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Short Communication

Clinico-Biochemical Effects of Xylazine-Ketamine and Isoflurane on Rabbits Undergoing Ovariohysterectomy

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ABSTRACT

The serum biochemical and cardiovascular effects of total intravenous anesthesia, xylzine-ketamine and inhalation anesthesia, isoflurane was clinically compared in healthy female rabbits (n=16) undergoing ovariohysterectomy. One group of 8 rabbits was anesthetized with combination of xylazine (3mg/kg) and ketamine (10mg/kg, I/V), while the second group (n=8) was pre-anesthetized with xylazine, followed by treatment with isoflurane (4%) with oxygen flow rate of 1L/min. Rabbits administered with xylazine-ketamine anesthesia showed significant increase ($P<0.05$) in serum alanine aminotrasferase, aspartate transaminase and blood urea nitrogen as compared to isoflurane anesthesia; whereas, no statistical difference ($P>0.05$) was observed for alkaline phosphatase, creatinine and bilirubin between the two groups. Heart rate, blood pressure and saturation of oxygen were decreased in both groups but this decline was significantly lesser ($P<0.05$) in isoflurane administered group compared to xylazine-ketamine administered group. Both xylazine-ketamine and isoflurane proved to be suitable anesthetic agents for successful ovariohysterectomy in rabbits. As injectable anesthesia caused significant effect on serum biochemical and cardiovascular parameters when compared to inhalation, so, it is a less safe choice during lengthy surgical procedures like ovariohysterectomy.

Rabbits are very subtle and sensitive animals. Any malhandling can cause deleterious effects. For emergency surgery patients, careful selection and precise calculation of an anesthetic agent optimizes recovery chances by imparting minimum effects on biological systems. Many anesthetic drugs are used alone or in cocktail form (*e.g.,* xylazine, ketamine), propofol, isoflurane, ketamine and diazepam (Durrani *et al*., 2008).

Ketamine, due to its favorable pharmacological properties has been used extensively for the induction and maintenance of anesthesia (Bovill, 2008) and is referred as dissociative anesthetic agent and lacks cardiopulmonary depression, amnesia and anesthesia of stage 3 anesthesia. It acts as N-methyl-D-aspartate (NMDA) receptor antagonist which is site for the action of excitatory neurotransmitters in central nervous system (CNS). Due to some undesirable effects like poor muscle relaxation activity and muscle tremors, it is always used in combination with other anesthetic agents such as alpha-2-adrenergic drugs, diazepam or xylazine, according to the species of animals (Durrani *et al*., 2008). Ketamine in combination with xylazine has been extensively used in many species of animals from a long period as an intravenous or intramuscular anesthesia (Yershov *et al*., 2007).

Similarly, the recently introduced volatile anesthetics, especially isoflurane allows less side effects on cardiovascular, renal and hepatic system (Asokan *et al*., 2006). Inhalation anesthesia provides rapid adjustment of anesthetic depth, but most inhalant gasses are hypotensive and their analgesic potency is generally low. Regardless of easiness in the administration of injectable anesthesia, they may be associated with cardiovascular and respiratory side effects (Offinger *et al*., 2012). Pharmacokinetic properties of xylazine-ketamine and isoflurane have not been analyzed during lengthy surgical procedures such like, ovariohysterectomy (Akinea *et al*., 2001). With the

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passage of time, extensive use of phencyclidine and the increased experiments on rabbits, ideal anesthetic agent having minimum deleterious effects such as isoflurane became apparent (Nishiyama and Kazou, 2004). Rabbits are used in biochemical studies because of being docile and manageable but are more sensitive to surgery involving the gastrointestinal tract and reproductive tract (Jang *et al*., 2017). In the past due to less availability of safe anesthetic agents, it was difficult for veterinarians to evaluate and monitor their rabbits during surgical interventions. Now a day's surgical procedures are less complicated in rabbits due to the availability of effect and safe anesthetic drugs.

The rationale of this study is to make an assessment on the justification of the approach taken towards the use of xylazine-ketamine and isoflurane in rabbits undergoing ovariohysterectomy and to introduce an anesthetic modality based on statistics as use of isoflurane is more effective as compared to xylazine-ketamine, for anesthesia with respect to effects on cardiovascular, renal and hepatic functions/dynamics.

Materials and methods

Sixteen adult female rabbits (*Oryctolagus cuniculus*) were housed in individual stainless-steel cages (38 cm × 51 cm × 36 cm) with collection pans beneath each cage in a room with controlled environmental conditions. All animals were clinically healthy prior to the study. Animals were tested to be serologically negative for infectious pathogens. The rabbits were fed a standard rabbit diet (150 g daily), and fresh water was provided *ad libitum*. Sixteen female rabbits were allocated in two groups (n=8 each). The effects of different anesthetics were observed and recorded in both groups during ovariohysterectomy. For this, both groups were first pre-medicated using Atropine Sulphate @ 0.02 mg/kg body weight. However, induction and maintenance of anesthesia in Group A was achieved using a cocktail containing xylazine HCl (Xylaz®, Farvet, Holland @3mg/kg) and ketamine (Ketarol® Global Pharm, Pakistan @10mg/kg) adjusted to the ratio of 1:2, respectively, for induction of anesthesia. The solution was injected intravenously through the cephalic vein of the animals. In Group B, however, after premedication and sedation with atropine and xylazine HCl, induction and maintenance was done using, isoflurane (4%) (Forane®, Abbott, Pakistan) inhalation anesthesia having oxygen flow of 1L/min. Ovariohysterectomy was performed in each animal, and blood samples were collected from the cephalic vein at time 0 min (before the surgery), 10 min, 20 min and 30 min during the surgery, and after the surgery, respectively. The blood samples were analyzed using automated hematological analyzer (Abacus Junior Vet 5, Diatron, Austria) for complete biochemical profile including renal function tests (creatinine, blood urea nitrogen (BUN)) and liver function tests (serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)). Cardiovascular hemodynamics was, likewise, assessed using cardiovascular monitor for blood pressure, heart rate and saturation of oxygen (SpO_2) 10 min, 20 min and 30 min after administration of anesthesia.

Results and discussion

The selection of an appropriate anesthetic poses a considerable challenge for successful surgical intervention in rodents and rabbit, because numerous anesthetic agents and procedures described earlier for use in these species are ineffective, impractical or produce a high risk of anesthetic complications. This study showed the effects of xylazine-ketamine and isoflurane anesthesia on cardiovascular and serum biochemical parameters during ovariohysterectomy surgery in rabbits.

Table I.- Effect of xylazine-ketamine and isoflurane on mean arterial pressure (MAP) (mm Hg), heart rate (HR) (beats/min), partial pressure of oxygen (PO_2) (%), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and blood urea nitrogen (BUN) of rabbits undergoing ovariohysterectomy.

Parameters	Xylazine-ketamine group				Isoflurane group			
	0 min	15 min	30 min	45 min	0 min	15 min	30 min	45 min
MAP	170.00±2.67	153.75±2.63	163.75±2.27	161.25±1.83	171.25±2.95^{NS}	163.75±4.20^{NS}	172.50±1.64**	172.50±2.50**
HR	220.13±4.32	198.75±3.58	190.13±4.32	185.13±4.32	218.25±4.10^{NS}	200.13±3.76^{NS}	209.13±3.76**	201.75±3.45**
PO_2	98.25±0.89	97.75±1.28	98.88±0.35	99.00±0.00	97.50±0.53^{NS}	98.88±0.35*	98.63±0.52^{NS}	98.63±0.52^{NS}
ALT	77.61±4.97	93.98±1.88	91.50±1.98	88.50±1.98	77.85±4.46^{NS}	91.98±1.87^{NS}	89.43±2.02^{NS}	85.18±2.06**
AST	12.69±2.72	22.69±2.72	20.70±2.72	30.71±2.70	12.58±2.54^{NS}	18.82±2.61*	17.95±1.91	22.58±2.54**
Urea	15.75±2.38	20.92±2.31	23.54±2.43	22.15±1.86	16.88±1.64^{NS}	18.58±1.93*	19.39±1.80**	17.64±1.38**
BUN	15.75±2.38	21.05±2.28	23.54±2.43	22.15±1.86	16.88±1.64^{NS}	16.82±2.04**	15.53±1.19**	17.14±1.67**

**, Highly significant difference at $P \leq 0.010$.

Results showed decreased systolic blood pressure in both anesthetized groups (xylazine-ketamine, isoflurane) 5 and 10 min after injection. Inter-group comparisons showed significant ($P<0.05$) lower systolic blood pressure in the xylazine-ketamine group, as compared to the isoflurane group, 30 and 45 min after administration of anesthesia, respectively (Table 1).

Heart rate (HR) also decreased in both groups. However, 5 min after administration of anesthesia bradycardia was more pronounced ($P< 0.01$) isoflurane group compared to xylazine-ketamine group. While, after 30 and 45 min, group administered with isoflurane showed significant increase ($P<0.05$) in HR as compared to those administered with xylazine-ketamine. At 5 and 15 min isoflurane group of rabbits showed significantly higher ($P<0.05$) PO_2 as compared to those administered with xylazine-ketamine (Table 1).

The depression of cardiovascular functions during current study was attributed to be associated with depression of vasomotor center in the brain due to the effect of anesthetic agents, as also documented by Yershov *et al*. (2007). Similarly, the decrease in mean arterial pressure (MAP) and HR in the xylazine-ketamine treated group was attributed to peripheral vasodilation associated with depression of vasomotor center and bradycardia due to the alpha-2 adrenergic agonist effect of xylazine, as also narrated by Grubb *et al*. (1997). Although ketamine itself causes increase in HR and blood pressure, these changes associated with ketamine are due to the secondary stimulation of sympathetic nervous system (Grubb *et al*., 1997). Contrarily, depression of MAP and HR associated with isoflurane could be explained due to a reduction in the cardiac output and total peripheral resistance due to reduction in sympathetic tone (Constantinides *et al*., 2011). Likewise, the decrease in the value of sPO_2 in both groups was attributed to xylazine-associated depression of respiratory system (Offinger *et al*., 2012) and hypoxia during apnoiec period during isoflurane anesthesia (Hedenqvist, 2008). This hypoxic condition rebounded towards normal at 5, 15, 30 and 45 min, when oxygen was supplied along with isoflurane. In the isoflurane group, the sPO_2 values were significant at 5 and 15 min ($P\leq0.05$), respectively.

Serum ALT levels increased in both groups at 15, 30 and 45 min. However, the increase in ALT was more pronounced in xylazine-ketamine group as compared to the isoflurane group. Serum AST levels were significantly elevated ($P<0.05$) in the xylazine-ketamine group at 15 and 30 min, while at 45 min, the increase in ALT was highly significant, as compared to the isoflurane group. Likewise, serum ALP concentrations were high in both groups administered with xylazine-ketamine and isoflurane anesthesia. Bilirubin was significantly high ($P<0.05$) in both groups administered with xylazine-ketamine and isoflurane anesthesia. This rise in serum level is statistically non-significant.

Assessment of the biochemical profile depicted an increase in serum ALT and AST concentrations during induction of anesthesia in both groups; however, this increase was less pronounced and within normal range in the animals treated with isoflurane group as compared to those animals treated with xylazine-ketamine group. Significant increase ($P<0.05$) in ALT values in isoflurane group was attributed to xylazine-associated changes in blood pressure (hypertension followed by hypotension), as ketamine itself does not significantly alter hepatic blood flow (Gil *et al*., 2002). The increase in the values of ALP and bilirubin indicate that xylazine-ketamine may affect liver more as compared to isoflurane (Gil *et al*., 2002).

Serum BUN concentrations were significantly increased at 15 ($P\leq0.050$), 30, and 45 minutes ($P\leq0.010$), in the isoflurane group. Similarly, serum creatinine concentrations were significantly higher in isoflurane group at 15 min ($P\leq0.050$), 30 and 45 min ($P\leq0.010$), as compared to the xylazine-ketamine group.

The renal profile depicted significant increase ($P<0.05$) in serum creatinine and BUN concentrations at 15, 30 and 45 min, respectively. Alteration in the values of both variables, were hypothesized to occur due to changes in blood pressure after ketamine-xylazine injection, since it results in decreased renal blood flow, thereby decreasing the glomerular filtration rate and, consequently, raising BUN and creatinine levels (Gil *et al*., 2002). These changes were less pronounced with isoflurane anesthesia. Since, isoflurane has less blood solubility and causes less renal and hepatic injuries (Hikasa *et al*., 2000).

Conclusion

Despite extensive use of xylazine-ketamine injectable anesthetic combination in rabbits, isoflurane inhalation anesthesia can be opted as efficacious option during major surgical procedures, because of its fewer post-operative complications. Moreover, the findings of this study may confound the experimental results from rabbits treated with general anesthesia.

Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article**.**

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