EFFECT OF DIFFERENT METHODS OF ANESTHESIA ON PHYSIO-BIOCHEMICAL PARAMETERS IN LABORATORY MALE RATS

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ABSTRACT

The present study was designed to compare different anesthetic methods commonly used by Iraqi researchers and students. Twenty four adult male rats were divided into four groups (six for each) as following: control group was represented by collecting blood without anesthesia. (Ket.Xylz.) group: was represented by anaesthetizing of rats by injection ketamine / xylazine cocktail (87.5mg/Kg Ketamine, 12.5 mg/kg Xylazine) Intraperitoneally (IP)., while the chloroform group was represented by anaesthetizing of rats by 1% inhaled chloroform (0.05ml/liter of container volume). The di ethyl ether group: was represented by anaesthetizing of rats by 1.9% inhaled diethyl ether (0.08 ml / liter of container volume). Blood samples were collected after anaesthetizing of animals using cardiac puncture to evaluate some hematological and biochemical parameters (RBC, Hb, PCV, WBC، ALT، AST enzymes, creatinine and urea). The results revealed there were significant variable differences in Hb and RBC values, while the animals appeared to increase significantly in WBC count for all the anaesthetized group compared with control group. The deleterious effect of chloroform as inhaled anesthesia appeared on ALT, AST, creatinine, and urea. These results revealed that use of di ethyl ether as inhaled anesthesia for laboratory rats was the easiest anesthetic methods for laboratory rats.

INTRODUCTION

Rats are one of the most widely used organisms in medical researches and student projects for their advantages in easy breeding and treatment as well as organs isolation, blood inspiration and special disease or syndrome induction[1] . Although of all the above advantages, the student still faced the challenges when using rats in their experiments, especially if there is interval collection of blood which may be important for anesthetizing the animal. Therefore, many universities and association protocols issued to deal with laboratory animals through anesthesia [2, 3], all of these protocols recommended to use of Isoflurane as inhaled anesthesia and Ketamine□Xylazine for intraperitoneal (IP) injection anesthesia. [4]

Our observation for the student in University of Basrah referred to used chloroform or diethyl ether as inhaled anesthesia and some researchers also used Ketamine□Xylazine as injectable anesthesia for rats although no protocols are indicated for any of these methods in rat anesthesia [3, 4]. [5] observed that 10 ppm chloroform inhalation in mice leads to liver toxicity. The major effect of human exposure to chloroform from acute inhalation is depression of the central nervous system. Chloroform exposure at very high levels (40,000 ppm) can cause death, with concentrations in the range of 1,500 to 30,000 ppm producing anesthesia, and lower concentrations (<1,500 ppm) resulting in dizziness, headache, tiredness, and other effects [6,7]. Effects noted in humans exposed to chloroform via anesthesia include changes in respiratory rate, cardiac effects, gastrointestinal effects such as nausea and vomiting, and effects on hepatic and kidney symptoms. Currently chloroform is not used as a surgical anesthesia [7].

While Animal Care and Use Committee [6] referred to safety used of di ethyl ether as inhalant anesthetic for rodent although it flammable, it is relatively safer agents to use by open drop because they volatilize poorly, and it takes longer to anesthetize animal, greatly increasing the safety margin [8].

Ketamine in most animals produces immobility, and can be administered by intramuscular, intraperitoneal and intravenous routes. In most species it causes only moderate respiratory depression, and increases blood pressure [9]. Ketamine can be given as a single injection with medetomide, xylazine, or acepromazine and the combination administered as a single injection. Long-term administration of ketamine can result in urinary bladder Irritation [10].The aim of this

study is to investigate the effect of common anesthetic methods of rats on some physiological and biochemical parameters to recognize the recommended methods of anesthesia.

MATERIAL AND METHODS

This experiment was conducted in the Faculty of Veterinary Medicine College/ University of Basrah. Twenty four adult male rats (6 for each group) weighing 180-210gm were used and housed under control temperature (22±2°C) and humidity (50-60%) with ventilation was installed and its lighting was kept constant regular 12 h dark/light cycle (light on at 7 a.m. and off at7 p.m.) at laboratory animal house of Veterinary Medicine College/ University of Basrah.

All experimental animals were given free access to standard dietary ration and water. They were divided into Four groups (n=6 / group) depend on method of anesthesia for blood collection as following:

1-Control group: Blood collecting without anesthesia.

2- Ket.Xylz. Group: rats were anaesthetized by intraperitoneal injection of ketamine / xylazine cocktail (87.5 mg/kg Ketamine, 12.5 mg/kg Xylazine) 0.1ml/20gm B.W.IP (4).

3- Chloroform Group: rats were anaesthetized by inhaled chloroform1% (0.05ml/liter of container volume) as anesthetic dose for blood collection [6].

4-Ether group: rats were anaesthetized by inhaled diethyl Ether 1.9% (0.08 ml / Liter of container volume) as anesthesia for blood collection [6].

Blood samples were collected via cardiac puncture after anaesthetized animal and drain blood by using 5ml disposable syringe according to Hoff and Raltg method[9]. Then a part of blood put in special tube (with EDTA) for hematological parameter, other part of blood put in sterile labeled tubes (Clot Activator with Gel) and centrifuged (3000 rpm/15 min.) and put in -4°C for serum preparation and biochemical measurement(ALT,AST, Urea and Creatinine).

All anaesthetized animals were followed to observe the anesthetic stages, time need to anesthesia, weak up period, and mortality rate.

RESULTS AND DISCUSSION

Observation during the experiment for animals that anesthetized by different methods (table 1) showed variant challenges especially in control group to blood drench because difficult handling of animal and uses of injection directly to the heart, the same challenges appeared in ketamine and xylazine method because it need injection for two time and researchers must need assistance to complete blood collection. Whereas, inhaled methods were appeared to be easier than injection method especially for ether that showed no effect on liver and kidney indicators. These results agreed with [3].

Hematological parameters represented by Hb, RBC count , PCV and WBC count in figure (2,3,4,5) showed elevation significantly ($P<0.05$) in WBC count for all animal groups that anaesthetized before blood collection when compared with control without anesthesia applied on animals through blood collection. While Hb and RBC values appeared variable among studied groups, significant reductions in their values in chloroform group animals compared with control group animals were recorded. The cause of total WBC elevation attributed to suppression of cellular immunity is a major response of the host to surgical stress, which may be deleterious to host defense mechanism along with the overproduction of inflammatory mediators [12].These results also agreed with [13].

The effect of different methods of anesthesia of rat blood collection on liver and kidney function indicated by measuring the activity of ALT and AST enzymes activity and creatinine and urea values , respectively figure 5,6,7,8 . The results showed significant increase ($P<0.05$) ALT and AST enzymes activity in chloroform anesthetized group when compared with other studied groups and also results revealed significant increase ($P<0.05$) in creatinine and urea values for chloroform anesthetized group when compared with other methods of anesthesia groups which appeared no significant effect when compared with animal of control group.

The results above agreed with [14] that recorded significant increase of ALT and AST enzymes activity after 30 and 60 minute of exposure to chloroform. While, [5] investigated the exposure to inhaled chloroform led to liver toxicity in female rats. Whereas, Animal Care and Use Committee [6] approved uses of inhaled di ethyle ether as anesthesia for laboratory animals and it was safer agents to use by open drop because they volatilize poorly, and it takes a longer

time for animals to become anesthetized, greatly increasing the margin of safety. , In contrast, IACUC (4): Megan [11] recommended using of ketamine and xylazine as injectable anesthesia for rats.

Table 1: Observations of the anesthetic lab. rat

Group	**Time of Anesthesia** (Min.)	**Time of Wake-up** (Min.)	**Notes during experiment**
Ket \xylz.	1.5-2.5	30-45	More difficult in preparation for anesthesia due to handling and injection
Chloroform	1-1.5	3 -5	Stressed animal led to 3 animal died
Diethyl ether	1.5-2	3.5-6	Easy for blood collection and treatment
Control	----	----	Very difficult dealing through blood collection

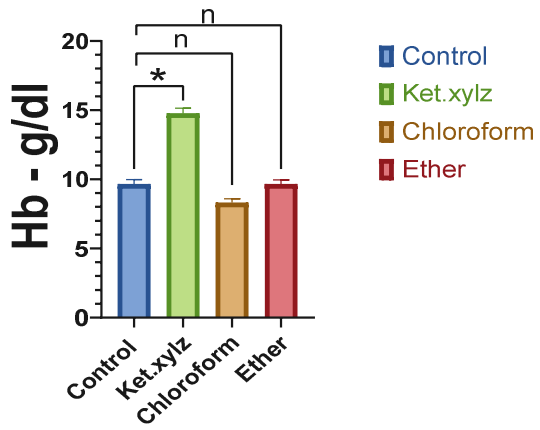

Fig 1: Hemoglobin level associated with different methods of anesthesia. Significance (p-value: *<0.05, **<0.01, ***<0.005, ****<0.001) was determined by using one-way ANOVA.

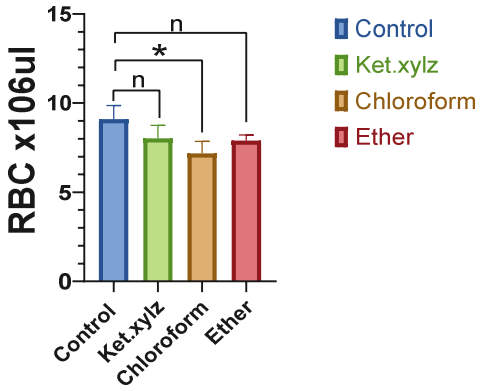

Fig 2: Red blood cells count associated with different methods of anesthesia. Significance (p-value: *<0.05, **<0.01, ***<0.005, ****<0.001) was determined by using one-way ANOVA.

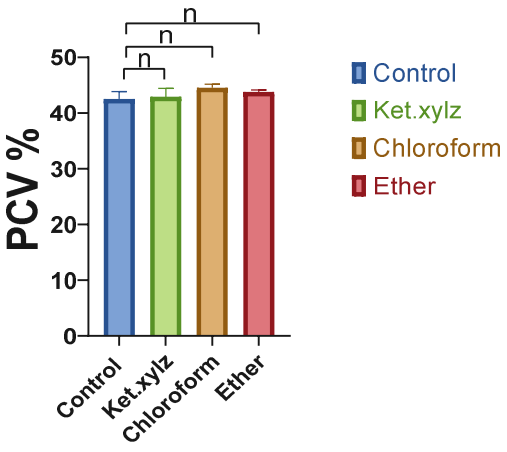

Fig 3: PCV level associated with different methods of anesthesia. Significance (p-value: *<0.05, **<0.01, ***<0.005, ****<0.001) was determined by using one-way ANOVA.

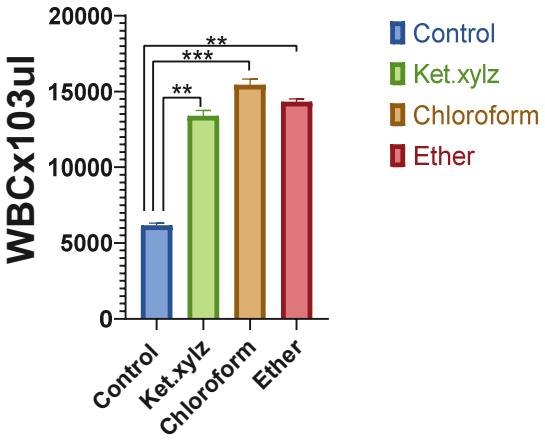

Fig 4: White blood cells count associated with different methods of anesthesia. Significance (p-value: *<0.05, **<0.01, ***< 0.005, ****< 0.001) was determined by using one-way ANOVA.

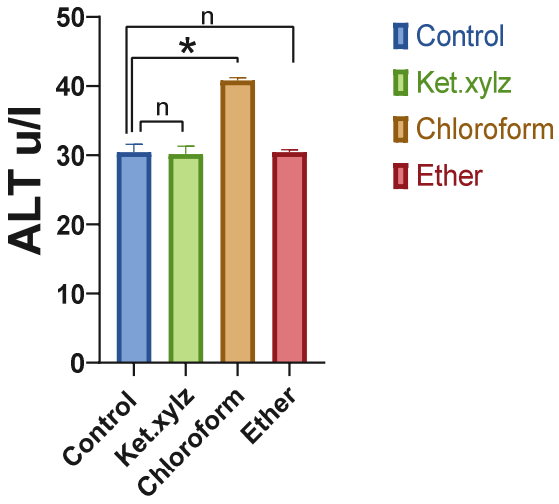

Fig 6: Alanine transaminase level associated with different methods of anesthesia. Significance (p-value: *<0.05, **<0.01, ***< 0.005, ****<0.001) was determined by using one-way ANOVA.

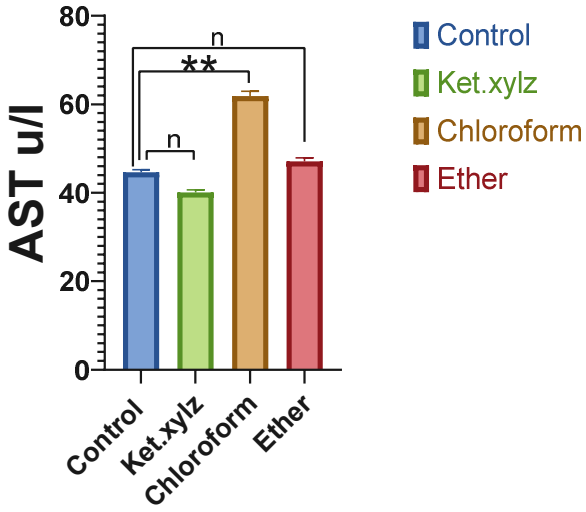

Fig 7: Aspartate transaminase level associated with different methods of anesthesia. Significance (p-value: *<0.05, **<0.01, ***<0.005, ****<0.001) was determined by using one-way ANOVA.

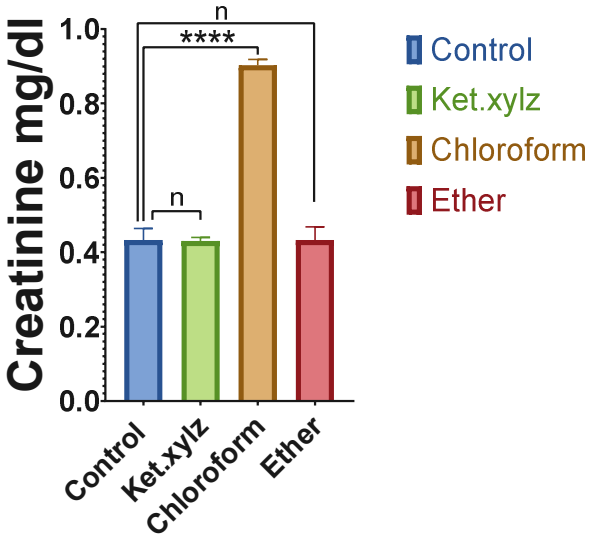

Fig 8: Creatinine level associated with different methods of anesthesia. Significance (p-value: *<0.05, **<0.01, ***<0.005, ****<0.001) was determined by using one-way ANOVA.

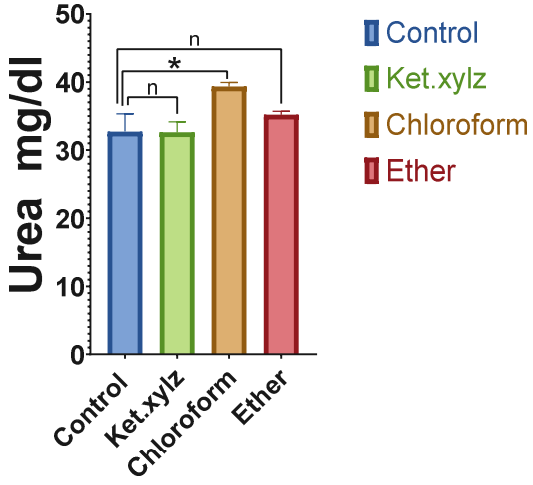

Fig 9: Urea level associated with different methods of anesthesia. Significance (p-value: *<0.05, **<0.01, ***<0.005, ****<0.001) was determined by using one-way ANOVA.

تاثير الطرق المختلفة للتخدير على المعايير الفسيولوجية والكيموحيوية لذكور الجرذان المختبرية

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الخلاصة

صممت الدراسة الحالية للمقارنة بين طرق التخدير الشائعة المختلفة والمستخدمة من قبل الباحثين وطلبة الدراسات في العراق. تم استخدام أربعة وعشرون من ذكور الجرذان البالغة التي قسمت الى أربع مجموعات (ستة لكل منها) على النحو التالي: مجموعة السيطرة التي تم جمع عينات الدم منها دون استخدام التخدير مجموعة Ket.Xylz. تم تخدير الجرذان بواسطة مزيج الكيتامين / زيلازين١٠% (٨٧.٥ملغ /كغم كيتامين، ١٢.٥ ملغ/كغم زيلازين) عن طريق الحقن في الخلب. مجموعة الكلوروفورم: تم تخدير الجرذان باستخدام الكلوروفورم المستنشق ١٪ (٠.٠٥ مل / لتر من حجم الحاوية) ومجموعة الايثر: تم

تخدير الفئران باستنشاق داي إيثيل الأيثر ١.٩٪ (٠.٠٨ مل / لتر من حجم الحاوية). جمعت عينات الدم عن طريق القلب مباشرة بعد تخدير الحيوانات لتقييم عدد كريات الدم الحمرRBC، الهيموغلوبين Hb، حجم الخلايا المرصوصة PCV وعدد كريات الدم البيض WBC بالاضافة الى قياس فعالية الإنزيمات الناقلة للامين ALT و AST وقياس الكرياتينين Creatinine واليوريا Urea بين حيوانات التجربة .

اظهرت النتائج وجود فروق معنوية متباينة في قيم Hb وRBC،في حين اظهرت الحيوانات زيادة كبيرة في عدد خلايا الدم البيضاء (WBC)لجميع مجموعات المعاملة بطرق التخدير المختلفة مقارنة مع مجموعة السيطرة.فيما اظهرت مجموعة الكلوروفورم المستنشق زيادة معنوية على فعالية انزيمات (AST،ALT) وقيم الكرياتينين واليوريا عند مقارنتها مع مجموعة السيطرة ومجاميع الدراسة الاخرى .اظهرت نتائج الدراسة إلى ان استخدام التخدير داي ايثيل ايثرالمستنشق كان أسهل طريقة للتخدير في الجرذان المختبرية

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