EXPLORE THE SYNERGISM OF SEDATIVE DOSE MEDETOMIDINE WITH TRAMADOL TO INDUCE ANALGESIC EFFECTS IN RABBITS

Mahmood. B. Mahmood

Department of Pharmacology, College of Veterinary Medicine, University of Duhok , Duhok, Iraq

(Received 27 December 2015 ; Accepted 21 February 2016)
Key word: *Medetomidine, Tramadol, Analgesia* ,Rabbits

ABSTRACT

The study was designed to evaluate the analgesic effects of Medetomidine and Tramadol in rabbits, to detect the best dose (as onset and duration) for antinociceptive in this model, also evaluate the antinociceptive effect as sedative doses in these drugs as a mixture by using electrical stimulator. Administration of Medetomidine alone at 200 µg\kg B.W. (I.M) and Tramadol alone at 2 mg \ kg B.W. (I.P) were the best doses for relief pain induced by electrical stimuli. There was increased in the voltage change for pain (15, 30, 45, 60, 75, 90 minutes) in comparison with control and other doses for each drug. Administration of Medetomidine at 50 µg\ kg B.W. (I.M) with Tramadol at 0.5 mg\ kg B.W. I.P) significantly referred to synergism of the antinociceptive effect which induced analgesia in 100 % of the rabbits in comparison with other groups for each drug alone (at the same analgesic doses) without any overt side effects and without differences in glucose, glutathione, ALT and AST level in animals. The data of this study demonstrated the mixture of Medetomidine and Tramadol at low doses (subanalgesic doses) had a typical synergistic effect (super-additive) for inducing good and safe analgesia as well as its skeletal muscle relaxation in rabbits.

INTRODUCTION

Medetomedine(Domitor)® is a potent and highly specific alpha 2 –adrenoceptor agonist that induce sedation, analgesia and muscle relaxation, and anxiolytic, as well as decrease in the anesthetic requirements of injectable and inhalant agents in wild animals, dogs and cats (1; 2; and 3).Its widely used to treat moderate and severe pain (4; 5) the drug also had clinical uses in small ruminants such as sheep and goat (6; 7; 8; and 9) horses (10) camel (11) buffalo (12) and birds (13 ; 14) and chicks (15) The mechanism of action of Medetomidine to induce

antinociceptive on presynaptic alpha 2 – adrenoceptors in peripheral and central nervous system (CNS) led to decrease catecholamine release and turnover and subsequently inducing depression of the brain (16) .

Tramadol is mostly commonly used in human and animals (17; 18; and 19). The antinociceptive effect of Tramadol were resulted from dual action, first, it binding for Mu-opioid receptor in CNS and second, it inhibited the presynaptic reuptake of norepinephrine and serotonin (20). It's used for reduction of the mostly signs of aneuralgia, depression, anxiety (21) and treat post- surgical chronic pain in dogs, ruminant and laboratory animals as rat and mice (22; 23 and 24). The inhibitory effects were contributed as analgesic effect of Tramadol by inhibiting pain reflex in the CNS (25 and 26) .

Because widely used of these drugs in veterinary medicine as sedative and analgesics agents alone or as combination, there for,

The aim of study to evaluate the best analgesic dose , the maximal analgesic effect response by synergistic modulation of sub analgesic dose of Medetomidine with Tramadol ,the analgesic effect estimate through electrical stimulator of pain indiction in rabbits and the efficacy and efficiency improved checking adverse effects of analgesia of synergism (27). Electrical stimulator test is a pain assessment method used in animals to measurement the pain by using a voltage frequency set points .

MATERIALS AND METHODS

Animals:

Sixty four of both sexes rabbits weighing 1-2 kg were used. The animals were obtained from animal house of Veterinary Medicine, University of Dohok, The animals were housed at 20 ± 1°C ,12 h light / dark cycle and fed standard diet (put origin or type) and water and Libitum

Experiments:

First Experiment: Detection the best analgesic dose of Medetomidine and Tramadol in Rabbits.

A- Detection the best analgesic dose of Medetomidine in rabbits:

The animals were randomly divided into 4 groups; each group consisted of 4 animals**.** First group of animals (control group) were injected with physiological saline (I.M), whereas; the other groups (2^{nd}, 3^{rd} and 4^{th}) were injected with Medetomidine (1 µg\ mL, Domitor , Orion corporation , Turku, Finland) at 50 , 100 , 200 µg\kg (B.W) I.M respectively.

B- Detection the best analgesic dose of Tramadol in rabbits:

The animals were randomly divided into 4 groups; each group consisted of 4 animals**.** First group of animals (control group) were injected with physiological saline (I.P), whereas; the other groups (2^{nd}, 3^{rd} and 4^{th}) were injected Tramadol (50 mg\ml, Mepha, Ltd Aesh-Basel, Swits-land) at 0.5, 1 and 2 mg \ kg of body weight I.P, respectively.

The voltage that induced pain via electrical stimulation by electrical stimulator device (100- Bio science , England) which is specific for muscle stimulator after modified to induce pain in rabbit (15) was measured at time zero (pre-treatment),15 , 30 , 45 , 60 , 75 , 90 minutes of injection with different analgesic drugs (Medetomidine and Tramadol) and record the changes in voltage (pain threshold) with the same drugs.

Second Experiment: The effect of sedative dose of Medetomidine or Tramadol to induce analgesia in Rabbits.

The rabbits were divided to the 4 groups each group has 4 animals**:**

1- First group (control) injected with Physiological saline I.M.

2- Second group injected with Medetomidine at 50 µg \kg B.W I.M.

3- Third group injected with Tramadol at 0.5 mg \ kg B.W I.P.

4- Forth group injected with Medetomidine at 50 µg \ kg I.M with directly injected with Tramadol at 0.5 mg \ kg I.P .

After injection the animals with drugs according to above groups, detect the onset of sedation (minute), the duration of sedation (minute) and the duration of analgesia after 30 minutes of injection (calculate the percentage of analgesia in each group).

In preliminary experiments those doses were observed to induce mild sedation in rabbits but not induce any relief of pain (analgesia) after 30 minutes of injection of any drug alone (the voltage that cause pain to each animal was determined before injection of any drug and then repeated the pain test by using electrical stimulator device after 30 minutes of injection at the same voltage of each animal as previously clarify.

After 30 minutes of injection of each animal groups were sacrificed to obtain blood samples by anticoagulant tubes to be used in measuring the glucose level, AST and ALT by using kits after that opening the abdominal wall of the animals ,the liver exercised and used in measuring of glutathione level by using alternative Elman's method (28) to evaluate the glutathione level in the liver tissue.

Statistical analysis:

The parametric data were subjected to analysis of variance, followed by the least significant difference test, and the non-parametric data (percentage data) were analysis by Fisher Exact probability (29 ; 30; 31 and 32). The level of significance was $P < 0.05$.

RESULTS

First Experiment: Detection the best analgesic dose of Medetomidine and Tramadol in Rabbits.

A- Detection the best analgesic dose of Medetomidine (I.M) in rabbits:

Medetomidine at 200 µg \kg B.W (I.M) was caused significantly increased in pain voltage in animals at time (15, 30, 45, 60, 75, 90) minutes compared with time zero within same group and with control group and groups at 50 ,100 µg \kg B.W (I.M), respectively at the same periods (table 1), whereas; the dose at 50 µg \kg B.W.(I.M) was failed to induce analgesia in rabbits in all period of the test(Table 1).

Table 1 investigate the best dose of Medetomidine at 200 µg/ kg B.W (I.M) to induce analgesia at time (15, 30, 45, 60, 75, 90) minutes of injection compared with other groups.

Table 1: Detection the best onset of analgesia and the best analgesic dose of intra muscular injection of Medetomidine in Rabbits.

Groups	Voltage in time zero (pre treatment)	Voltage after 15 min. of inj.	Voltage after 30 min. of inj.	Voltage after 45 min. of inj.	Voltage after 60 min. of inj.	Voltage after 75 min. of inj.	Voltage after 90 min. of inj.
Control (Physiological salt solution)	2.4 ± 0.2	2.4 ± 0.2	2.4 ± 0.2	2.4 ± 0.2	2.4 ± 0.2	2.4 ± 0.2	2.4 ± 0.2
Medetomidine 50µg/kg I.M	2.5 ± 0.24	2.6 ± 0.24	2.6 ± 0.24	2.5 ±0.24	2.6 ± 0.24	2.6 ± 0.24	2.2 ± 0.29
Medetomidine 100µg/kg I.M	2.5 ± 0.4	2.4 ± 0.5	3.3 ± 0.7	4.7 ±0.6 $^{*\,a\,c\,d\,e}$	4.7 ± 0.5 $^{*\,a\,c\,d\,e}$	4.6 ± 0.5 $^{*\,a\,c\,d\,e}$	2.4 ± 0.6 $^{f\,g\,h}$
Medetomidine 200µg/kg I.M	2.1 ± 0.5	8.4 ± 0.8 $^{*\,a\,b\,c}$	8.1 ± 0.8 $^{*\,a\,b\,c}$	8.8 ± 0.5 $^{*\,a\,b\,c}$	8.2 ± 0.7 $^{*\,a\,b\,c}$	8.2 ± 0.8 $^{*\,a\,b\,c}$	8.5 ± 0.7 $^{*\,a\,b\,c}$

Value are presented as the mean ± SE of 4 rabbits/ group

*: Significantly different than the control group at same times, $P < 0.05$.
^{a}: Significantly different than the 50 µ g / kg of Medetomidine , $P < 0.05$.
^{b}: Significantly different than the 100 µg / kg of Medetomidine , $P < 0.05$.
^{c} : Significantly different than the time zero at same dose , $P < 0.05$.
^{d}: Significantly different than the time 15 at same dose , $P < 0.05$.
^{e} : Significantly different than the time 30 at same dose , $P < 0.05$.
^{f} : Significantly different than the time 45 at same dose , $P < 0.05$.
^{g}: Significantly different than the time 60 at same dose , $P < 0.05$.
^{h}: Significantly different than the time 75 at same dose , $P < 0.05$.

B- Detection the best analgesic dose of Tramadol (I.P) in rabbits:

Tramadol at 2 mg \kg B.W (I.P) was rabidly induce analgesia than other doses (0.5 , 1 mg \kg) B.W. (I.P) until 90 minutes after injection (last time of experiment) (Table 2). It (2 mg/ kg) was caused significantly increased in voltage that caused pain in animals at (15, 30, 45, 60, 75, 90) minutes of injection compared with control group at the same periods and with the time zero (before injection) in same dose (Table2), also significantly increased compared with Tramadol at 0.5 mg\kg in (15, 30, 45, 75, 90) minutes of injection at the same periods also in 15, 90 minutes of injection compared with Tramadol at 1 mg \ kg at same periods (Table 2).

Table 2 investigated the best dose of Tramadol at 200 mg \kg B.W (I.P) to induce analgesia (increasing pain threshold) at (15, 30, 45, 60, 75, 90) minutes of injection compared with the other groups.

Table 2: Detection the best onset of analgesia and the best of analgesic dose of intra peritoneal injection of Tramadol in Rabbits.

Groups	Voltage in time zero (pre treatment)	Voltage after 15 min. of inj.	Voltage after 30 min. of inj.	Voltage after 45 min. of inj.	Voltage after 60 min. of inj.	Voltage after 75 min. of inj.	Voltage after 90 min. of inj.
Control (Physiological salt solution)	2.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.2
Tramadol 0.5 mg \kg I.P	2.7 ± 0.2	2.7 ± 0.2	2.6 ± 0.2	3.5 ± 0.4 $^{c\,d\,e}$	3.9 ± 0.3 $^{*\,c\,d\,e}$	3.8 ± 0.3 $^{*\,c\,d\,e}$	2.8 ± 0.5 *
Tramadol 1 mg \kg I.P	2.3 ± 0.3	2.7 ± 0.3	3.7 ± 0.3 $^{a\,c\,d}$	4.2 ± 0.4 $^{*\,a\,c\,d}$	4.1 ± 0.2 $^{*\,c\,d}$	4.4 ± 0.3 $^{*\,a\,c\,d\,e}$	2.4 ± 0.3 $^{e\,f\,g\,h}$
Tramadol 2 mg \kg I.P	2.3 ± 0.4	4.1 ± 0.6 $^{*\,a\,b\,c}$	4.4± 0.3 $^{*\,a\,c}$	5.0 ± 0.5 $^{*\,a\,c}$	4.5 ± 0.6 $^{*\,c}$	4.5 ± 0.2 $^{*\,a\,c}$	4.1 ± 0.3 $^{*\,a\,b\,c}$

Value are presented as the mean ± SE of 4 rabbits/ group

*: Significantly different than the control group at same times, $P < 0.05$.
^{a}: Significantly different than the 0.5 mg / kg of Tramadol , $P < 0.05$.
^{b}: Significantly different than the 1 mg / kg of Tramadol , $P < 0.05$.
^{c} : Significantly different than the time zero at same dose , $P < 0.05$.
^{d}: Significantly different than the time 15 at same dose , $P < 0.05$.
^{e} : Significantly different than the time 30 at same dose , $P < 0.05$.
^{f} : Significantly different than the time 45 at same dose , $P < 0.05$.
^{g}: Significantly different than the time 60 at same dose , $P < 0.05$.
^{h}: Significantly different than the time 75 at same dose , $P < 0.05$.

Second Experiment:

The effects of sedative dose of Medetomidine and Tramadol to induce analgesia and in glutathione concentration in liver, glucose level and AST, ALT activities in Rabbits.

Administration of Medetomidine at 50 µg \kg B.W (IM) and Tramadol at 0.5 mg \ kg B.W (I.P) both of them induce sedation in rabbits without causing analgesia after 30 minutes of injection. There were significant no difference ($p \geq (0.05)$difference between groups in onset and duration of sedation (Table 3). The clinical signs of sedation were represented as dropping of head, hair errection, ataxia, urination, defecation, salivation lacrimation, depress and

decumbency. whereas; Medetomidine with Tramadol together at 50 µg / kg B.w (I.M) with 0.5 mg \ kg B.w (I.P) were leds to induce analgesia at (100 %), respectively, in rabbits compared with control group and with groups of Medetomidine alone and Tramadol alone without significantly affecting on onset and duration of sedation (Table 3).

Table 3: Effect of sedative dose of Medetomidine and Tramadol to induce analgesia

Value are presented as the mean ± SE of 4 rabbits/ group

* Significantly different than the other groups, $P < 0.05$.

All drugs used in this study as alone or combined (mixture) at the above doses have not significantly effects in glutathione level in the liver and in glucose level, AST, ALT activities in plasma of rabbits after 30 minutes of injection (Table 4).

Treatments	Sedative onset(min)	Sedative duration (min)	% of analgesia after 30 (min) of injection
Control (Physiological saline)	**0.0 ± 0.0**	**0.0 ± 0.0**	**0%**
Medetomidine 50 µg/kg I.M	**1.5 ± 0.5**	**20 ± 0.6**	**0%**
Tramadol 0.5 mg/kg I.P	**3.7 ± 0.7**	**17.8 ± 0.7**	**0 %**
Medetomidine 50 µg /kg , I,M + Tramadol 0.5mg/kg, I.P	**3.2 ± 1.3**	**16.7 ± 1.3**	**100 %** *

Table 4: The sedative effect dose of Medetomidine or Tramadol alone and together in glutathione concentration in liver or glucose level, ALT and AST activity in plasma. after 30 minutes of injection in rabbits

Treatments	Glutathione in liver (Micromol / g)	Glucose (mg/ 100 ml)	ALTactivity (i.u /L)	ASTactivity (i.u/ L)
Control (Physiological saline)	3.81± 1.5	199.05± 4.5	5.61 ± 1.6	212.65 ±8.5
Medetomidine 50 µg \ kg I.M	2.29 ± 0.8	193.20 ± 6.5	6.82 ± 2.1	229.65 ± 9.9
Tramadol 0.5 mg/kg I.P	3.67 ± 15	213.20 ± 8.8	9.09 ±0.9	218. ± 7.8
Medetomidine 50 µg/kg I.M +Tramadol 0.5 mg/ kg I.P	2.35 ± 0.6	195.68 ± 9.8	6.02 ± 1.1	216.22 ± 1.3

Value are presented as the mean ± SE of 4 rabbits/ group

DISCUSSION

Many reports have been published an analgesic combination such as Ketoprofen with Acetaminophen, Tramadol with Acetaminophen (19 and 20), it had manifested these combinations of drugs acting on different receptors may produce super or sub additive interaction in antinociceptive effects (17) In the present study had been used electrical stimulator for indice pain then detect the analgesic pain tolerance analgesic induction of and to evaluate analgesic effect of Medetomidine or Tramadol alone or as a combination quantitively and qualitatively in rabbits .This device was used firstly by Al-Mashhadany to evaluate the analgesia by Medetomidine in goats (33) also used to evaluate the analgesic effects of xylazine in chicks (15) , In this study used electrical stimulator to evaluate the analgesic action of some analgesic drugs such as Medetomidine and Tramadol and to detect the best onset and duration of analgesia, also to detect the best analgesic dose. The results were indicate the Medetomidine at 100 , 200 micro g \ kg B.W. (I.M) and Tramadol at 0.5, 1, 2 mg\ kg B.W. (I.P) in rabbit to relief of pain sensation as dose dependent response to electrical stimulation by electrical stimulant via voltage increasing (increase of pain threshold) compared with its voltage before injection and control . The best analgesic doses of Medetomidine and Tramadol at 200 micro g \ kg B.W.(I.M) and 2 mg \ kg B.W. (I.P), respectively, induced rapid significantly analgesia compared with other doses in each of them and persist more than 90 minutes of injection, these results of Medetomidine

were agreement with previous studies in goats (33) chicks (15) and Tramadol results were agreement with previous studies in chicks (34), rats (35 and 36) and mice (37). The analgesic effects of Medetomidine due to activation of pre-synaptic alpha 2- adrenoceptor in the peripheral and central nervous system leading to decrease of catecholamine release and turnover (13 and 38) but analgesic effects of Tramadol result from dual action, first; it has affinity to binding with μu-opioid receptor in the CNS and the second; it had inhibited effect of pre-synaptic reuptake of nor epinephrine and serotonin (20).

The clinical signs in this study were manifested by ataxia, hair erecting, urination, defecation, lacrimation, closed eye depress and decumbency , they were agreement with other studies in analgesic effects of Medetomidine in goats (33) ,chicks (15), camel (11) , cats (3), horses (10) and in analgesic effects of Tramadol in chicks (34and 15) , dogs (2) and in mice (39).

In the present study evaluating the analgesic effects of drug interaction (synergism) between sedative doses of Medetomidine at 50 micro g / kg B.W. (I.M) with Tramadol 0.5 mg \ kg B.W. (I.P) leading to excellent analgesic effect as 100 % of treated rabbits compared with each of them given alone (not have analgesic effects) and the causing of this synergism revert to may be due to Tramadol has weak opioid agonist with antinociceptive effects through its action on Mu-receptor or by inhibiting the neural reuptake of both noradrenalin and serotonin (26) .Many studies demonstrated the Tramadol has analgesic effect through acting on descending noradrenergic pathways and this play a role in analgesic properties of the non- opioid, stimulating this pathway produces antinociceptive effect from the activation of the spinal alpha 2- adrenergic receptor by noradrenergic neuron (39). Previous study suggested that the coexpression of the synergistic receptor pair alpha 2- adrenoceptor and Mu- receptor on primary afferent nociceptive fibers may representing on substrate for analgesic synergy, perhaps as a result of interacting between neural G protein coupled receptor (40) . Alpha 2- adrenergic receptor coupled to sensitive inhibitory G– protein that causing inhibition of adenylyl cyclase which result in decrease $_{C}$AMP formation, is an important consequence of alpha 2- adrenoceptor activation (41),and the drug interaction in sedative dose was not manifested any significantly differences in onset and duration of sedation in animals when given as a single or combined dose , and not appeared any CNS side effects as well as, not induced any significant difference in glucose, glutathione , AST and ALT levels in rabbits.

تاثيرات الجرعه تحت المسكنه (المسدره) للميديتوميدين والترامدول معا لاحداث التسكين في الارانب

محمود بشير محمود
فرع الادويه ،كليه الطب البيطري ،جامعه دهوك ، دهوك ، العراق

الخلاصة

كان الهدف من الدراسة الحاليه هو تحديد أفضل جرعة مسكنة للالم (وقت بدء التسكين ومدته) لبعض المسكنات كالمديتومدين والترامادول في الارانب عند معاملتها بالجرع المسكنه. فضلا عن تقييم الفعل المسكن للجرع المسدره (تحت المسكنه) لهذه المسكنات عند إعطاءها معا بشكل مركبات وباستخدام جهاز المحفز الكهربائي . وكانت أفضل جرعة مسكنه للالم لعقار الميدينومدين لوحده هي 200 مايكروغرام \ كغم من وزن الجسم بالعضلة وللترامادول لوحده هي 2 ملغم / كغم من وزن الجسم بالخلب ، حيث ادت الى زياده معنوية في الفولتية المسببة للالم عند الاوقات (15، 30، 45، 60، 75، 90) دقيقة من الحقن مع مجموعة السيطرة والمجاميع الاخرى لكل عقار. في حين أدى إعطاء عقار المديتومدين والترامادول معاً بجرعة مسدره (تحت المسكنه) عند 50 مايكروغرام/ كغم بالعضلة و 0.5 ملغم / كغم بالخلب على التوالي الى إحداث تسكين ممتاز من الالم وبنسبة 100 % مقارنة مع المجاميع المعاملة بكل عقار لوحده (عند الجرع تحت المسدره نفسها) . لم تظهر هناك تأثيرات جانبية ضاره على الحيوانات بالاضافة الى عدم حصول تغييرات معنوية في مستوى كل من الكلوكوز والكلوتاثيون ونشاط كل من خميرة ALT و AST في الحيوانات المحقونة بالجرع المسدره معا. لقد أظهرت الدراسة بان المزيج (التداخل الدوائي) بين الميديتومين والترامادول في الجرع الواطئة هو تآزري ويعد هذا المزيج مثاليا مناسبا لإحداث تسكين آمن وجيد من الالم بالاضافة الى ارتخاء للعضلات الهيكلية في الارانب.

REFERENCES

1- Short, C.E. (1992).Alpha 2-Agents in Animals. Sedation, Analgesia and Anesthesia. Veterinary Practice Company, Santa Barbra, CA.

2- Murrell, J.C. and Hellebrekers, L.J. (2005). Medetomidine and dexmedetomidine: a review of cardiovascular effects and antinociceptive properties in the dog.Vet. Anesth. Analg., 32:117-127.

3- Granholm, M., Mckusick, B.C. and Aspegren, J.C. (2006). Evaluation of the clinical efficacy and safety of dexmedetomidine or medetomidine in cats and their reversal with atipamizole. Vet. Anaesth.Analg., 33: 214-223.

4- Dayer, P., Desmeules,J.(1997). Pharmacology of Tramadol. Drugs. 53:18-24.

5- Adams, HR. and Sousa, JP. (2001). Veterinary Pharmacology and Therapeutic: 8th ed., Lowa State. University Press., Ames Lowa., 314-321.

6- Mohammed, F.K., Zangana, I.K. and Abdul-Latif, A.R. (1993). Medetomidine sedation in sheep. Zentralbl. Veterinarmed. A., 40: 328-331.

7- Kastner, S.B.R., Pakarinen, S.M., Ramela, M.P., Kutter, A.P.N., Boller, J. and Huhtinen, M.K.(2006). Comparative pharmacokinetics of Medetomidine enantiomers in goats and sheep during sevoflurane anesthesia. J. Vet. Pharmacol. Ther., 29:63-66.

8- Carroll, G.L., Hartsfield, S.M., Champney, T.H., Geller, S.C.,Martinez, E.A. and Haley, E.L.(2005).Effect of Medetomidine and its antagonism with atipamizole on stress-related hormones, metabolite, physiologic responses, sedation, and mechanical threshold in goats. Vet. Anaesth. Analg., 32:147-157.

9- Kastner, S.B.R.(2006). Alpha 2- agonists in sheep: areview. Vet.Anaesth.Analg., 33: 79-96.

10- Ringer. S.K., Kalchofner. K., Boller, J., Fürst, A, and Bettschart-Wolfensberger, R. (2007). A clinical comparison of two anaesthetic protocols using lidocaine or medetomidine in horses. Vet Anaesth Analg. ;34 :257-68.

11-Peshin, P.K., Beniwal, J., Sharma, D.K.; Kumar, A.; Sharma, C. K. and Singh, S.R (2011). Clinical evaluation of medetomidine hydrochloride as a sedative and its reversal with atipamizole in camels. J. of Camelid Sci. 4 : 83–84.

12- Singh V., Amarpal, K. P., Pratap, K. and Aithal ,H.P.(2003). Comparison of xylazine and medetomidine with and without ketamine for epidural analgesia in buffalo calves. Indian J. Vet.Surg.24:76 - 82

13- Gross, M. E. (2001). Tranquilizers, α2-Adrenergic agonists and therapeutics. 8th ed., Lowa State University Press, Ames: pp, 299-234.

14- Lumigi, J. T. (2003). Medetomidine-Ketamine and Diazepam-Ketamine Anesthesia in Racing Pigeons (*Columba livia domestica*)—A Comparative Study. Medetomidine-Ketamine and Diazepam-Ketamine Anesthesia in Racing Pigeons (*Columba livia domestica*)—A Comparative Study. J. Avian Med. and Surg.: 17:191-196.

15- Shaban, -Kh. A. and Faris, G. A-M.(2012). Evaluation of the analgesic effect of xylazine, dipyrone and tramadol in asingle dose or as a combination in chicks. J. Al- Anbar. Vet.Sci.,5: 197-209.

16- Curro, T.J., Okeson, D., Zimmerman, D., Armstrong, DL. And Simmons, L.G. (2004). Xylazine midazolam-Ketamine versus Medetomidine midazolam- Ketamine anesthesia in captive Siberian tigers (Panthers tigers altaica). J. Zoo wild Med., 35:320-327.

17- Valle, M.; Garrido, M. J., Pavon, J. M., Calvo, R. and Troconiz, I. F. (2000). Pharmacokinetic-Pharmacodynamic modeling of the antinociceptive effect of main active metabolites of tramadol, (+) O-desmethyltramadol and (-) O-desmethyltramadol, in rats. J. Pharmacol. Exp. Ther., 293: 646-653.

18- Natalini, C. C. and Robinson, E. P. (2000). Evaluation of analgesic effect of epidudurally administered morphine, alfentanil, butorphanol, tramadol and U50488H in horses. Am. J. Vet. Res., 61:1579-1586.

19- Finkel, J. C., Rose, J. B. and Schmitz, M. L. (2002). An evaluation of the efficacy and tolerability of oral tramadol hydrochloride tablet for the treatment of postsurgical pain in children. Anesth. Analg., 94: 1469-1473.

20- Finkel, R., Clark, M. A. and Cubeddu, L. X. (2009). Opioids, In : drugs affecting central nervous system. Lippincott's illustrated reviews: Pharmacology, 4th ed., PP.159-171

21- Bamigbade, T. A. and Hangford, R. M. (1998). Tramadol hydrochloride: an overview of current use. Pain Rev., 5:155-182.

22- Miranda, H. F. and Pinardi, G. (1998). Antinociception, tolerance and physical dependence comparison between morphine and tramadol. Pharmacol. Bioch. Behav., 61:357-360.

23- Natalini, C. C., Polydoro, A. S. and Crosignani, N. (2007). Antinociceptive effects of epidural tramadol administration in dogs as an analgesic technique for experimental stifle surgery. ACLa Sci. Vet., 35:189-195.

24- Sliva, M. A. G., Pollastri, E., Pantaleao, J. A. S., DeCarvalho, A. N. B., Henriques, H. N., Camara, N. R.; Pacheco, J. T. and Boaventura, T. G. (2007). Tramadol minimizes potential pain during postoophor-ectomy for Wister rats. Proc. 6th World Congress on Alternative and Animals Use in the Life Sciences. August 21-25, Tokuo, Japan. AATEX 14 Special Issue, PP. 91-92.

25- Collart, I., Lythy, C. and Dayer, P. (2008). Multimodal analgesic effect of Tramadol .Clin.Pharmacol.Therap. 53: 223-229.

26- Raffa, R.B., Friderichs, E. and Reimann, W.(2009). Opioid and nonopiod components independently contribute to the mechanism of action of Tramadol, an "atypical" opiod analgesic .J Clin. Pharm. Exp. Therap., 260: 275-285.

27- Raffa, S.M. and Abdulwahib, M.B. (2001) . Pharmacology oral combination analgesics: rational therapy for pain. J. Clin. Pharma. Therap., 26: 257-264.

28- Moron, M.S.,Depirre,J.W. and Mennerrik. B.(1979)Levels of glutathione, glutathione reductase and glutathione S-transfarase activities in rats lung and liver . Biochem. Biophs.Acta., 582:67-78.

29- Runyon, R. P. (1977). Non-parametric statistics: A contemporary approach. Addison-wesley publishing Co., Reading, Massachusetts, pp. 212-217.

30- Bluman, A. G. (2007). Elementary statistics, Astep by step approach. 6th ed., McGraw-Hill companies, Newyork, USA.

31- Cleophas, T. J., Zwinderman, A. H., Cleophas, T. F. and Cleophas, E. P. (2009). Statistics applied to clinical Trials. 4th ed., PP. 375-477.

32- Daniel,W.W. (2010). Biostatics basic concepts and methodology for health sciences. Weily J Son. INC 9th edition. P: 356.

33- Al-Mashhadany, M.B. (2000) Neuropharmacological effects of Medetomidine relate with physiological changes in goats. Msc. Thesis. University of Mosul, Mosul, Iraq.

34- Tawfik, N. O.; Taqa, G. A. and Alsandook, T.A. (2009). Evaluation of antinociceptive effect of tramadol in chicks. Iraqi J. Vet. Sci., 23:19-22.

35- Garlick, J., Dorazil-Dudzik, M., Wordlicz, J. and Przewlocka, B.(2006). Effect of intraarticular tramadol administration in the rat model of knee joint inflammation. Pharmacol. Res., 58:672-679.

36- Bonjardim, L. R., Silva, A. P., Tambeli, C. H.; Veiga, M. C. F. A. & Gameiro, G. H. (2009) .Nociceptive behavior induced by mustard oil injection into the temporo- mandibular joint is blocked by a peripheral non-opiod analgesic and a central opioid analgesic. Pharmacol. Bioch. Behav., 91:321-326.

37- Al-Jader, G. H. M. 2011. Study the effects of diphenhydramine (H1-receptor antagonist) on tramadol analgesic effect in mice. MS.c thesis, Mosul University, Mosul, Iraq.

38 Papich, M. G. (2007). Saunders Handbook of Veterinary Drugs. 2nd ed., North Carolina State University, Raleigh, North Carolina.

39- Taqa, G. A. 2012. Synergism of the analgesic activities of tramadol with α2-adrenoceptor agonist xylazine in mice. Iraqi J. Vet. Sci. 65-640.

40- Riedl, MS., Shnell SA., Overland AC., Chabot-Dore AG., Tylor AM., Riberio, DA., Elde RP., Wilcox GL. and Stone LA.(2009). Coexpression of alpha2-adrenergic and opioid

receptor in substance P- containing terminal in rats dorsal horn. J. Comp. Neuro., 513: 385-398.

41- Yagiela,AG., Jone, FG., Dowed, FJ., Johnson, BS., Mariotti, AJ. And Neidle, EA. (2011). Pharmacology and therapeutics for dentistry. American Dental association. New-York 6th ed.P78-161.