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EFFECT OF IMMOBILIZATION AND ANAESTHESIA ON CERTAIN HAEMATO-BIOCHEMICAL PARAMETERS IN ASIATIC LIONS *(PANTHERA LEO PERSICA)*

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ABSTRACT

The study was conducted to assess the impact of chemical immobilization and anaesthesia on certain haematological and biochemical parameters in Asiatic lions intended for diagnostic and surgical procedure. The impact of anaesthetic and ancillary drugs was studied in 8 Asiatic and hybrid lions (*Panthera leo persica*) in 12 trials. In all the trials the lions were immobilized with xylazine and ketamine (1.00 and 2.50 mg/kg BW), induced with intravenous administration of ketamine (2.00 mg/kg) in trial I and propofol (1.00 mg/kg BW) in trial II and both trial I and II lions were maintained with isoflurane at a vaporizer setting of 0.5 to 2.00 in 100 oxygen. The mean haemoglobin and packed cell volume decreased significantly ($p < 0.01$) in both the trials when compared with the mean of after immobilization. Similarly leucocytosis and neutrophilia with corresponding lympopenia ($p < 0.01$) was noticed in both the trials after induction, during maintenance and before recovery and the trend simulated the "classical stress leucogram". The mean blood glucose and blood urea nitrogen level elevated ($p < 0.05$) in both the trials after induction, during maintenance and before recovery. No statistically significant variations could be observed in serum creatinine, ALT and AST. Though the haemato-biochemical parameters in Asiatic lions immobilized with xylazine-ketamine, induced with ketamine or propofol and maintained with isoflurane varied, it did not have clinical significance as the values remained within the normal clinical level.

INTRODUCTION

Anaesthetics and ancillary drugs and their metabolites induce alterations in haemato-chemical parameters, either by their direct or indirect actions (Kim *et al.,* 2013) which may have post-anaesthetic impact resulting in stress (Nermien Waly et al., 2013) morbidity and mortality (Cok *et al.,* 2011). The alterations in haemato-biochemical parameters could be due to peripheral vasodilatation as induced by acepromazine (Emory *et al.,* 2015), shift of interstitial fluids into circulating compartment as induced by xylazine (Stephen *et al.,* 2014), splenic sequestration of erythrocytes as induced by thiopentone sodium (Nermien Waly et al., 2013), reduction in renal blood flow as induced by thiamylal (Shiga *et al.,* 2003), direct hepatotoxic effect as induced by halothane (Kim *et al.,* 2013), respiratory and metabolic acid-base imbalance as induced by hypoventilation (Lak and Araghizadeh, 2009) and other associated causes. Further, sympathetic stimulation, decrease in blood perfusion, underlying diseases, sepsis, drug reactions, side effects and damage from surgeries (O'Connor *et al.,* 2010). It may not possible to assess the impact of immobilization, anaesthesia on vital organs and associated functional changes in wild felids during the post anaesthetic period and after recovery. Monitoring of haemato-biochemical parameters during chemical immobilization and anaesthesia is beneficial as biomarkers is to predict whether the changes are transient or changes that warrants initiation of suitable treatment before recovery from anaesthesia. Asiatic lion (*Panthera leo persica*) listed in schedule-I of the Wildlife Protection Act, 1972 and today approximately 250 Asiatic lions occupy a 1400 square kilometers area that includes the Gir Forest Sanctuary and the surrounding forest in the Gujarat State in Western India (O'Brien *et al.,* 1987). Chemical immobilization and anaesthesia is an integral component in conservation of lions in wild and captivity. Ketamine and tiletamine are commonly used anaesthetics for chemical immobilization and induction in lions, which often induce convulsions in lions. Ketamine has a shorter duration of action

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and has the disadvantages of poor muscle relaxation, excessive salivation. Alpha-2 adrenergic agonists are combined as adjunct to promote the analgesic action, muscle relaxation, increase the duration of immobilization and to reduce the dose of ketamine. Propofol is an alkyl phenol, ultra-short acting non-barbiturate, detoxified by tissue metabolism and readily induce reliable anaesthesia in one arm-brain circulation, which is to be administered necessarily through intravenous route. Administration of inhalant anaesthetic is the safest procedure for long term anaesthesia due to the merit of less stress, smooth and short recovery, ease in change of depth of anaesthesia and possibility of adopting resuscitative methods at times of emergency. Isoflurane is an inhalant agent used in veterinary anaesthesia, which induces minimal changes in cardiovascular and respiratory functions and advocated for long term anaesthesia as it is non-reactive with rubber and carbon dioxide canister (Murugan Bharathidasan *et al.,* 2016).
In the present study the magnitude of alteration in certain haemato-biochemical parameters induced following xylazine-ketamine immobilization, ketamine or propofol induction and maintenance with isoflurane was studied in Asiatic and hybrid lions.

MATERIALS AND METHODS

Ethical committee Approval

Ethical committee approval was not required as the case reported in this article was a clinical case as the lions included in the study were clinical cases referred for diagnostic and surgical procedures. However the study was conducted in accordance with Indian Wild Animal Protection Law 1972, amended in 1992. All the procedures were approved by the Director of Clinics and Dean, Faculty of Veterinary Science, Tamil Nadu Veterinary and Animal Sciences University.

Study population and trials

The study was conducted on 8 Asiatic lions (Panthera leo persica) and hybrid lions housed in Arignar Anna Zoological Park, Vandalur, Chennai which were referred for radiography of hip, thorax, digits and tibia, wound dressing and bandaging and surgical procedures like vasectomy, tail amputation and phacoemulsification and extraction of cataract lens to the Department of Veterinary Surgery and Radiology, Madras Veterinary College Teaching Hospital for diagnostic and surgical procedures. The lions were maintained in the Zoological Park. Previous history of any ailment, deworming schedule and vaccination schedule were taken into account. The weight of the lions ranged from 90 to 145 kg. A total of 12 trials were conducted in the 8 lions and the trials were allotted to treatment I and II irrespective of sex of the animals and care was taken not to allot the same lion repeatedly to the same trial.

Immobilization and Anaesthesia

The lions were confined in cages 24 hours prior to the procedure and feed was withheld for 12 hours and free access to water was allowed (Epstein *et al.,* 2002). All the lions were immobilized with a mixture of xylazine hydrochloride (1.00 mg/kg) and ketamine hydrochloride (2.50 mg/kg), intramuscularly on the upper hind limb or shoulder area using blow gun (Wenger *et al.,* 2010) (Fig.1). If required, incremental doses of xylazine and ketamine were administered to achieve recumbency and immobilization characterized by absence of ear flick reflex (Bharathidasan *et al.,* 2014). The immobilized lions were blindfolded and the fore limbs were hobbled (Kock and Burroughs, 2012) (Fig. 2). Intravenous catheters (18 gauges) were fixed either in cephalic or saphenous vein. The actual weights of the lions were recorded and the lions were transported to the Zoo Veterinary Hospital for further procedures.

In trial I, the immobilized lions were induced with the intravenous administration of ketamine at the rate of 2.00 mg/kg body weight to achieve sufficient jaw muscle relaxation. In trial II, the immobilized lions were induced with the intravenous administration of propofol at the rate of 1.00 mg/kg body weight sufficient to achieve jaw muscle relaxation. The lions were positioned in sternal recumbency and the neck was extended cranially. The jaws were opened with the help of straps sufficient to open and visualize the glottis (Fig. 3) and the lions were intubated by either by visualization of glottis or by digital palpation of the glottis (Murugan Bharathidasan *et al.,* 2016) (Fig. 4) which situated between 5^{th} to 6^{th} cervical vertebrae. Isoflurane was used as maintenance agent in both the trials with the vaporizer setting between 0.5 to 2 per cent and100 per cent oxygen was used as carrier gas. The flow rate was maintained at the rate of 15 ml/kg body weight as tidal volume. Anaesthesia was maintained to complete the procedure and monitored throughout the procedure.

Haemato-biochemical parameters

Venous blood samples were collected after immobilization, after induction, during maintenance and before release into the cage (recovery) and compared between trials. The haematological parameters assessed were total erythrocyte count (millions/cu.mm), total leucocyte count (thousands/cu.mm), packed cell volume (per cent) and differential count in per cent (Kakel, 2013). The biochemical parameters estimated were blood urea nitrogen (BUN) in mg/dl, creatinine in mg/dl, blood sugar in mg/dl, total serum protein (TSP) in g/dl, alanine aminotransferase (ALT) in IU/L and aspartate aminotransferase (AST) in IU/L (Erasmus, 2008).

RESULTS

Haematological parameters

The results of haemato-biochemical parameters were tabulated in Table-1. The mean erythrocyte count decrease significantly ($p < 0.01$) after induction, during maintenance and before recovery when compared with after immobilization in both the trials. Similarly the mean packed cell volume decreased after induction, during anaesthesia and before recovery in both the trials but the decrease was not significant ($p > 0.01$). The mean neutrophil per cent revealed significant increase after induction, during maintenance and before recovery significantly ($p < 0.01$) in both the trials. Correspondingly the mean leucocyte count decreased significant ($p < 0.01$) in both

the trials when compared with the mean of after immobilization. The mean percentage of eosinophil fluctuated between 1.53±0.21and 6.00±0.58 and basophil between 0.95±0.05 and 1.56±0.53 in both the trials at various stages of study intervals.

Biochemical Parameters

The mean blood glucose level revealed significant ($p < 0.05$) increase in both the trials after induction, during maintenance and before recovery when compared with the mean of after immobilization in both the trials.

Table 1. Mean ±SE of Haemato-biochemical Parameters in Trial I and II

Parameters	Trials	After Immobilization	After Induction	During Maintenance	Before Recovery	F – value
Total erythrocyte count ($x10^6/mm^3$)	I	7.93^{bc}±0.19	6.95^{a}±0.24	7.27^{a}±0.20	7.37^{ab}±0.23	3.313**
	II	8.02^{c}±0.45	6.87^{a}±0.13	6.97^{a}±0.24	7.18^{a}±0.08	
Total leucocyte count ($x10^3/mm^3$)	I	11.67^{a}±0. 93	13.83^{bc}±0. 36	14.20^{c}±0.29	14.27^{c}±0.11	5.214*
	II	12.60^{ab}±0.57	14.43^{c}±0.19	14.40^{c}±0.15	13.77^{b}±0.27	
Packed cell volume (%)	I	44.00±3.52	41.83±3.54	40.83±2.501	41.50±2.26	0.427
	II	45.00±3.87	41.67±2.69	36.33±2.17	40.17±1.83	
Neutrophil (%)	I	65.67^{a}±1.56	68.33^{ab}±1.92	70.83^{b}±2.26	70.67^{b}±2.23	2.940*
	II	62.83^{a}±1.64	68.17^{a}±2.36	72.17^{bc}±2.29	74.67^{c}±2.65	
Lymphocyte (%)	I	27.83^{c}±0.79	25.33^{ab}±0.42	24.50^{a}±0.96	24.83^{a}±0.60	7.409*
	II	29.33^{c}±0.95	27.33^{bc}±0.61	23.83^{a}±0.40	24.17^{a}±0.91	
Eosinophil (%)	I	5.00^{bc}±0.97	5.00^{bc}±0.37	4.00^{b}±0.45	4.00^{b}±0.00	7.064*
	II	6.00^{c}±0.58	4.00^{b}±0.37	4.00^{b}±0.37	1.53^{a}±0.21	
Basophil (%)	I	1.50±0.39	1.23±0.43	1.33±0.49	1.32±0.17	0.395
	II	1.56±0.53	1.00±0.18	1.00±0.45	0.95±0.05	
Blood glucose (mg/dl)	I	92.17^{ab}±3.77	98.00^{b}±3.64	101.67^{c}±3.07	103.17^{c}±1.62	3.171*
	II	89.00^{a}±3.08	99.17^{bc}±13.27	101.67^{c}±1.05	101.50^{c}±2.14	
Blood urea nitrogen (mg/dl)	I	33.67^{a}±1.36	35.17^{a}±1.50	36.17^{a}±1.33	37.83^{b}±1.47	1.637*
	II	34.67^{a}±0.95	35.33^{a}±0.95	37.00^{ab}±1.12	38.17^{b}±1.05	
Creatinine (mg/dl)	I	2.17±0.39	2.27±0.45	2.22±0.43	2.42±0.44	0.392
	II	2.12±0.27	2.12±0.29	2.30±0.49	2.25±0.37	
ALT (U/L)	I	38.17±2.32	37.67±1.52	38.33±1.56	38.33±2.81	0.077
	II	37.50±2.37	38.67±1.33	37.00±1.51	37.50±2.33	
AST (U/L)	I	31.00±3.06	31.50±2.19	31.33±1.96	32.50±2.25	0.054
	II	31.00±2.95	32.17±2.07	31.83±1.92	31.83±2.64	
Total serum protein (g/dl)	I	8.00±0.58	6.83±0.54	6.50±0.50	6.83±0.31	1.674
	II	8.00±0.45	7.17±0.48	6.50±0.50	6.83±0.31	

Means bearing different superscripts in a parameter differ significantly
** Highly significant ($p < 0.01$);
* Significant ($p < 0.05$)

Fig. 1. Asiatic lioness darted on the upper hind limb

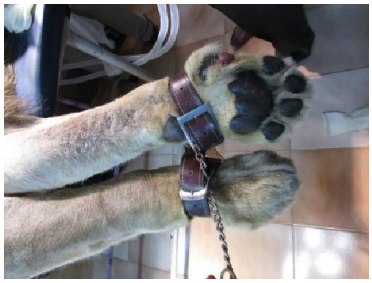

Fig. 2. Asiatic lion hobbled after immobilization

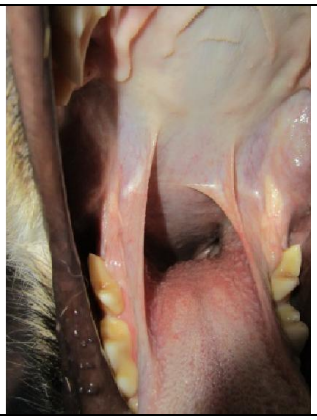

Fig. 3. Visualization of larynx in Asiatic lion

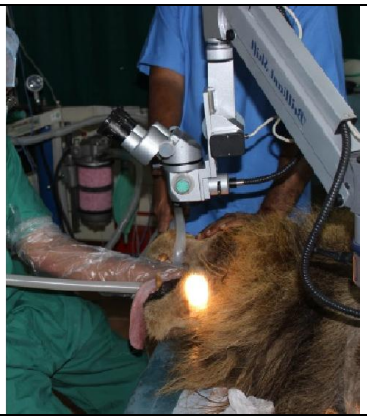

Fig. 4. Digital palpation of glottis and intubation in a lion intended for ocular surgery

The mean blood urea nitrogen before recovery elevated significantly ($p < 0.05$) in both the trials when compared with the other stages of the study. The mean creatinine level fluctuated between 2.12 and 2.42 mg/dl in both the trials at different stages of the study without significant variations ($p > 0.05$). The mean ALT level fluctuated between 3.77.00 and 38.33 U/L in both the trials without significant ($p > 0.05$) variation at different stages of the study. The mean AST also fluctuated between 31.00 and 32.50 U/L at different stages of the study without statistical significance ($p > 0.05$). The mean TSP level decreased after induction, during maintenance and before recovery in both the trials, but the variations were not statistically ($p > 0.05$).

DISCUSSION

Haematological parameters

The normal erythrocyte count and packed cell volume reported for African lion were $8.97x10^6/mm^3$ and 42.38 per cent in African lions (*Panthera leo*) (Maria *et al.,* 2015) and the values recorded in Asiatic lions ranged between 7.93 and $8.02x10^6/mm^3$ and 36.33 and 45.00 per cent which was comparable with the present values. However the mean erythrocyte count and packed cell volume reduced which could be attributed to pooling of erythrocyte in the spleen as a result of adrenocortical stimulation (Nermien Waly *et al.,* 2013) and migration of interstitial fluid into the circulating compartment due to the actions of xylazine, ketamine, propofol and isoflurane (Ali, 2005 and Behera et al., 2013). The study revealed that the anaesthetics and ancillary drugs did not have significant impact on erythrocyte and packed cell volume as the values were within the clinical limits. The total leucocyte count increased in both the trials of lions after induction, during maintenance and before recovery and the mean values fluctuated between 11.67±0.93 and 14.43±0.19 $x10^3/mm^3$. The leucocyte count recorded in African lion was 9.37 $x10^3/mm^3$ (Maria *et al.,* 2015). The mean percentage of neutrophil and lymphocyte recorded in African lions were also fluctuated between 62.83±1.64 and 74.67±2.65 with significant increase after induction, during maintenance and before recovery. The lymphocyte percentage decreased after induction, during maintenance and before recovery and the mean percentages fluctuated between 23.83±0.40 and 27.83±0.79. The values were comparable with the neutrophil and lymphocyte values recorded for African lions (Maria *et al.,* 2015). Perusal of mean values indicated significant ($p < 0.01$) leucocytosis and neutrophilia with corresponding lympopenia and the trend simulated the "classical stress leucogram". Cortisol produced marked neutrophilia which usually occurred without increase in band and other immature forms. The neutrophilia resulted from increased influx of neutrophils from the bone marrow storage and decreased migration of neutrophils from the circulation into the tissues. The findings of the present study concurred (Ali, 2005; Behera *et al.,* 2013).

Biochemical parameters

The biochemical constituents and enzymes of African lion (*Panthera leo*) reported were 76.06 ± 3.20 to 81.83 ± 4.53 mg/dl glucose, 7.77 ± 0.14 to 7.90 ± 0.14 g/dl total protein, 137.09 ± 12.10 to 145.54 ± 6.56 mg/dl urea, 1.48 ± 0.06 to 1.63 ± 0.14 mg/dl creatinine, 17.40 ± 1.87 to 20.02 ± 2.20 IU/L aspartate aminotransferase and 12.39 ± 1.35 to 13.76 ±1.78 IU/L alanine aminotransferase (Behera *et al.,* 2013). The normal biochemical values in clinically healthy 44 Asian lions and reported were 95.04 ± 4.27 mg/dl of blood glucose, 32.14 ± 2.52 mg/dl of blood urea nitrogen, 2.35 ± 0.11 mg/dl of creatinine (Jani and Sabapara, 2010). In the present study the mean glucose level in mg/dl increased to 103.17±1.62 in lions induced with ketamine and 101.50±2.14 in lions induced with propofol. Increase in blood glucose level could be attributed to increase level of glucocortical hormone, inhibition of insulin secretion, decreased membrane transport of glucose, decreased renal excretion and decreased glucose utilization (Muir and Hubell, 1988) due to the effect of xylazine, ketamine, propofol and isoflurane. Reduction ($p > 0.05$) in mean TSP level in both the trials after induction, during maintenance and before recovery which could be attributed to migration of interstitial fluids into the circulation (Ali, 2005; Behera *et al.,* 2013; Jani and Sabapara, 2010). The mean ALT values in the present study fluctuated between 31.00±3.06 and 32.50±2.25 IU/L and AST between 37.00±1.51 and 38.33±2.81 IU/L ALT respectively without any significant difference, indicating no interference to hepatic blood flow and lack of insult to hepatocytes and muscle fibres (Jani and Sabapara, 2010).

Conclusion

Haemato-biochemical parameters in Asiatic lions immobilized with xylazine-ketamine at the dose of 1.00 and 2.5 mg/kg intramuscularly, induced with ketamine at a dose of 2.00 mg/kg intravenously or propofol at a dose rate of 1.00 mg/kg and maintained with isoflurane at a vaporizer setting between 0.5 to 2.00 per cent induced alterations suggesting haemodilution, hyperglycaemia, leucocytosis and neutrophilia with corresponding lymphopenia; the alterations are within clinical limits.

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REFERENCES

Ali, M. 2005. Propofol or ketamine as maintenance agents in xylazine-ketamine induced captive lions. *M. V. Sc* thesis submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.

Behera, P. C., Dash, D., Senapati, M. R., Bisoi P. C. and Parija, S. C. 2013. Effect of sex and season on serum biochemical constituents and enzymes of *Panthera leo* of Nandankanan Zoological Park. *IOSR J. Agri. Vet. Sci*., 4(2): 68-72.

Bharathidasan, M., Thirumurugan, R., William, B. J., George, R. S., Arunprasad, A., Kannan T. A. and Viramuthu, S. 2014. Xylazine-ketamine immobilization and propofol anaesthesia for surgical excision of sebaceous adenoma in a jaguar (Panthera onca). *Vet World,* 7(11): 986-990.

Cok, O.Y.O.Z., Pasaoglu, H. and Yardim, S. 2011. Glucose response during craniotomy: Propofol-remifentanil versus isoflurane-remifentanil. *Minerva. Anesthesiol.,* 77 (12): 1141-8, 2011.

Epstein, A., White, R., Horowtiz, I.H., Hass P.H., and Ofri, R. 2002. Effects of propofol as an anaesthetic agent in adult lions (*Panthera leo*): a comparison with two established protocols. *Res. Vet. Sci.,* 72: 137-140.

Erasmus, H. L. 2008. Determination of some blood parameters in the African lion (*Panthera leo*). Magister Scientiae in Agriculturae, Dissertation submitted to University of Free State, Bloemfontein.

Hélène Amory, Brieuc de Moffarts, Valeria Busoni, Didier Serteyn and Charlotte Sandersen, 2015. Evaluation of acepromazine-induced hemodynamic alterations and reversal with norepinephrine infusion in standing horses. *Can. Vet. J.*, 54(2): 150–156.

Jani, R. G. and Sabapara, R. H. 2010. Serum biochemical study of major elements of captive Asiatic Lions (*Panthera leo persica*). *J. Threat. Taxa,* 2(2): 724.

Ji Wook Kim, Joo Duck Kim, Soo Bong Yu and Sie Jeong Ryu. 2013. Comparison of hepatic and renal function between inhalation anesthesia with sevoflurane and remifentanil and total intravenous anesthesia with propofol and remifentanil for thyroidectomy. *Korean J. Anesthesiol.,* 64(2): 112-116.

Kakel, S. J. 2013. The evaluation of traditional and automatic Coulter method in estimation of haematological parameters in adult rats. *Beni-Suef Uni. J. Basic Applied Sci*., 2(1): 31-35.

Kock, D. and Burroughs, R. 2012. Chemical and physical restraint of wild animals: A training and field manual for African species. Edn.II. IWVS, South Africa.

Lak, M. and Araghizadeh, H. 2009. Acid-Base Disturbances and Related Factors in Patients under General Anesthesia. *Iranian Red Crescent Medical Journal (IRCMJ)* 11(1):28-31.

Maria Helena Matiko, Akao Larsson Patrícia, Lourenzon do Espírito Santo, Regina Mieko Sakata Mirandola, José Daniel Luzes Fedullo, Fumio Honma Ito, Paula Hiromi Itikawa and Rebecca Bastos Pessoa, 2015. Hematologic Parameters of Captive Lions (*Panthera leo*) and Siberian Tigers (*Panthera tigris altaica*) *Acta Scientiae Veterinariae*, 43: 1311.

Muir, W. W. and Hubell, J. A. E. 1988. Cardiopulmonary and anaesthetic effect of ketamine and its enantiomers. *Am. J. Vet. Res*., 49: 530-534.

Murugan Bharathidasan, Benjamin Justin William, Ramamurthy Jayaprakash, Thandavan Arthanari Kannan, Rajarthanam Thirumurugan and Ravi Sundar George. 2016. Immobilization and Anaesthesia in Asiatic Lions (*Panthera leo persica*). *AAVS* 4 (3) 134-144.

Nermien Waly, Waleed S.H. Farrag and Ashraf Hasaballa. 2013. Alteration of Hematological and Plasma Physiological Parameters Following a Standard Protocol of General Anesthesia: A Pilot Study in Assiut University Hospitals Med., *J. Cairo Univ*., 81 (1)797-800,

O'Brien, S. J., Joslin, P., Smith, G. L., Wolfe, R., Schaffer, N., Heath, E., Joslin, J. O., Rawal, P. P., Bhattacharjee, K. K. and Martenson, J. S. (1987). Evidence for African origins of founders of the Asiatic lion species survival plan. *Zoo Biol.,* 6: 99-116.

O'Connor, C. J., Rothenberg, D.M. and Tuman, K.J. 2010. Anesthesia and the hepatobiliary system. In: Miller's Anesthesia. 7th ed. Edited by Miller RD: Philadelphia, Elsevier Churchill Livingstone., pp 2135-49.

Shiga, Y., Minami, K., Uezono, Y., Segawa, K., Nagaoka, E., Shiraishi, M., Noguchi, T. and Shigematsu, A. 2003. Effects of the intravenously administered anaesthetics ketamine, propofol, and thiamylal on the cortical renal blood flow in rats. *Pharmacol*., 68(1):17-23.

Stephen B. Hladky and Margery A. Barrand, 2014. Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. *Fluids and Barriers of the CNS*., 11, 2-32.

Wenger, S., Buss, P., Joubert, L., Steenkamp, J., Shikwamb, P. and Hatt, J. M. 2010. Evaluation of butorphanol, medetomidine and midazolam as a reversible narcotic combination in free-ranging African lions (*Panthera leo*). *Vet. Anaesth. Analg*., 37(6): 491-500.

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