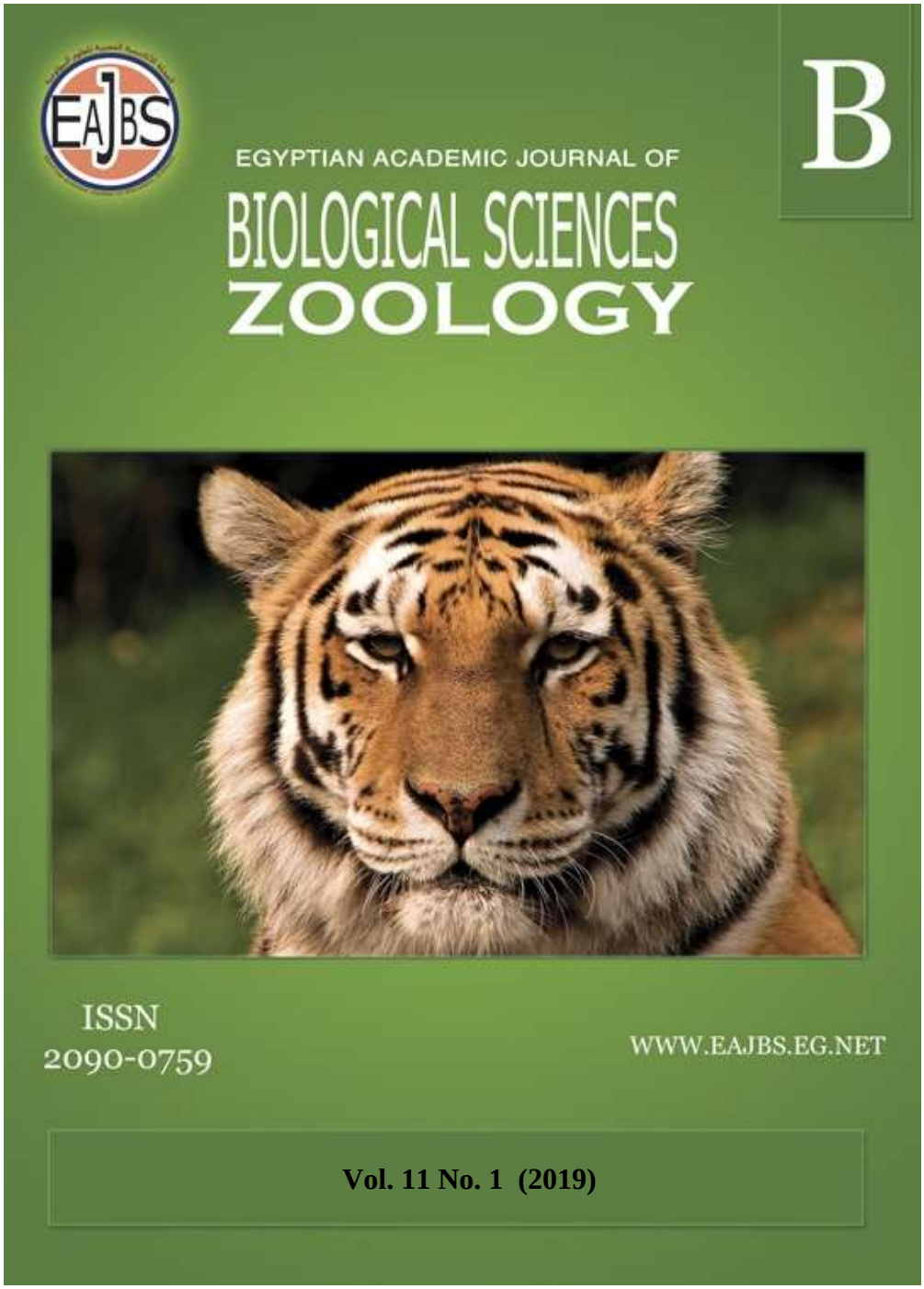

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society of Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

The Journal publishes original research papers and reviews from any zoological discipline or from directly allied fields in ecology, behavioral biology, physiology & biochemistry.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 11(1): 13- 18 (2019)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
www.eajbsz.journals.ekb.eg

Optimization of Xylazine-Ketamine Anesthetic Dose in Mice with Chronic Liver Injury

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ARTICLE INFO
Article History
Received:8 /12/2018
Accepted:13/2/2019

***Keywords*:**
Anesthesia, Intraperitoneal, Rats, Ketamine, Xylazine

ABSTRACT

Objective: The aim of the present study was to find the safest and appropriate intraperitoneal injection dose of Ketamine-Xylazine cocktail for short to the medium-duration surgical procedure (ultrasound-guided liver biopsy) in rats suffering chronic liver injury.

Methods: Four anesthetic doses of Ketamine-Xylazine combination were compared for their safety and efficacy (death rate and surgical tolerance), using observations and reflex tests. Anesthesia evaluated during ultrasound-guided liver biopsy procedure. The reactions of physiological parameters to surgical stimuli were used to determine anesthesia depth and were correlated with reflex test results.

Results: Full dose of Ketamine-Xylazine (87 mg/ kg- 13 mg/ kg) rapidly induced a deep state of anesthesia that lasted for about 70 minutes followed by complete cessation of respiration and death. Three-quarters dose of the cocktail also, rapidly induced a deep state of anesthesia that lasted 45 minutes. Anesthesia was adequate to perform the procedure. Recovery was long. No postoperative complications detected. Half dose of the Ketamine-Xylazine cocktail was short acting. Very good analgesia and muscle relaxation were recorded. Anesthesia lasted for about 30 minutes that was adequate for performing the procedure. Physiological parameters decreased followed by rapid stabilization. Smooth recovery noted. No postoperative complication recorded. Quarter dose produced a state of sedation. Analgesia and muscle relaxation were poor. Animals showed pain during manipulation. The procedure could not be achieved.

Conclusions: The optimal intraperitoneal dose of Ketamine-Xylazine cocktail for balanced anesthesia in lab rats suffering chronic liver injury is (43.5 mg/kg and 6.5 mg/kg) respectively.

INTRODUCTION

Mice and rats are served as the preferable lab animal for biomedical research model due to their small size, ease of handling, short life span and similarity in anatomical, physiological and genetic disciplines to humans. They have been used for in vivo studying of mechanisms of liver fibrosis and its possible treatments. Carbon tetrachloride induced liver fibrosis resembles human liver fibrosis and serves as an attractive model for chronic liver intoxication on the molecular levels (*Nabeshima et al.*, 2006; Normann *et al.*, 2007). Completion of certain research may require keeping

all participated animals alive, despite of frustrated procedures of examination or samples collection. These procedures are performed in anesthetized mice in order to facilitate the procedure and enable the operator to perform safely and accurately. General anesthesia has been described in lab mice. Ether and isoflurane inhalant agents have been reported for inducing general anesthesia in mice (Flecknell, 2009). Several injectable protocols have been described. Xylazine 10mg/kg- Ketamine 100mg/kg, Xylazine 10 mg/kg- Ketamine 100 mg/kg- Acetyl promazine 3 mg/kg and Diazepam 5mg/kg -Ketamine 200 mg/kg mixtures have been designated for survival procedures in mice (Arras *et al.* 2001; Welberg et al. 2006; Fish *et al.*, 2008; Flecknell, 2009; Plumb, 2018). The combination of ketamine and xylazine is still the most widely used ketamine combination in mice, providing good immobilization with some degree of analgesia. Several different dosage combinations of the ketamine/xylazine mixture have been reported for mice in the medical literature, varying from 65/4 to 100/13 mg/kg. The large variability of the recommended dosages depends on differences related to strain, sex, age, and type of experimental procedure (Chari *et al*. 2001; Roth *et al.* 2001; Janseen *et al.* 2004; Schaefer et al. 2005; Buitrago et al. 2008). Injectable anesthetics may be associated with cardiovascular and respiratory depression, prolonged recoveries, the lower margin of safety and hard to control the depth of anesthesia. Both xylazine and ketamine are metabolized by liver enzymes (Giroux *et al.*, 2016). Animals with end-stage liver disease are at significant risk of mortality during and after anesthesia and surgery (Maze and Bass, 2000). In the text below, we studied four doses of Ketamine-Xylazine mixture, and we report our own experience in the selection of a safe and reliable anesthetic protocol.

MATERIALS AND METHODS

Wistar rats used in this study were selected from the reservoir rats dedicated to research designed for studying the effect of mesenchymal stem cells on chronic liver injury. Rats were of both sexes and weighing 180-200gm. They were all treated with carbon tetrachloride (CCL4), eight doses (2 ml/ kg / dose), two weeks apart. Acute liver injury was confirmed and rats were left for chronic liver disease progression. Rats were divided into four equal groups (A, B, C and D) with five for each (η=5). All mice were prepared for short to the medium-duration surgical procedure (ultrasound-guided liver biopsy) under general anesthesia. At the time of surgery, group A received freshly prepared Ketamine-Xylazine (87 mg/ kg- 13 mg/ kg) mixture (dose A), the dose was according to (Huerkamp, 1995 and Plumb, 2018), group B received, K 66 mg/kg- X 10 mg/kg (Dose B), group C received; K 43.5 mg/kg- X 6.5 mg/kg (Dose C) and group D administered; K 22mg/kg- X 4mg/kg (Dose D). The four doses were presented as a full dose (A), three-quarters dose (B), half dose (C) and quarter dose (D). The anesthetic mixture was injected intraperitoneal IP. Criteria of assessment were; induction time, analgesia, anesthesia, duration of action, recovery time, and post-anesthetic complications. Criteria and assessment are tabulated in table 1.

Table 1. clinical criteria to evaluate anesthetic dose

Criteria		Assessment
Induction time	Measured in minutes from the onset of IP injection to effect	**Ultrashort** (in less than a minute) **Short** (in less than 5 minutes) **Long** (in more than 5 minutes)
Analgesia	Measured on the visual analog scale of pain response to pin break	**Mild** (rats respond to any stimuli on the whole body) **Moderate** (rats respond to stimuli on highly sensitive areas) **Profound** (no response to stimuli)
Anesthesia	Depth of anesthesia	**Shallow** (rats show some movements during biopsy procedure) **Deep** (no response during the whole procedure)
Duration of action	Measured in minutes from unconsciousness to recovery	**Ultra-short** (that lasts less than five minutes) **Short** (that lasts less than thirty minutes) **Long** (lasts more than thirty minutes)
Recovery time	Measured in minutes from regaining consciousness to full strength movements	**Short** (less than ten minutes) **Moderate** (from ten to twenty minutes) **Long** (more than twenty minutes)
Postanesthetic complications		

Ultrasound-guided liver biopsy was performed to all rats. Reliability of the anesthetic protocol was also evaluated for its suitability to short/ medium-duration surgical intervention.

RESULTS

Rats of the group (A) that received the full dose of (K 87 mg/ kg- X13 mg/ kg) have shown rapid induction of the deep state of anesthesia. Profound analgesia and muscle relaxation were induced with severe bradycardia and shallow very slow respiration. Rectal temperature decreased and respiratory rate remained shallow and slow. Bradycardia also persists. No reflexes recorded during anesthesia. All rats of group A died few hours after the procedure.

Rats of the group (B) that received three-quarters dose (ketamine 66 mg/kg and xylazine 10 mg/kg) have also shown rapid induction. Analgesia was profound, good muscle relaxation was predicted with a long recovery period. Anesthesia remained 44± 1 minutes. The physiological parameters decreased along with anesthesia and begin to increase at the recovery.

Rats of the group (C) that received a half dose (ketamine 43.5 mg/kg and xylazine 6.5 mg/kg) have shown short induction period. Analgesia was good, no pain detected during the procedure. Stabilization of physiological parameters and prober analgesia in combination with good muscle relaxation indicates balanced anesthesia. The recovery period was short. All Rats of this group regained normal status in a short period. No post-anesthetic complications detected.

Rats of the group (D) that received a quarter dose (ketamine 22 mg/kg and xylazine 4 mg/kg) have a good sedative effect. Analgesia and muscle relaxation were poor. General anesthesia was not induced in any animal of this group. Physiological parameters decreased firstly then raised after stimulation.

Table 2. the clinical effect of four different doses of Ketamine-Xylazine cocktail

	Group A	**Group B**	**Group C**	**Group D**
Induction time	5±1 m	7±1 m	10±2 m	---
Analgesia	Profound	Profound	Profound	Very mild
Depth of anesthesia	Very deep	Deep	Deep	Shallow
Duration of anesthesia	70 m	45 m	35 m	---
Recovery time	---	15±3 m	9±1 m	2±1 m
Post-operative complications	Death after the procedure	No complication	No complication	Procedure could not be achieved

DISCUSSION

To the authors' knowledge, this study represents the first clinical evaluation and optimization of intraperitoneal ketamine-xylazine anesthetic dose in rats suffering chronic liver injury.

The goal of the present study was to provide the reliable, safe and adjustable anesthetic dose of Ketamine-Xylazine combination suitable for short to medium-duration surgical procedures in liver diseased rat models, a dose that is reliable to adapt the needs of a wide range of researchers.

Ketamine alone in high doses was used as a sole anesthetic agent (Gardner et al., 1995), it produced only sedation without analgesia that altered induction and recovery behaviors. The results of this study support the use of anesthetic combination. Results of the present study are supportive to the former studies demonstrating the reliable effect of Ketamine-Xylazine mixture as a general anesthetic for short to medium-duration surgical procedures. Ketamine-Xylazine combination has been used with various doses in almost all species of animals and birds (Burke, 1999; Sindak et al, 2010). Regarding the findings of previous literature, the reviewed doses have a narrow therapeutic range and poor safety margin (Smith, 1993; Flecknell, 1996; Arras et al., 2001). To obtain reliable anesthetic status and increase the safety and surgical tolerance of anesthesia, variable doses of the combination were examined. At a parallel line, Arras et al. (2001) reported that the addition of some sedatives to the combination in combination with decreasing the doses resulted in more reliable and safe anesthesia. Lack of some sedatives may limit the capability for obtaining similar results

A study conducted by Suliburk et al (2005) showed that ketamine has a liver protective effect over isoflurane. This result plus other technical facts support the use of ketamine rather than inhalation anesthesia. Despite these supportive results, both Ketamine and Xylazine are bio-transformed in liver (Lavoie et al., 2012; Dinis-Oliveira, 2017) with high hepatic clearance ratio (Li et al., 2015), the initial dose is to be reduced (Delcò et al., 2005). Drug information service released a bulletin on (2013) recommended decreasing the dose of high hepatic clearance drugs such as ketamine and xylazine by 50%. Animals with liver dysfunction are considered at greater risk for complications associated with general anesthesia as reported by Weil (2011). Several studies were conducted to optimize Ketamine-Xylazine anesthetic dose for preclinical imaging (Gargiulo et al., 2012) and medium-duration surgical procedures (Arras et al., 2001), the establishment of anesthesia optimization study in rats suffering chronic liver disease will help researchers in their biomedical daily tasks.

The most appropriate and highest safety margin was associated with the three-quarters dose for medium-duration and a half dose for short-duration procedures, doses that are not widely known. Wide range dosage reported indicates that strain, administration rout, and health status of the lab rat are principle rules in dose adaptation. The three quarters and half dose regimens were adapted for induction of surgical plane of anesthesia. Those protocols provided surgical tolerance for 45 minutes that is adequate for medium-duration surgical procedures and 35 minutes suitable for short-duration surgical procedures as transcutaneous liver biopsy performed here.

Conclusion

The appropriate intraperitoneal dose of Ketamine-Xylazine combination to obtain balanced anesthesia in lab rats suffering chronic liver injury is (43.5 mg/kg and 6.5 mg/kg).

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