

Evaluation the Effect of Single and Multiple Administration of Tramadol on Sedation and Analgesia in Sheep's

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ABSTRACT

Objective: The aim of this study was to investigate effect of single (acute) and multiple-dose(chronic) of tramadol intramuscular injection on pain threshold after stimulation by electrical stimulator.

Martials and Methods: Eight male sheep were used in the study were randomly divide into two groups. Group1 (control): receiving 0.9% normal saline intramuscular. Group2 (single-dose administration): receiving tramadol 5mg/kg intramuscular. The animals in Group 1 and 2 were treated daily with intramuscular injection of normal saline IM (5 mg/kg) and tramadol IM (5 mg/kg) respectively for three days. The pain threshold were evaluated at 0, 15, 30, 45, 60 minute after administration single dose in all group by using electrical stimulator. The intensity of pain response (analgesia) were recorded and sedation were evaluated.

Results: The estimated showed significant increase in pain threshold between pre and post treatment that which represent the analgesic activity of tramadol. Increase in pain threshold were significantly higher than baseline values at all estimation times after administration of tramadol and were lower tonear to the baseline after 60 minute. The peak of analgesic activity was at 45 minute in single and multiple administration and there is no significant difference in peak of analgesic activity between single and multiple administration. Sedation are the adverse effects were observed in single and multiple administration but the degree of sedation is more in multiple dose than in single dose after administration of tramadol. Results showed that no significant differences between single I.M administration of tramadol at 5mg/kg and multiple-dose regimen for three days on bone marker (Specific Bone alkaline phosphatase (BALP) and Osteocalcin (OC)).

Conclusions: Single and multiple administration of tramadol produce good and intensity analgesic activity, thus can be use tramadol in sheep as a good analgesic drug with little side effects and without effect on bone density.

Key words: Antinociceptive effect, Tramadol, sheep, Pain threshold, Sedation score.

INTRODUCTION

Pain is one of the most common reasons for which people seek medical attention, thus, analgesics are the most commonly prescribed medication in clinical practice (Curtero et al., 2006). Several prescription analgesic medications are taken either as single or multiple dose. Tramadol is one of the most prescribed analgesics in the world. It is extensively used to control mild and moderate pain in humans, the use of tramadol in veterinary patients is relatively recent (Natalini and Robinson, 2000). Tramadol is an opioid analgesic drug widely used in recent years that prescribed for patient suffering from moderate and severe pain or chronic pain, including postoperative, gynecologic, and obstetric pain, as well as pain of various other origins, including cancer (Lee et al., 1993, Finkel et al., 2002).

Tramadol has been used in veterinary anesthesia as a pre-anesthetic (Kongara et al., 2012) to improve the quality of anesthetic properties (Ajadi et al., 2009). Tramadol is a synthetic analgesic of opioid class (Collart et al., 1993). It is a centrally acting analgesic whose mechanism of action is predominantly based on blockade of serotonin reuptake. Also it has been found to inhibit norepinephrine transporter function, μ -Opioidergic and monoaminergic (5-hydroxytryptamine and

noradrenalin) pathways and prostaglandin-dependent mechanisms are individually important in the modulation of pain (Yaksh and Malmberg, 1994). Tramadol is partially antagonized by naloxone, because it is believed to be only a weak μ -receptor agonist (Rashidpouret al., 2012). Because it is only partially antagonized by naloxone, it is believed to be only a weak μ -receptor agonist.

The goals of the present investigation was undertaken to study the effect of single (acute) and multiple dose (chronic) regimen of tramadol on pain in sheep and determination duration of action of analgesia and evaluate the effect of tramadol on Specific Bone alkaline phosphatase (BALP) and Osteocalcin (OC).

MATERIALS AND METHODS

Eight male healthy ewes sheep from local market weighing ranging 35-40 kg with aged about one to 1.5 years were selected for the study. The animals were housed and fed with standard diet and water and kept in standard animal housing condition with the room temperature of $28 \pm 1^\circ\text{C}$. The animals were approved from animal care housed in Dentistry Collage of Mosul University in Iraq. The sheep were divided into 2 groups, each group consist of four animals. Group 1 served as a control was given normal saline 0.9% (ml/kg, intramuscular), group 2 received (5 mg /Kg, intramuscular) tramadol ((Mepha Ltd Aesh-Basel swit-zerland). The animals in Group 1 and 2 were treated daily with intramuscular injection of normal saline IM (5 mg/kg) and tramadol IM (5 mg/kg) respectively for three days.

Antinociceptive effect of tramadol in sheep was evaluated depending on increased in pain threshold after stimulating the nasal mucous membrane by electrical stimulation (noxious stimulation) because this area is sensitive to electrical pain stimulation. The nociception threshold for each sheep was determined after attachment the electrical polar of electrical stimulation to the nasal mucous membrane and record the volt that which the sheep moved the head far for the source of stimulation. Pain threshold was evaluated for each sheep before treatment and consider as a base line. The nociception threshold volt were recorded before and (15, 30, 45 and 60 min.) following IM administration of tramadol. The increased in volt compared with the values of the pretreatment were used for comparison. Pain threshold volt was assessed after single injection of tramadol and with continuous injection for 3 days.

Sedation score for sheep were evaluated pre and after 15, 30, 45, 60 from tramadol injection in control and after single and multiple dose regimen according to (Vaha Vahe, 1991) as follows :

- 0: Normal (no sedation).
- 1: Drooping of head with normal walking.
- 2: Ataxia
- 3: Lying but able to rise.
- 4: Recumbance and loss of righting reflex.

Evaluate the **Effect of single and multiple-dose regimen of tramadol on bone marker (Specific Bone alkaline phosphatase (BALP) and Osteocalcin (OC)) in blood:**

After 45 minutes of the injection in two groups blood samples (5ml) was taken from the jugular vein from each animal. The blood sample was collected into sterile plane tube and allowed for 30 minutes at room temperature for clotting then centrifuged at 3000rpm for 10 minutes in centrifuge, to isolate the serum from whole blood. Serum was transferred to Eppendorf tube and stored in deep freeze at -20°C till time of analysis (within 5 days) analysis by ELISA to evaluate the effect of single injection tramadol on bone marker (Specific Bone alkaline phosphatase (BALP) and Osteocalcin (OC)) in blood. Another blood samples were taken from the jugular vein after three days of continuous treatment to evaluate the effect of multiple injection dose of tramadol on same previous parameter of blood.

RESULTS

Determination the antinociceptive effect of tramadol:

The I.M administration of tramadol at 5mg/kg lead to significant increase in pain threshold volt between pre and post treatment at $p \leq 0.05$. The onset of drug action start at 15 min after drug administration and prolong the increased in the pain threshold volt for more than one hour compare to the pretreatment (figure 1).

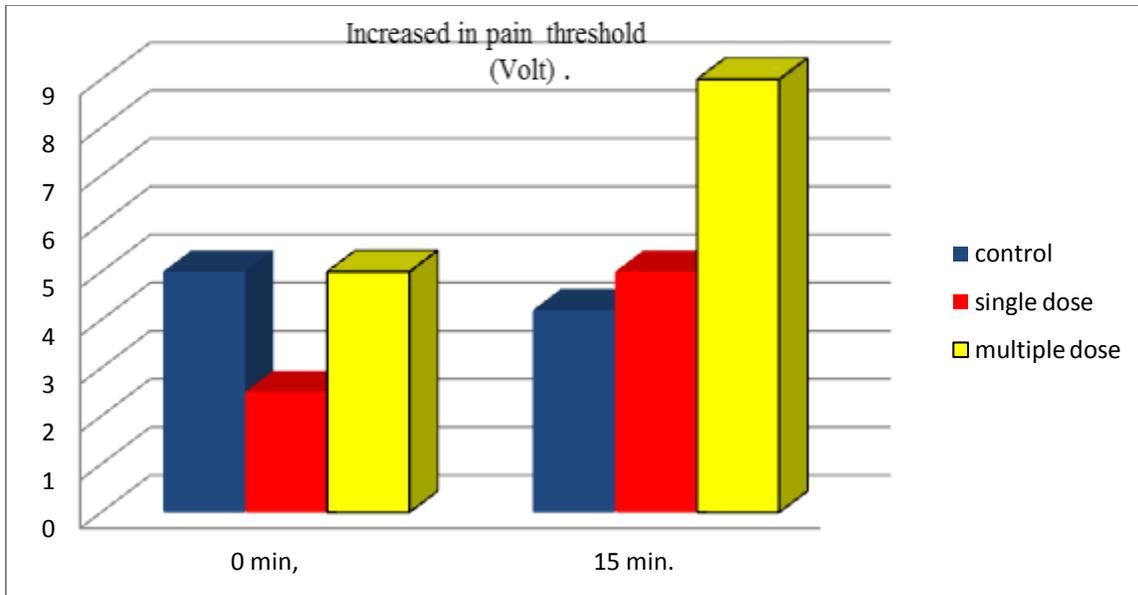


Fig (1): Duncan's multiple range test of onset of tramadol analgesia

Pain was reversed by tramadol after acute treatment (single injection 5mg/kg) start at 15 minute from injection in comparison to the control and last the duration of analgesia to one hour. The maximum effect of analgesic effect of tramadol at 45 minute this indicate that the best time of tramadol action, and the severity of analgesic effect was start decline after one hour. (Figure 2).

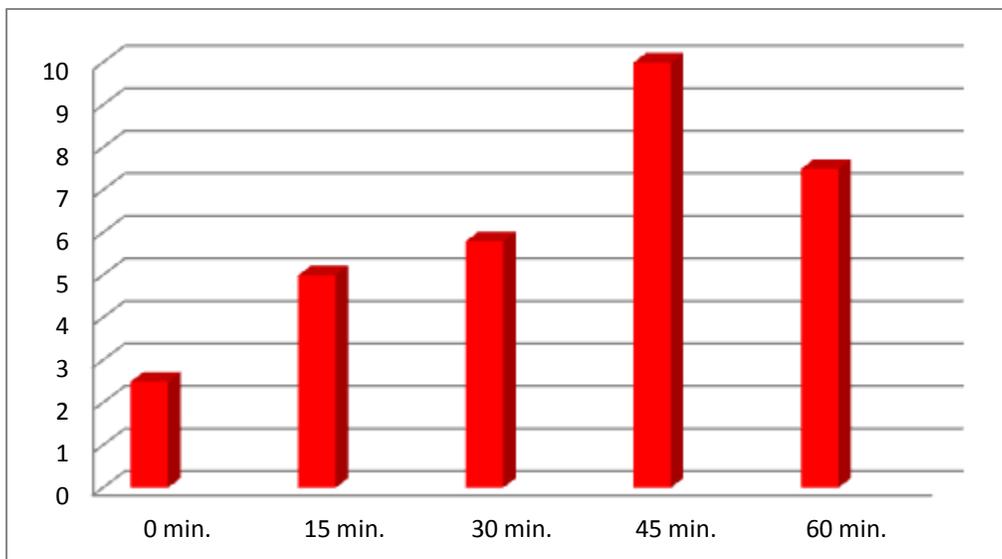


Fig (2): duration of analgesia and the maximum effect of tramadol single dose.

In the multiple administration (chronic) we found that tramadol also significant produce analgesic effect in comparison to control at $p \leq 0.05$ whereas the analgesic effect no significant difference between single and multiple dose except at 45 minute.

And the reversal of the electrical hyperalgesia persisted throughout a period of chronic tramadol treatment 5 mg/kg per day, with continuous injection for 3 days from 15 to 60 minute also same as single injection (Figure 3).

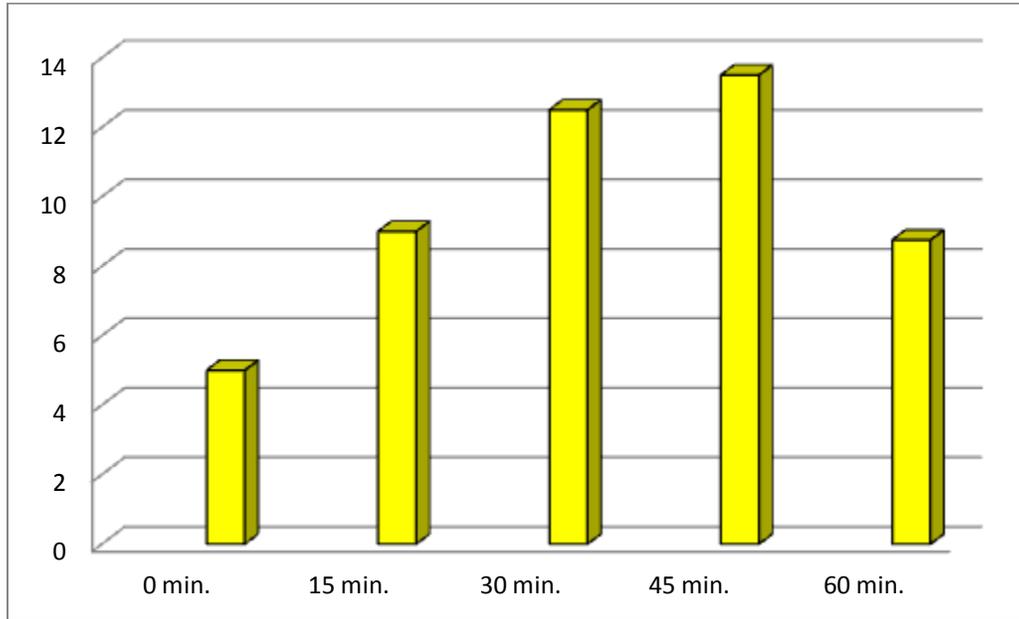


Fig (3): duration of analgesia and the maximum effect of tramadol in Multiple dose

The multiple-dose regimen for three days reduced the maximal intensity of electrical stimulating pain in sheep in comparison to the control. While no significant difference at $p \leq 0.05$ among acute treatment group and chronic group in time 30,40,50,60 minute after administration.(figure 4).

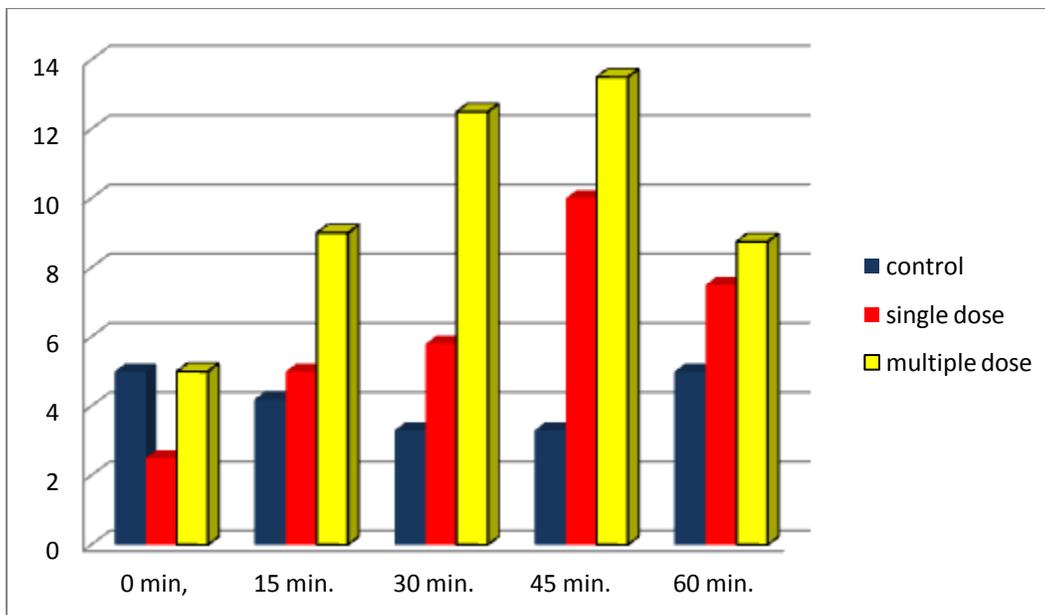


Fig (4): The increase in pain reaction time threshold of tramadol in sheep

Effect of single and multiple-dose regimen on Bone alkaline phosphatase (BALP)

Results showed that no significant differences at $p \leq 0.05$ between single I.M administration of tramadol at 5mg/kg and multiple-dose regimen for three days on bone marker (Specific Bone alkaline phosphatase (BALP)) (5.10 ± 0.66) (7.73 ± 0.66) pg/ml respectively. (Figure 5).

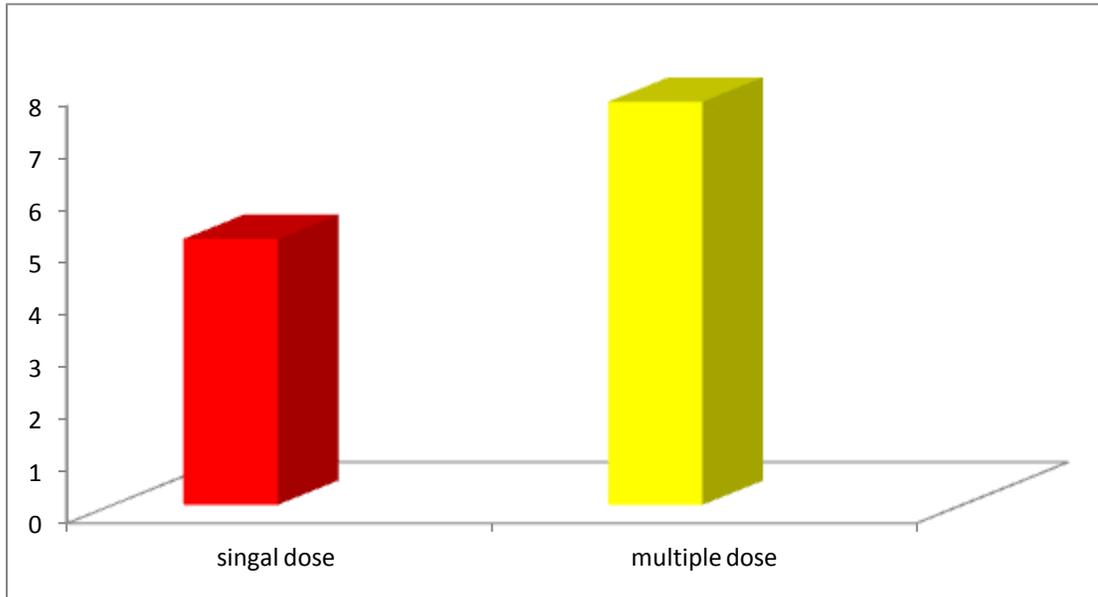
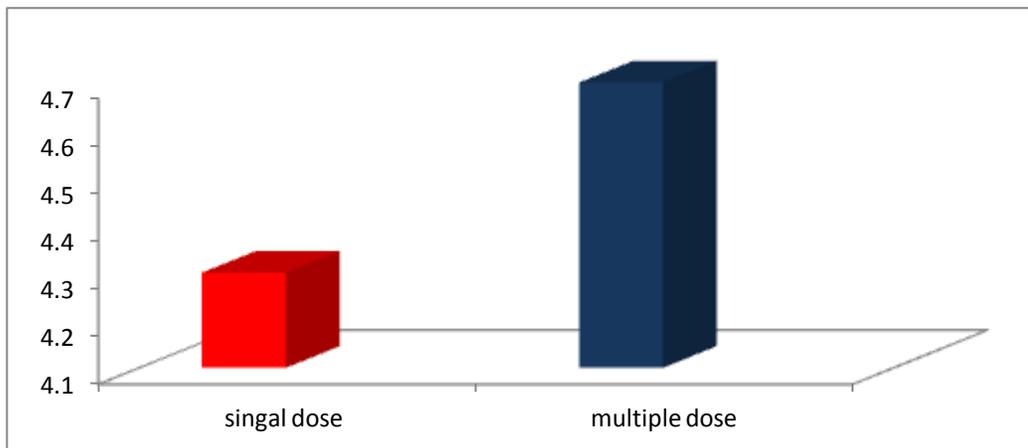


Fig (5): Means of Bone alkaline phosphatase in both single and multiple-dose Tramadol treatment.

Effect of single and multiple-dose regimen on Osteocalcin (OC)

Results showed that no significant differences at $p \leq 0.05$ between single I.M administration of tramadol at 5mg/kg and multiple-dose regimen for three days on bone marker Osteocalcin (OC) (4.30 ± 0.85) , (4.78 ± 0.60) pg/ml respectively (Figure 6):.



Fig(6): Means of Osteocalcin in both single and multiple-dose Tramadol treatment.

Statistical analysis

The data were expressed as mean ± SD, difference between 2 experimental groups were statistically analyzed by One – way ANOVA test followed by Duncan's Multiple Range Test was applied to show any correlation of parametric values. and Independent Sample T-Test was applied to show any correlation of parametric values. The level of significance was at $p < 0.05$.

DISCUSSION

Opioids are the most potent and effective analgesics available and is increased nowadays to treat acute, cancer and non-cancer chronic pain (Collet 2001).

In the present study, tramadol produced a potent antinociception effect after single and multiple dose administration in sheep. This result is in agreement with other study suggested that tramadol produce sedative and antinociceptive action in camels (Al-Mubarak, 2013).

Pain was reversed by tramadol after single intramuscular injection with 5 mg/kg. The reversal of the thermal hyperalgesia that induced by electrical stimulation persisted throughout a period of chronic tramadol treatment (multiple treatment) of 5 mg/kg for 3 days, through intramuscular injection are significantly produced a good analgesia, this result is in agreement with other study suggested that tramadol is a better choice for treatment of chronic neuropathic pain. (Yu-Chuan Tsai and Shen-Jeu Won, 2001). The maximum analgesic effect of tramadol in sheep at 45 minute after single and multiple administration this result is in agreement with previous study suggested that tramadol give the maximum analgesic effect after 45 minute in mice (Al-Jader and Taqa, 2014).

Tramadol is a racemic mixture consisting of two isomers (\pm) enantiomer, (Hui-chen et al., 2004), (+) enantiomer (M1) has a highly affinity for μ -opioid and M1 has 200 times higher affinity than the parent (\pm) tramadol (Gillen et al., 2000, Wu et al., 2002) while (-) enantiomer is responsible for other mechanism (non-opioid) of action that inhibit noradrenalin and serotonin reuptake (Scott and Perry, 2000). which are mainly involved in the inhibition of pain. (Sacerdote et al., 1997). The

effect of the non-opioid component of tramadol is mediated through α_2 -agonistic and serotonergic activities, by inhibiting the re-uptake of norepinephrine and 5-hydroxytryptamine and, possibly, by displacing stored 5-hydroxytryptamine from nerve endings. (James et al 1996) that

involvement the 5-HT_{1A} autoreceptors from the raphe nuclei and spinal 5-HT_{1A} receptors in the antinociceptive effect. (Berrocoso et al., 2006) Tramadol is rapid and complete absorption after intramuscular injection and over 30 minutes is extent to the of systemic availability (Lintz et al., 1999). This fact acceptable with the result in the present study that found tramadol is start produce analgesic effect after administration in single and repeated dose and the maximum analgesic and sedative effect reach the maximum after 45 minute after injection the drug, this is because tramadol is converted to more active metabolites M1 and M2 and the most analgesic effect is produce from M1 metabolite (Seo, et al 2011, Al-mubarak, 2013).

Tramadol hydrochloride (5 mg/kg, IM) significantly increased the pain threshold for sheep after single and multiple administration this result in the present study is in agreement with other study found that administration tramadol in Amazon parrots is significantly increased the thermal nociception threshold with no observed adverse effects. (Geelen et al., 2013).

Bone Alkaline Phosphatase, Osteocalcin

In the present study, changes in bone density were not significant difference in both serum level of BALP, OC in both single and multiple administration sheep. Therefore the present study, is in line with previous reports that underline that carried out to determine the ability of bone turnover markers to monitor the Bone Density during bone healing, based on the levels of those markers, no effects were seen on bone formation (Vestergaard et al., 2012; Schnabel et al., 2013; Oh et al., 2014). This can be explained by the fact that these markers can effectively detect changes in bone formation after 3-6 months from the changes in Bone Density (Marques et al., 2013). Osteocalcin is considered a parameter of osteoblast activity and bone formation (Murata et al., 2002). With respect to non significant differences in OC levels during study times in in both single and multiple animals administration which is possible explanation to be a more specific marker of bone resorption may participate in this phenomenon. and circulate in blood with short half life of approximately 20 min in the circulation, as its rapidly eliminated by the kidneys. (Taylor et al., 2002). In this study there were no significant elevations in serum levels of BALP, OC this result may be return to that μ -opioid receptor agonists inhibit the synthesis of BALP and OC by osteoblast cells and causing reduced bone formation, this fact give Another explanation about not affected bone marker by tramadol because tramadol is a weak μ -opioid receptor agonists it will not induce such effect on bone markers (Aghili et al., 2013).

CONCLUSION

Over all, data revealed that tramadol considered a suitable analgesic drugs in sheep when administration in single and multiple regimen with little side effects and no effect in serum levels of bone marker (Specific Bone alkaline phosphatase (BALP) and Osteocalcin (OC)).

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