Niger. J. Physiol. Sci. 28(June 2013) 051 –056
www.njps.com.ng

Niger. J. Physiol. Sci.

Evaluation of tribromoethanol, tribromoethanol-buprenorphine and ketamine-xylazine combinations for anaesthesia in Sprague-Dawley rats undergoing ovariectomy

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Summary: Effect of premedication with buprenorphine (BP) on efficacy and safety of tribromoethanol (TBE) - induced anaesthesia was evaluated and compared with anaesthesia induced by ketamine (K) and xylazine (X) combination in rats undergoing ovariectomy. Fifteen Sprague -Dawley rats (mean weight 246.5 ± 13.1g) were randomly divided into three groups. Group (TBE) received tribromoethanol solution (250mg/kg). Group (TBE+BP) was premedicated with BP (0.02mg/kg) and 30 minutes later with TBE (250mg/kg). Group KX was anaesthetized with mixture of K (43.5mg/kg) and X (6.5mg/kg). All injections were administered intraperitoneally. Anaesthetic parameters determined were onset of anaesthesia (OAN), duration of antinociception (DAN), duration of sleep (DSP) and recovery time (RCT). Rectal temperatures (RT) and respiratory rates (RR) were recorded immediately after loss of righting reflex and at ten minute interval up to 90 minute. In addition, rats were monitored for adverse signs up to one week after ovariectomy. Anaesthetic indices were compared using Student's t-test, while RR and RT were compared using analysis of variance (ANOVA). Two rats in TBE group and one rat in TBE+BP group died three days after ovariectomy. Duration of antinociception (DAN) was significantly (P= 0.0015) longer in TBE than in KX anaesthetized rats but not significantly (P= 0.054) different between TBE and TBE-BP anaesthetized rats. Also, DSP was significantly (P=0.001) longer in KX anaesthetized rats than TBE- anaesthetized rats. Similarly, the DSP was significantly (P= 0.013) shorter in TBE group than TBE+ BP anaesthetized rats. Both RR and RT decreased significantly (P< 0.0001) with time following anaesthesia in all groups. It was concluded that KX mixture provided better anaesthesia than TBE and TBE+BP, and addition of BP to TBE did not have any beneficial effect.

Keywords*:* Tribromoethanol, Buprenorphine, Ketamine, Xylazine, Anaesthesia, Rats, Ovariectomy

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Manuscript Accepted: April, 2013

INTRODUCTION

Surgical manipulations are important aspect of many biomedical researches designed to evaluate osteoarthritis, neurobiology, reproductive biology, pharmacology, and behaviour. For instance, ovariectomized rats are used as model for the study of osteoarthritis and the role of estrogen in the development of osteoarthritis in post-menopausal women (Schaller *et al.*, 2005). Similarly, ovariectomized rats have been used to study the effect of pharmacological agents on luteinizing hormone (LH) secretion since the regulatory effect of estrogen has been removed (Furuta *et al.*, 2002). However, the tissue damage that accompanies any surgical procedure often leads to pain and distress which can increase morbidity and mortality in rats. Thus anaesthetic agents are desired to provide pain relief and alleviate the stress associated with surgery in laboratory animals. The ideal agent/or technique should provide rapid and smooth induction of anaesthesia, adequate analgesia within the duration of the surgery, minimal cardiopulmonary depression and a rapid and smooth recovery. In addition, the agent must be cheap and readily available and be easy to administer with minimal use of equipment to justify use in laboratory rodents. Also the agent must be free from adverse reactions during the period of anaesthesia and thereafter.

Tribromoethanol is widely used in laboratory rodents for short duration surgical procedures and offers the advantage of not being a federally controlled drug. Ease of administration, rapid induction, good muscle relaxation, and fast recovery

are considered as other advantages associated with its use (Gopalan *et al.,* 2005). However, the drug is reported with varying degree of morbidity and mortality especially at high doses, adverse reactions such as peritonitis, fibrous adhesions in the abdominal cavity, irritation, hepatic and renal injury, splenic lymphocyte injury and ileus (Arras *et al.,* 2001; Thompson *et al.,* 2002; Lieggi *et al.,* 2005). The other problem associated with the use of tribromoethanol also include difficulty in the preparation and storage of the solution as it dissociates over time resulting in increased frequency of adverse effect (Lieggi *et al.,* 2005). In-spite of this reported set-back, a number of laboratories still use tribromoethanol to provide anaesthesia for surgery of short duration.

Aside from the adverse effects associated with the use of tribromoethanol in rats, another major setback is the short duration of analgesia produce by single injection of the drug. This often necessitates repeated injections when surgical procedure is prolonged, which further increase the risk of adverse reaction. The need for repeated injection may be improved by preoperative administration of opioid analgesics. Previous studies have shown that preoperative administration of analgesics is a more effective means of providing post-surgical anaesthesia (Roughan *et al.,* 1999). In addition, the agent is likely to potentiate the effects of the anaesthetics, thus enabling the dosage of the anaesthesia required for surgical anaesthesia to be reduced. For instance, premedication with buprenorphine reportedly increased the duration of anaesthesia induced by ketamine-medetomidine combination in rabbits (Murphy *et al*, 2010). It is thus hypothesized that premedication with buprenorphine will significantly increase the duration of anaesthesia following administration of tribromoethanol in rats. The aim of this study therefore was to evaluate the effect of premedication with a single injection of buprenorphine on the efficacy and safety of tribromoethanol induced anaesthesia in rats undergoing ovariectomy. We also compared these two anaesthetic techniques with the combination of ketamine and xylazine.

MATERIALS AND METHODS

Fifteen female Sprague-Dawley rats (mean weight 246.5 ± 13.1g) bred in-house were used. The experiment was part of a study evaluating the effect of *Garcinia kola* extract on reproductive functions in ovariectomized rats, approved by the Experimental Animal Committee of St. Cloud State University, St. Cloud, Minnesota, USA and was done in accordance with the National Institute for Health (NIH), USA guideline for use of laboratory animals in experimental research. The animals were maintained in the Animal Research facilities at the Department of Biological Sciences, St. Cloud State University, St. Cloud, Minnesota throughout the duration of the experiment. They were housed individually in plastic cages in a room maintained at 25^0C with a 12-hour/12-hour light/dark cycle and given pelletized rat feed (Global diets, Madison, USA) and fresh water *ad-libitum* in a graduated glass water bottle. The study was carried out in an air-conditioned room with temperature maintained at 25^0C throughout the duration of the study.

Drugs

Tribromoethanol was prepared by dissolving 5g of tribromoethanol (T48402, Sigma Aldrich, St. Louis, Mo) in 5ml of butanol (tertiary amyl alcohol, 240486, Sigma Aldrich) in a bottle to obtain a stock solution of 1g/ml. The bottle was then covered with aluminum foil and stored in a dark room at 4^0C as described by Gopalan *et al.,* (2005). A 2.5% solution was made by reconstituting 1ml of the stock solution in 40 mls of distil water. The resultant solution was also kept in a sterile bottle covered with aluminum foil and stored in a dark room at 4^0C. The resultant solution was administered at the rate of 250mg/kg body weight of the rats. The ketamine-xylazine mixture was prepared by mixing 1.5 ml of 10% (100mg/ml) xylazine hydrochloride (Anased, Lloyd Laboratories, Shenandoah, IA) and 10 mls of 10% (100mg/ml) ketamine hydrochloride (KetaVed, Vedco Inc, St. Joseph, MO). The resultant solution was then administered at the rate of 43.5 mg/kg of ketamine and 6.5mg/kg of xylazine. Buprenorphine was administered as a 0.005mg/ml clear colourless solution of buprenorphine hydrochloride (Bedford Labs, Bedford, OH).

Design

This study used a simple randomized blinded design. The rats were randomly divided into three groups of five rats each using randomization chart. The first group (TBE) received 250 mg/kg of 2.5% solution of tribromoethanol solution intraperitoneally. The second group (TBE+BP) was first premedicated with intraperitoneal injection 0.02 mg/kg of buprenorphine and 30 minutes later with 250 mg/kg of 2.5% tribromoethanol solution. The third group (KX) was anaesthetized with intraperitoneal injection of a mixture of ketamine (43.5mg/kg) and xylazine (6.5mg/kg). The rats were restrained manually for the intraperitoneal injections which was made using size 26 gauge needle.

Experimental procedure

The rats were first weighed with a sensitive weighing balance (Satorius Basic, Satorius Corporation, Bohema, NY). Thereafter, they were restrained manually and the ventral abdomen disinfected with alcohol swab (BD Consumer Healthcare, Franklin Lakes, NJ). Following administration of the anaesthetic agent, the rats were kept in a plastic cage and observed until there was loss of righting reflex.

Thereafter, the rats' abdomen was prepared aseptically for laparotomy. Following the disappearance of pedal withdrawal reflex, the rats were positioned in dorsal recumbency for laparotomy and ovariectomy. Thereafter, the rats were monitored until full recovery. During the ovariectomy procedure, antinociception was tested by applying a mosquito artery forceps at the interdigital space and closed to the first ratchet. This was repeated at 2 minutes interval until it evokes a response of pedal withdrawal.

The following anaesthetic parameters were determined during the course of this study. The onset of anaesthesia (OAN) was determined as the time interval between the end of drug administration and the loss of righting reflex. The duration of antinociception (DAN) was determined as the time interval between the loss of pedal withdrawal reflex and the return of pedal withdrawal reflex. The duration of sleep (DSP) was determined as the time interval between the loss of righting reflex and the return of righting reflex while, the recovery time (RCT) was determined as the time interval between the return of righting reflex and the return of normal activity. Normal activity in the rats was defined as the time when the rats run around in the cage, or made attempt to groom itself or eat. All anaesthetic parameters were recorded in minutes using a stop watch.

The rectal temperatures (RT) and the respiratory rates (RR) of the rats were determined immediately after the loss of righting reflex and at ten minutes interval up to 90 minutes after induction of anaesthesia. The rectal temperature was determined in centigrade using a clinical mercury thermometer, while the respiratory rates were counted in breath/min using abdominal excursion. The rats were also monitored for adverse signs related to the administration of the anaesthetic agents during the surgery, following recovery from anaesthesia and up to one week after the administration of the anaesthetic agents. Observations noted include quality of anaesthetic induction or recovery, evidence of pain following surgery, quality of appetite, death during and after surgery. In addition, postmortem examination was performed on dead rats to determine the cause/causes of death. Additional buprenorphine injection was given post-operatively if rats were observed to show pain, moderate to severe pain signs.

Statistical analysis:

Data distribution was tested for normality by constructing frequency histograms of the data series. Data with normal distribution were expressed as mean (standard deviation). Anaesthetic indices between treatments were compared using Student's t-test. Rectal temperatures and respiratory rates were compared both for differences within and between treatments using analysis of variance (ANOVA) for repeated measures. Least square difference was used for post hoc analysis. All statistical analysis was performed using SAS 9.1 (TS1M3) software (SAS Institute Inc., Cary, NC, USA). A value of $P< 0.05$ was considered significant.

RESULTS

The quality of anaesthesia was considered excellent in all the three groups. Following premedication with buprenorphine, rats in the (TBE+BP) group were observed to have increased activity. In all the groups, the rats were observed to arch their back for few minutes during recovery from anaesthesia. No adverse effect was observed during anaesthesia and up to the recovery period. However, two rats died in the TBE and one rat in the TBE+BP group three days after ovariectomy. None of the rats in the KX died up to one week after ovariectomy. Necropsy of the dead rats showed that two of the rats had moderate peritonitis, adhesions around the liver and stomach and ileus, while a rat in the TBE group had haemoperitoneum and clot measuring up to 1cm around the area where the left ovary was removed.

Results of the anaesthetic indices of the rats are shown in Table 1. The onset of anaesthesia (OAN) was not significantly different between the three groups. Duration of antinociception (DAN) was significantly ($P= 0.0015$) longer in the KX anaesthetized rats than TBE anaesthetized rats, however there was so significant ($P= 0.054$) difference in the DAN between TBE anaesthetized rats and TBE+ BP anaesthetized rats. The duration of surgery did not differ between the three groups of rats. The duration of sleep (DSP) was significantly ($P=0.001$) longer in the KX group and shortest in the TBE group. However, the recovery time (RCT) did not differ in the three groups.

Table 1. Comparison of anaesthetic indices following either intraperitoneal injections of tribromoethanol (TBE) or ketamine-xylazine (KX) combination in Sprague Dawley rats undergoing ovariectomy

Anaesthetic Indices (Min)	*TBE (n=5)*	*TBE + BP (n=5)*	*KX (n=5)*
Onset of Anaesthesia (OA)	1.2 ± 0.2	1.4 ± 0.2	1.8 ± 0.4
Duration of Antinociception (DAN)	25.2 ± 2.6	19.8 ± 0.9	31.4 ± 1.5*
Duration of Surgery (DS)	25.2 ± 0.6	21.6 ± 2.0	26.8 ± 1.4
Duration of Sleep (DSP)	53.6 ± 5.2	84.4 ± 7.8	108. 8 ± 9.0**
Recovery time (RCT)	18.2 ± 1.6	22.0 ± 5.1	27.8 ± 3.4*

* P= 0.03 ** P= 0.0007

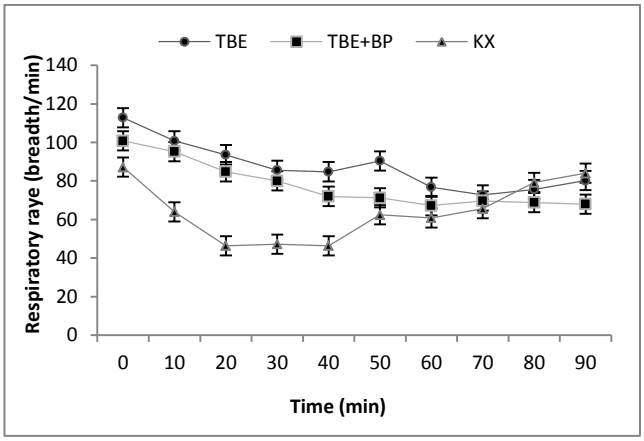

Figure 1. Respiratory rate following either intraperitoneal injections of tribromoethanol (TBE) or ketamine-xylazine (KX) combination in Sprague Dawley rats undergoing ovariectomy

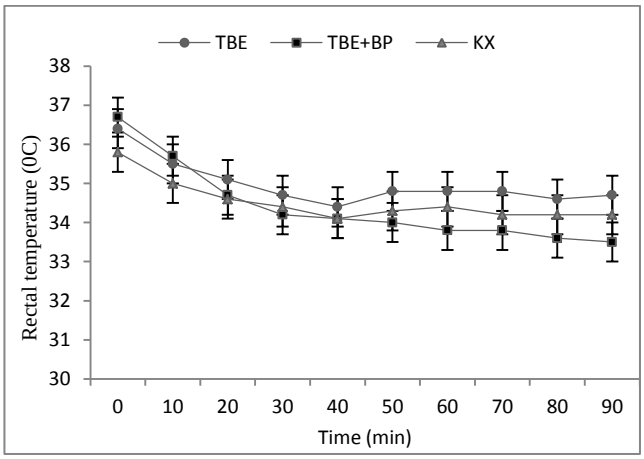

Figure 2. Anal temperature following either intraperitoneal injections of tribromoethanol (TBE) or ketamine-xylazine (KX) combination in Sprague Dawley rats undergoing ovariectomy

The changes in the respiratory rates (RR) and rectal temperatures (RT) following induction of anaesthesia and during recovery in the rat are shown in Figures 1 and 2. The rectal temperatures decreased significantly ($P< 0.0001$) with time following anaesthesia in all the three treatment groups. Similarly, the RT differs significantly ($P< 0.0001$) between the three treatment groups with the highest temperature maintained in the TBE group and the lowest temperature maintained in the TBE+ BP group. The RR decreased significantly ($P< 0.0001$) up to 40 min in the KX group and then increased steadily up to 90 minutes after induction of anaesthesia. However, in both the TBE and TBE+BP groups, the RR significantly ($P< 0.0005$) decreased throughout the 90 minutes following the induction of anaesthesia.

DISCUSSION

The field of experimental rodent surgery has grown significantly as transgenic technology has provided numerous rodent models suitable for surgical investigation (Lee-Parritz, 2007). Ovariectomized rats are now used as model to study the role of estrogen in cartilage turn-over and hence better understand the pathology of osteoarthritis in post-menopausal women (Hoegh-Andersen *et al.*, 2004). Ovariectomized rats can also be used to investigate the effect of a number of drugs or pharmacological extracts on the regulation and secretion of luteinizing hormone (Furuta *et al.,* 2002). While doing such procedures, ethical, scientific and regulatory consideration require effective use of sedatives, analgesics or anaesthetics for procedures which may cause more than momentary or slight pain. In addition, untreated pain increases catecholamine secretion and cause stress which may impair wound healing and immune function (Kona-Boun *et al.,* 2005; McGuire *et al.*, 2006).

Effective blockade of painful stimuli before surgery may significantly reduce the duration and intensity of postoperative pain by initially blocking the up-regulation of central pain processing pathways and thereby reducing subsequent mechanical allodynia (Ong *et al.,* 2005). Buprenorphine is the most widely used analgesic agent in the rodents and offer significant advantage over other narcotics. In this study, buprenorphine at the rate of 0.02mg/kg in the rats did not increase the duration of antinociception, although it significantly increased the duration of sleeping time. This finding will not appear satisfactory since the useful component of anaesthesia during surgery is the time when there is absence of nociception. In addition, there appeared to be no difference in the behavioural response of the rats in all the three groups after recovery as all the rats showed slight post-operative pain characterized by arching of the back. This lack of significant increase in the duration of antinociception may be associated with the lower dosage of buprenorphine used in this study or it may be that buprenorphine did not potentiate the analgesic effect of tribromoethanol in the rats.

The major drawback to the use of tribromoethanol in rodents is the frequency of the adverse effects. This has been associated with the dosage of the drug administered and the duration when the solution was prepared (Lieggi *et al.,* 2005). Thus, we assumed that lowering the dosage of the tribromoethanol in this study will significantly reduce the frequency of the adverse effect associated with its use. However, in this study, the frequency of adverse effect of tribromoethanol did not appear satisfactory and it does not appear to be affected by the dosage of the drug. Similarly, it does not appear that premedication with buprenorphine increased the frequency of adverse reaction associated with tribromoethanol use in rats. However, it will be recommended that care

should be taken while premedicating rats with buprenorphine prior to administration of tribromoethanol since previous study in rats receiving ketamine-xylazine combination following premedication with buprenorphine was associated with high frequency of adverse effects (Roughan *et al.,* 1999).

Pain assessment in rodents is somewhat difficult because of several aspect of rodent biology. Rats and mice are nocturnal and are therefore inactive, though arousable during the day. Assessment of spontaneous day time behaviour is therefore a poor indicator of well-being in this species (Perissin *et al.,* 2000). Vocalization is also not a reliable indicator of pain or distress because distress calls are inaudible to humans (Naito *et al.,* 2006). Behaviours such as arching of the back, writhe and poor gait though may be used to score pain objectively in the post-operative period is not applicable during the intra-operative period. Thus, the reaction of the rats to noxious stimuli is often used to assess the adequacy of analgesia during the intra-operative period. Pin-prick test and pedal withdrawal test are the two common techniques used to assess pain intra-operatively. In this study, we used the response of the rats to an artery forceps applied at the interdigital space to subjectively assess effective analgesia. Inadequate analgesia is defined as the withdrawal of the limb following application of the artery forceps. This test was repeated every two minutes so as to ensure that the maximum error in determining analgesia was reduced to two minutes. In this study, the duration of antinociception was longer in the rats anaesthetized with ketamine-xylazine combination compared with the other two combinations of tribromoethanol. This finding is in agreement with previous study (Thompson *et al.,* 2002; Sumitra *et al.,* 2004) and further confirmed that ketamine-xylazine combination is more effective than tribromoethanol for surgical procedure in rats. However, the prolonged sleeping time relative to the duration of antinociception in rats that received ketamine-xylazine or tribromoethanol-buprenorphine combinations will imply that the rats would have to be monitored for a longer period of time.

Monitoring of the cardiopulmonary functions and thermoregulation is very essential during surgery in animals. Although anaesthetic agents are known to cause respiratory and cardiovascular effects in large mammals, this has not been extensively investigated in rodents. This may be associated with the difficulty in assessing these parameters. For instance, while heart rates can be objectively measured in the large mammals using a stethoscope, the same is not true for laboratory rodents. However, in a previous study involving the use of ketamine-xylazine combination in rats, significant respiratory depression was associated reported (Sumitra *et al.,* 2004). Till now, there is dearth of information on the effect of tribromoethanol on respiration in rats. In this study, tribromoethanol and tribromoethanol-buprenorphine combination produced lesser decrease in the respiratory rates of the rats compared with ketamine-xylazine combination. In addition, premedication with buprenorphine appears to further decrease respiratory rate in tribromoethanol-anaesthetized rats. This finding is however expected since opioid analgesics have been reported to cause respiratory depression (Murphy *et al.,* 2010). Thus care should be exercised when premedicating rats with buprenorphine prior to tribromoethanol administration.

Thermoregulation and periodic evaluation of temperature in surgical patient is an important aspect of monitoring during anaesthesia. This is so because surgical patient under general anaesthesia can lose heat through two mechanisms (Sessler, 2008). The anaesthetic agent can depress the thalamus resulting in defective thermoregulation. On the other hand, the surgical patient can lose heat due to exposure of major body cavities to the atmospheric temperature which is lower than the core body temperature (Tan *et al.,* 2004). Decrease in body temperature of surgical patient has two important implications. There may be delay in recovery from anaesthesia, while the surgical patient may also die of hypothermic shock. Till now, there is dearth of information on the effect of tribromoethanol on body temperatures of rats. In this study, the rectal temperatures of the rats decreased significantly following anaesthesia in all the three groups. The highest decrease was seen in tribromoethanol and buprenorphine combination while the lowest decrease was seen in rats that received tribromoethanol alone. This suggests that premedication with buprenorphine produce a significant decrease in the body temperatures of the rats. It is therefore advised that body temperature should be monitored in rats undergoing major surgery and external source of warmth should be provided both during the intra-operative and recovery period as practised in larger mammals.

Finally, premedication with buprenorphine in rats anaesthetized with tribromoethanol was characterized with significant adverse reactions post anaesthesia, decreased respiratory rate and lowered rectal temperature. In addition, the combination did not appear to have improved intra-operative analgesia over rats anaesthetized with tribromoethanol alone except for the increased duration of sleep. It is therefore concluded that premedication with buprenorphine may not offer any beneficial effect in rats anaesthetized with tribromoethanol. However, further studies evaluating a higher dosage of buprenorphine is thus suggested.

REFERENCES

Arras, M., Autenried, P., Rettich, A., Spaeni, D., Rulicke, T. (2001). Optimization of intraperitoneal injection in mice: drugs, dosages, adverse effects and anaesthesia depth. Comp Med., 51: 443-456.

Furuta, M., Funabashi, T., Kimura, F. (2002). Suppressive action of orexin A on pulsatile luteinizing hormone secretion is potentiated by a low dose of estrogen in ovariectomized rats. Neuroendocrinology 75(3): 151-157

Gopalan, C., Hegade, C. M., Bay, T. N., Brown, S. R., Talcott, M. R. (2005). Tribromoethanol-Medetomidine combination provides a safe and reversible anaesthetic in Sprague Dawley rats. Contemporary Topics 44(1): 7-10.

Hoegh- Andersen, P., Tanko, L. B., Lundley, C. V., Mo, J. A., Heegaard, A., De Laisee, J., Christgau, S. (2004). Ovariectomized rats as a model of post-menopausal osteoarthritis: validation and application. Arthritis Res Ther 6(2): R169- 180.

Kona-Boun, J., Silim, A., Troncy E. (2005). Immunologic aspects of veterinary anaesthesia and analgesia. J. Am. Vet. Med. Assoc. 226: 355- 63.

Lee- Parritz, D. (2007). Analgesia for rodent experimental surgery. Isr J. Vet. Med. 62(3-4): 74-78.

Lieggi, C. C., Artwohl, J. E., Leszczynsk, J. K., Rodriguez, N. A., Fickbohm, B. L., Fortman, J. D. (2005). Efficacy and safety of stored and newly prepared tribromoethanol in ICR mice. Contemporary Topics 44(1): 11-22.

McGuire, L., Heffner, K., Glaser, R., Needleman, B., Mlarkey, W., Dickinson, S., Lemeshow, S., Cook, C., Muscarella, P., Melvin, W. S., Ellison, E. C., Kiecolt-Glaser, J. K. (2006). Pain and wound healing in surgical patients. Annals Behav Med., 31: 165-172.

Murphy, K. L., Roughan, J. V., Baxter, M. G., Flecknell, P. A. (2010). Anaesthesia with a combination of ketamine and medetomidine in the rabbit: effect of premedication with buprenorphine. Vet. Anaesth & Analg. 37: 222-229.

Naito, H., Okumura, T., Inouue, M., Suzuki, Y. (2006). Ultrasonic vocalization response elicited in adjuvant –induced arthritic rats as a useful method for evaluating analgesic drugs. Exp Anim 55: 125-129

Ong, K. S., Lirk, P., Seymour, R. A. (2005). The efficacy of preemptive analgesia for acute post-operative pain management: a meta-analysis. Anaesth & Analg. 100: 757-773.

Perissin, L., Facchin, P., Porro, C. A. (2000). Diurnal variations in tonic pain reactions in mice. Life Sciences 67: 1477- 1488.

Roughan, J. V., Ojeda, O. B., Flecknell, P. A. (1999). The influence of pre-anaesthetic administration of buprenorphine on the anaesthetic effects of ketamine medetomidine and pentobarbitone in rats and the consequences of repeated anaesthesia. Laboratory Animals 33: 234- 242.

Schaller, S., K. Henriksen, P. Hoegh-Andersen, B. Sondergaard, E. Sumer, L. Tanko, P. Qvist & M. Karsdal, (2005)**.** *In vitro*, *ex vivo*, and *in vivo* methodological approaches for studying therapeutic targets of osteoporosis and degenerative joint diseases: How biomarkers can assist? Assay and Drug Development Technologies, **3**: 553–580.

Sessler, D. I. (2008). Temperature monitoring and perioperative thermoregulation. Anaesthesiology 109(2): 318- 338.

Sumitra, M., Manikandan, P., Rao, K. V., Nayeem, M., Manohar, B. M., Puvanakrisnan, R. (2004). Cardiorespiratory effects of diazepam-ketamine, xylazine-ketamine and thiopentone anaesthesia in male Wistar rats: a comparative analysis. Life Sciences 75: 1887- 1896.

Tan, C., Govendar, M., Zaki, S., Miyake, Y., Packiarajah, P., Malik, R. (2004). Evaluation of four warming procedures to minimize heat loss induced by anaesthesia and surgery in dogs. Australian Vet J. 82(1-2): 65-68.

Thompson, J. S., Brown, S. A., Khurdayar, V., Zeynalzadedan, A., Sullivan, P. G., Schiff, S. W. (2002). Early effects of tribromoethanol, ketamine-xylazine, pentobarbitone and isoflurane anaesthesia on hepatic and lymphoid tissues in ICR mice, Comp Medicine 52(1): 63-67.