungal, drug and toxin or parasitic liver diseases). Values of ALT were also found to be affected by shock, low blood pressure (Evans, 2009).

No significant difference in plasma aspartate aminotransferase (AST) level. A similar result was reported by (Radi *et al*., 2011) in donkeys .In the present findings AST had been increased dramatically three hours post propofol injection then started to decrease to the normal value gradually in both experiment A (Anaethesia) and B (Surgery). Increase in experiment A (Anaesthesia) more than in experiment B (Surgery) when compared them with baseline values (Controls). Jassim. Khalaf *et al*, (2012) Reported that AST exist in most tissue (kidney, pancreas and erythrocytes) and increase in trauma , muscle injuries, cardiac muscle and hepatocellular injury.

ALT and AST may increase due to escape from the cell through the cellular membrane and these results in alteration in the permeability of the cell membrane (Vicker *et al*., 1984). (Hall *et al*.2001) reported that administration of large doses of diazepam in dogs did not affect on liver and kidney functions and diazepam has very low toxicity. (Ali *et al*, (1989) reported that intramuscular injection of xylazine in camels did not produce significant difference in AST into plasma. However, the mild increase of these enzymes in comparison with other reports by

Duke *et al*,(2006) and Elzubair *et al,* (2015) in which they suggested that lack of any clinical signs of myositis, lameness and myoglobinuria, indicate that there was minimal muscle damage.

There was no significant different in LDH in experiment A (Anaethesia) and experiment B (Surgery) compared with baseline values (Controls). But a small increase in LDH in both experiments, and there was a significant increasing after 24 hours from injection of propofol ($P<0.05$). In experiment B(Surgery) the increase was more than the increase which happened in experiment A(Anaesthesia). The results that suggested these increase were 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). (Oluwatos in and Adaramoye., *et al* (2013) in their study in rats reported that propofol at 2 and 4mg/kg significantly ($P< 0.05$) increased the activities of serum LDH.(Hayden and Tyagi., (2002) In their studies suggested that the increase in serum LDH of diabetic was due to cardiac muscular damage caused by the disease (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 elicited verse effects on the cardiac tissue.

Different reflexes were monitored during the period of experiments (A) and (B) in this study in attempt to measure reliability of these reflexes in monitoring anaesthesia and surgery, but although of these reflexes were used in the literature to monitor anaesthesia; palpebral and pedal reflex (Williams and Wyatt, 2007). Palpebral, corneal and eyelid reflex (Tammisto *et al*., 1981) spinal reflex as a sign of recovery. In this study all reflexes were affected in both experiments (A) and (B).

Anal reflex showed significant different at 5 minutes after injection of propofol ($P<0.01$), in experiment A (anaethesia) and experiment B (Surgery), there was a significant different ($P<0.01$) in tail reflex in experiment A (Anaethesia) and experiment B (Surgery). pedal reflex revealed a significant different ($P<0.01$) at 5, 10 minutes after injection of propofol in experiment A (Anaethesia) and experiment B (Surgery) **.**There was a significant different ($P<0.01$) in tongue reflex at 5, 10 minutes after injection of propofol, Tongue observed clearly protruding out site of mouth in both experiments A (anaethesia) and B (Surgery) slightly all the time of experiments and started to attain normal position inside the mouth. Lacrimation and salivation in experiment A (Anaethesia) and experiment B (Surgery) had seen in some donkeys but not all. There was a significant different ($P<0.01$) at 10 minutes after injection of propofol. Spinal reflex in both experiment A(Anaesthesia) and B(Surgery) showed significant different ($P<0.01$, $P<0.05$).

6.1 conclusion:

1-In this study usage of premedication (Xylazine 2% 0.25 mg/ kg and Diazepam 0.5% 0.25 mg/ kg) prior to propofol 1% injection 2 mg kg was found to be satisfactory to perform surgery because this combination produced smooth anaesthesia with good muscle relaxation and absence of untword effects during surgery.

In this study we used nine donkeys to conduct two experiments, the first is experiment A (Anaesthesia) we injected xylazine and diazepam firstly then after five minutes we injected propofol with the above mentioned doses, and the second experiment B(Surgery) we injected the combination mentioned above with the same doses and after the surgical incision in the left flank through the skin was performed and abdominal muscle incision was also carried out and we closed the wound by suturing of muscle using the usual practice for closing wounds. In both experiments A and B we recorded the following findings and we compared them to the baseline values (Controls).

2-Physiological parameters (Heart rate, Respiratory rate and Rectal temperature), heart rate showed an increase. Respiratory rate revealed a decreasing pattern ($P< 0.01$) with a decrease in body temperature which was also recorded.

3-Haematological parameters (RBCs, WBCs, Hb and PCV) revealed no significant difference but a very small variation was noticed when compared with the normal values.

4-Biochemical parameters (Glucose, Total protein, Albumin, ALT, AST and LDH) and plasma concentrations, glucose showed significant increase ($P<0.01$, $P<0.05$) at 3 and 24 hours after administered combination, total proteins remained with no significant difference however small decrease took place,

albumin showed a significant decrease ($P<0.01$, $P<0.05$) , ALT and AST revealed no significant difference in comparison with the controls but a small increase was observed. LDH revealed a significant increase ($P<0.05$) after 24 hours from injection of anaesthetic regimen.

Some of the selected reflexes showed no significant difference and the others showed a significant difference.

5-In conclusion during this session the experimental animals were kept in quite environment, stranger were kept away from the stable as much as possible to make the environment very standard, the regimen we used propofol 1% (2 mg/kg), xylazine 2% (0.25 mg/kg) and diazepam 0.5% (0.25 mg/kg) during both experiments, performed to assess the performance of the drugs and its combinations as the general anaesthetic when administer to the donkey as example of the equine family. Assessment was made in terms of drug toxicity the time for onset of the anaesthesia, duration of the anaesthesia and recovery it was found that 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.

6.2 Recommendations:

1-It is indeed very important to monitor the level of the blood gases and remove the accumulated CO_2 and ventilate the animal by giving O_2 to avoid the CO_2 drawback, the facilities we have didn't encourage such as precautions, we also wanted to encourage and consider the veterinary practitioner as user of this information and through some light on the type of complications in relation to the species that the practitioner might encounter.

2-It further important to consider the study for a better understanding of veterinary surgery and anaesthesia and as a part of training programe for the students and other people in concern.

3-Finally we anticipated that some readers would be using the study in research project in veterinary medicine or at medical centers. 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.

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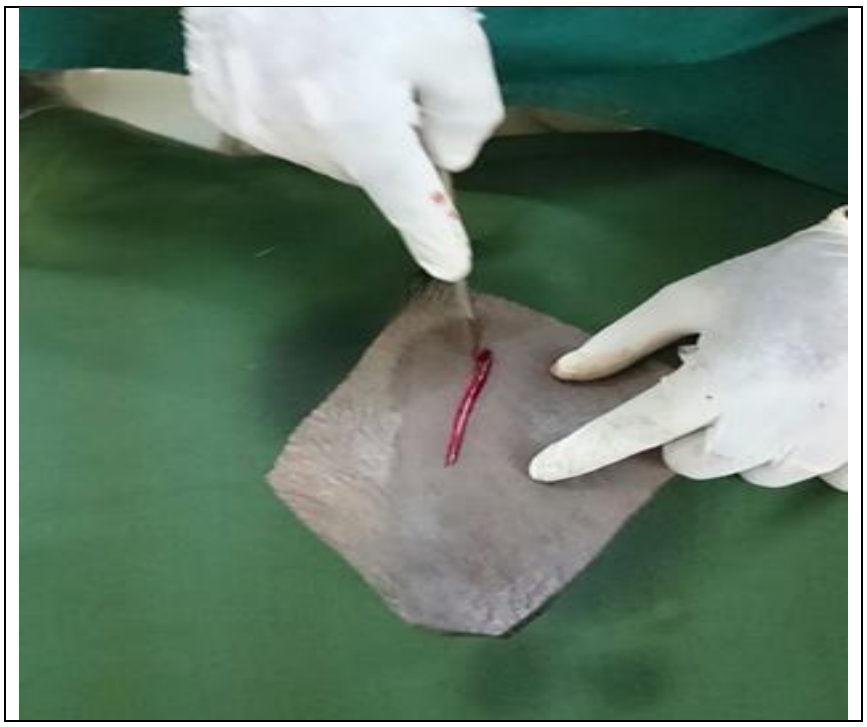
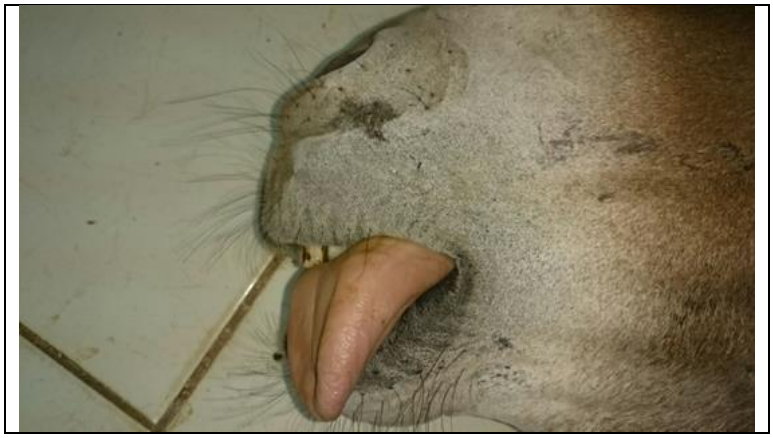


Appendix(1) : Incision through the flank region.

Appendix (2): Penile paralysis after injection of premedication.

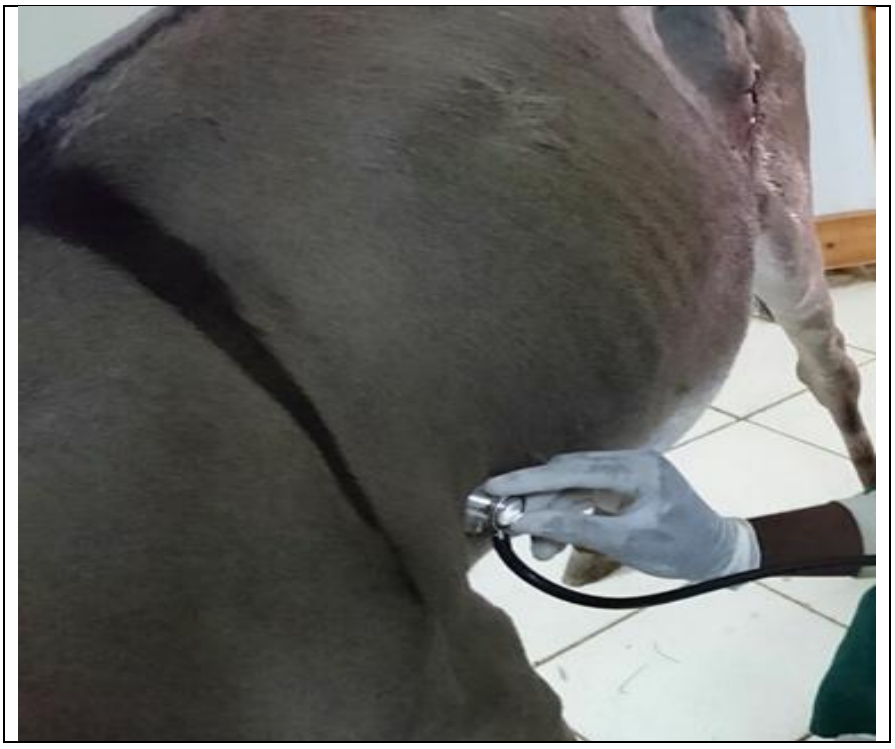
Appendix (3) : The response of introducing the finger in the anal sphincter to detect the anal reflex.



Appendix (4): Pulling the tongue out side the mouth to detect the tongue reflex.

Appendix (5) : The jaw relaxation after injection of anaesthetic regimen.

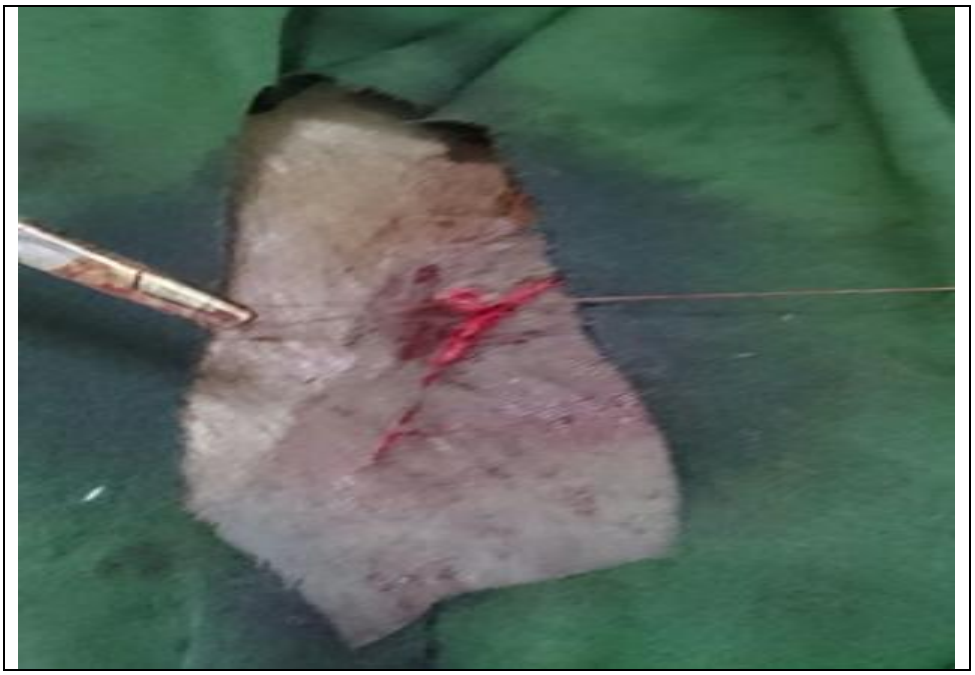
Appendix (6): Showed measuring the rectal temperature by using digital thermometer (quantitative measure).



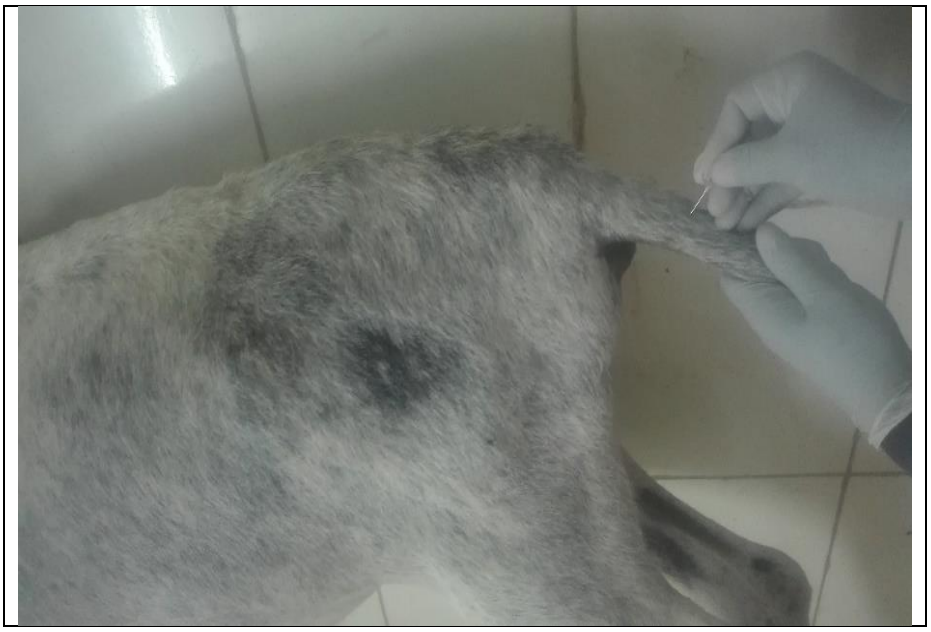
Appendix (7): Showed heart rate was taken from the intercostal space using stethoscope.

Appendix (8) : Pinching over the spinal canal using the needle to detect spinal reflex.

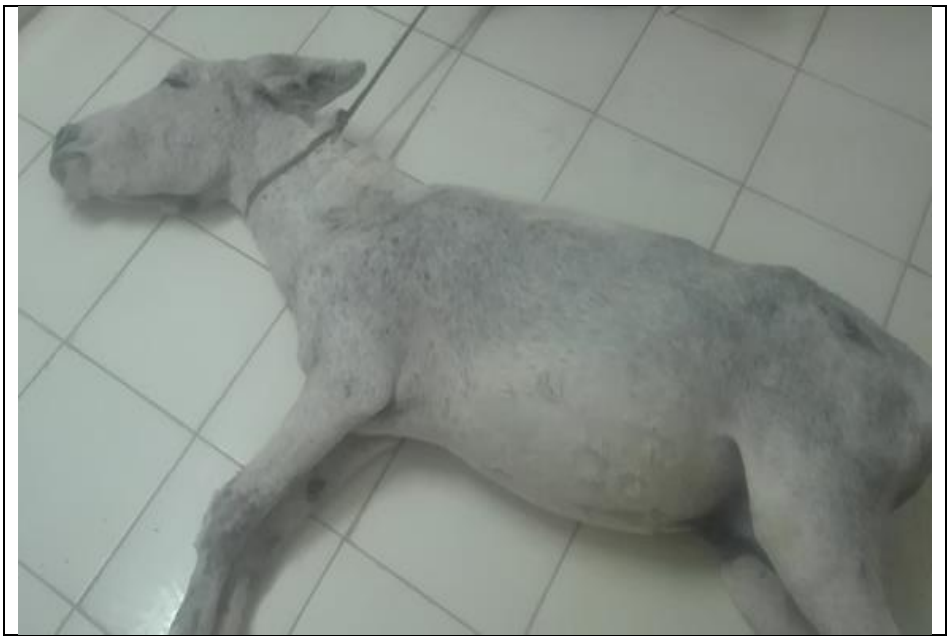
Appendix (9) : Pinching over the coronate using the needle to detect pedal reflex.



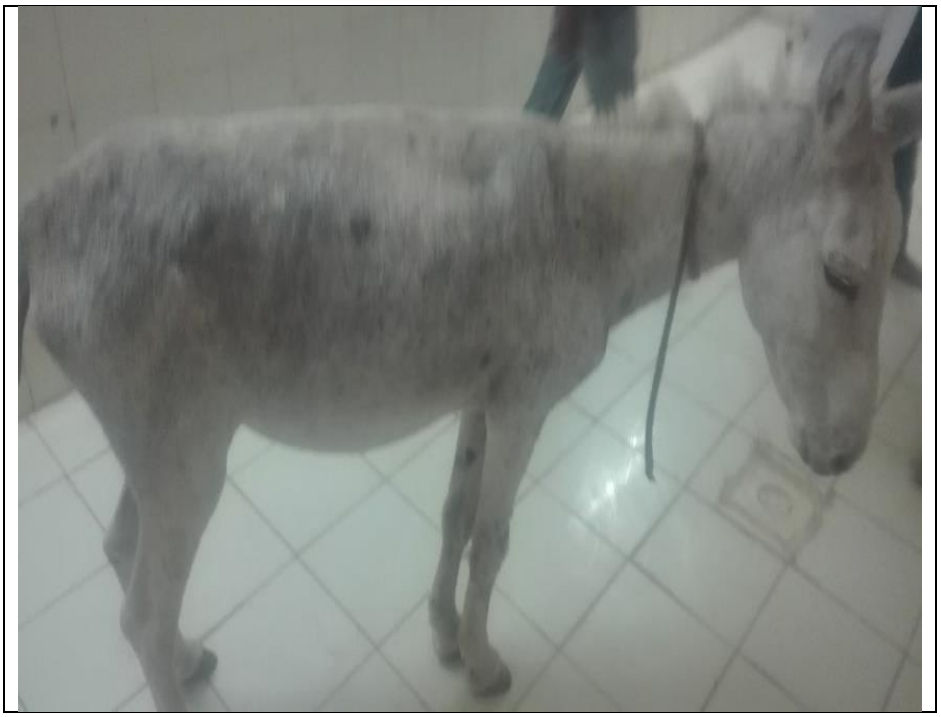
Appendix (10) : The usual practice for closing the wound.



Appendix (11) : Pinching over the tail using the needle to detect tail reflex



Appendix (12) : Lateral recumbency



Appendix (13) : Showed recovery phase with out any untword