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Effects of ketamine-midazolam and tiletamine-zolazepam as induction and isoflurane as maintenance anaesthetic agents on clinico-physiological and haemato-biochemical parameters in cats

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Abstract
The comparative anaesthetic study of ketamine-midazolam and tiletamine-zolazepam as induction and isoflurane as maintenance anaesthesia on clinico-physiological and haemato-biochemical parameters was conducted in eighteen clinical cases of cats requiring general anaesthesia and were randomly assigned into two groups (n=9 each). Anaesthesia was induced by intramuscular injection of ketamine @ 10 mg/kg b.wt. along with midazolam @ 1 mg/kg b.wt. in group I and tiletamine @ 5 mg/kg b.wt. along with zolazepam @ 5 mg/kg b.wt. in group II. After achieving desired depth of induction anaesthesia, animals were shifted on inhalation anaesthesia using isoflurane with 100% oxygen through face mask. Tiletamine-zolazepam anaesthetic combination produced minimum fluctuation of clinico-physiological and haemato-biochemical parameters as compared to ketamine-midazolam combination in cats.

Keywords: Cat, ketamine, midazolam, tiletamine-zolazepam, isoflurane, clinical-physiological, haemato-biochemical parameters

Introduction
Felines tend to be wild in nature hence, they are difficult to handle by physical method of restraining for routine clinical examination which increases the stress and decreases the well-being of handling personnel (Dugassa and Fromsa, 2018) [1]. They require sedation or general anaesthesia even for minor clinical procedures such as radiography, oral examination, blood collection or echocardiography (Moffat, 2008) [2]. Ketamine as dissociative anaesthetic drug is most commonly used in cats for general anaesthetic agent since long time (Shindle and Tewes, 2000) [3]. Tiletamine is new long-acting dissociative anaesthetic agent which is more potent than ketamine (Dugassa and Fromsa, 2018) [1].
Common complications of dissociative anaesthetics are catalepsy, convulsion, tachycardia and hypertension due to sympathetic nervous system stimulation which substitute by benzodiazepine. They provide sedative, anxiolytic, muscle relaxant, hypnotic and anticonvulsant effects (Riss *et al.*, 2008) [4] and (Reed *et al.*, 2019) [5]. Midazolam is a water-soluble benzodiazepine and has rapid onset of action, good absorption, minimum cardiovascular effect and rapid elimination after intramuscular injection (Ebner *et al.*, 2007) [6]; while, zolazepam has similar effect of midazolam, but it is 5-10 times more potent than midazolam and available in combination with tiletamine. Plasma half-life of zolazepam in cats is longer than tiletamine which leads to prolong and smooth recovery (Lin *et al.*, 1993) [7]. Isoflurane and sevoflurane are the most commonly used inhalant anaesthetics in small animal anaesthesia as it has less myocardial depressant properties along with short induction and recovery times (Altug *et al.*, 2009) [8]. Present anaesthetic study was undertaken to study comparative effect of ketamine-midazolam and tiletamine-zolazepam as induction and isoflurane as maintenance anaesthetic effect on clinico-physiological and haemato-biochemical parameters in cats.

Materials and Methods
Eighteen clinical case of cats requiring general anaesthesia for surgical protocols were randomly divided into two different anaesthetic groups (n=9 each) irrespective of age, breed, sex, body weight and surgical procedure. All animals were kept off feed for 12 hour and water was withheld for 6 hours prior to surgical interventions.

[1]Ketamine @ 10 mg/kg b.wt. along with [2]midazolam @ 1 mg/kg b.wt. was administered intramuscularly in group I and [3]tiletamine @ 5 mg/kg along with [3]zolazepam @ 5 mg/kg b.wt. was administered intramuscularly in group II. After achieving desired depth of induction anaesthesia, anaesthesia was maintained with [4]isoflurane using face mask in all the animals. Clinico-physiological parameters *viz.*; rectal temperature (ºF), pulse rate (beats/min), respiration rate (breaths/min), saturation of peripheral oxygen (%), systolic, diastolic and mean arterial blood pressure (mm Hg) were recorded prior to administration of any anaesthetic drug, 10th minutes after administration of anaesthetic combination and thereafter at every 10 minutes interval up to 50 minutes during maintenance anaesthesia using multipara monitor. Blood samples were collected prior to administration of any anaesthetic drug, 20th and 40th minutes after administration of anaesthetic combination. Haematological parameters *viz.*; haemoglobin (g/dl), packed cell volume (%), total erythrocyte count (million/cu.mm), total leucocyte count (thousand/cu.mm) and differential leucocyte count (%) were estimated using automatic haemato-analyzer. Blood glucose (mg/dl) was estimated using glucometer immediately after the collection of blood. Biochemical parameters *viz.*; total protein (g/dl), alanine amino transferase (IU/L), blood urea nitrogen (mg/dl) and creatinine (mg/dl) were quantified by using semi-automatic biochemical analyzer by standard diagnostic kits. Obtained statistical data were analyzed using R software version 4.0.3 to estimate the means and standard errors. Means were compared using analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT).

Results and Discussion

Mean values of rectal temperature were initially increased at 10th minute after administration of anaesthetic combination and thereafter gradually decreased throughout the observation period in both the groups. Highly significant difference in mean values of rectal temperature was observed within groups; whereas, non-significant difference was observed in between the groups at different time intervals (Table 1). Rectal temperature was decreased in the present study might be due to depression of the thermoregulatory center, reduction in basal metabolic rate and peripheral vasodilation (Bindu *et al.*, 2017) [9].

Mean values of pulse rate, systolic, diastolic and mean arterial pressure were initially increased after administration of anaesthetic combination and thereafter decreased throughout the maintenance period in both the groups. Highly significant difference was observed in mean values of pulse rate from 30th to 50th minute of administration of anaesthetic combination in between groups (Table 1). Similarly, increase in values of pulse rate was reported by Mosallanejad *et al.* (2020) [10]. Highly significant difference was observed in systolic and mean arterial pressure between 20th to 50th minute of observation period in between the groups; while, highly significant difference was observed in pulse rate, systolic, diastolic and mean arterial blood pressure within group in both the groups. Initial increase in pulse rate, systolic, diastolic and mean arterial blood pressure were observed after administration anaesthetic combination might be due to sympathetic nervous system stimulation as reported by Reed *et al.* (2019) [5].

Mean values of respiration rate were decrease; whereas, mean values of saturation of peripheral oxygen were increased throughout the observation period. No statistical difference was observed between groups; whereas, highly significant difference was observed within both the groups (Table 1). Similarly, decrease in respiration rate was reported by Mosallanejad *et al.* (2020) [10] after ketamine-midazolam and Nejamkin *et al.* (2020) [11] after tiletamine-zolazepam administration in cats which might be due to respiratory depressant effect of isoflurane and midazolam as reported by Heidari *et al.* (2017) [12]. Similar results were found by Akkerdaas *et al.* (2001) [13] and Bhowmik (2016) [14] in isoflurane-maintained cats. Increase in SpO_2 might be due to animals were shifted on inhalant anaesthesia using isoflurane with 100% oxygen through face mask.

Haemoglobin, packed cell volume and total erythrocytes count values were decreased after administration of anaesthetic combination in both the groups. Highly significant difference was observed in haemoglobin and packed cell volume in within group I; while, total erythrocyte count differed highly significantly within the groups (Table 2). Similar results were observed by Heidari *et al.* (2017) [12] after ketamine-midazolam and Spada *et al.* (2015) [15] after tiletamine-zolazepam administration in cats. Decrease in haemoglobin, packed cell volume and total erythrocyte count might be due to vasodilatation by general anaesthesia leading to shifting of fluid from extravascular to intravascular compartment to maintain normal cardiac output as reported by Hareesh (2016) [16]. No significant difference was observed in between the groups in above parameters. Total leucocyte count, neutrophils, monocytes values were decreased; whereas, lymphocytes and eosinophils values were increased after administration of anaesthetic combination at different time intervals in both the groups. Alternation in values of total leucocyte count, neutrophils and monocytes might be due to effect of glucocorticoid on blood cells or pooling of circulating blood cells in the spleen as reported by Nara *et al.* (1979) [17].

Blood glucose values were increased significantly at 20th minute followed by decrease at 40thminute after administration of anaesthetic combination in both the groups (Table 3). Similar findings were record by Heidari *et al.* (2017) [12] after ketamine-midazolam administration in cats. Anaesthetic as well as surgical stress stimulate adrenal grand to secrete glucocorticoid which in turn rises blood glucose levels as reported by Duggan *et al.* (2017) [18]. Total protein values were decreased after administration of anaesthetic combinations in both the groups. Highly significant difference was observed within group I. Decrease in ALT value might be due to effect on total hepatic blood flow resulting in lower production of liver enzymes as reported by Thomson *et al.* (1986) [19]. Blood urea nitrogen and creatinine levels were increased after administration of anaesthetic combination in both the groups. Blood urea nitrogen level showed highly significant difference; whereas, creatine showed significant difference within both the groups (Table 3). Changes in the levels of blood urea nitrogen and creatinine were observed due to temporary effect of anaesthetic drug on renal blood flow which leads to decrease in glomerular filtration rate and changes in cardiovascular blood supply as reported by Riviere and Papich (2018) [20]. Changes in haemato-biochemical parameters were within normal

[1] Ketamine: Inj. Zokent, Ketamine 50 mg/ml, Miracalus Pharma Ltd., Mumbai, Maharashtra.
[2] Midazolam: Inj. Napro-Mida-1, Midazolam 1 mg/ml, Naprod Life Science Pvt. Ltd., Thane, Maharashtra.
[3] Tiletamine+Zolazepam: Inj. Zoletil 50, Tiletamine and Zolazepam 50 mg/ml, Virbac S.A., Carros, France.
[4] Isoflurane: Isotroy ®30, 30 ml, Troikaa pharmaceuticals Ltd., Thol, Gujarat.

physiological range in both groups.

Tiletamine-zolazepam produced better stability with minimum fluctuation on clinico-physiological and haemato-biochemical parameters than ketamine-midazolam in cats and it is safer anaesthetic protocol for longer duration of surgical interventions in cats.

Table 1: Mean±SE values of clinico-physiological parameters at different time interval in cats

Parameters	Group	0 minute (Baseline)	Time interval (After administration of anaesthetic combination)					*p-value*
			10th minute	20thminute	30thminute	40thminute	50thminute	
Rectal temperature (F°)	I	100.84±0.59$^{a}_{A}$	101.22±0.59$^{a}_{A}$	100.00±0.43$^{ab}_{A}$	98.91±0.32$^{bc}_{A}$	98.09±0.35$^{c}_{A}$	97.28±0.33$^{c}_{A}$	0.0001
	II	100.94±0.33$^{ab}_{A}$	101.53±0.56$^{a}_{A}$	99.69±0.61$^{abc}_{A}$	99.11±0.59$^{bc}_{A}$	98.11±0.55$^{c}_{A}$	97.51±0.43$^{c}_{A}$	0.0001
	p-value	0.88	0.70	0.68	0.77	0.97	0.68	
Pulse rate (beats/min)	I	186.89±15.28$^{ab}_{A}$	214.89±15.64$^{a}_{A}$	198.33±13.20$^{ab}_{A}$	177.00±7.66$^{ab}_{B}$	164.11±5.37$^{b}_{B}$	152.11±5.19$^{b}_{B}$	0.003
	II	189.33±7.38$^{bc}_{A}$	222.44±6.17$^{a}_{A}$	205.11±5.39$^{ab}_{A}$	196.33±2.89$^{bc}_{A}$	186.67±2.38$^{bc}_{A}$	176.00±2.60$^{c}_{A}$	0.0001
	p-value	0.88	0.65	0.64	0.001	0.001	0.0008	
Respiration rate (breaths/min)	I	58.78±0.62$^{a}_{A}$	35.44±2.95$^{b}_{A}$	29.11±1.98$^{bc}_{A}$	26.00±1.56$^{c}_{A}$	22.67±0.75$^{c}_{A}$	22.11±2.12$^{c}_{A}$	0.0001
	II	56.44±5.30$^{a}_{A}$	42.22±4.33$^{b}_{A}$	28.11±2.65$^{c}_{A}$	26.67±1.89$^{c}_{A}$	24.11±2.35$^{c}_{A}$	20.00±1.77$^{c}_{A}$	0.0001
	p-value	0.66	0.21	0.76	0.78	0.56	0.45	
Saturation of peripheral oxygen (SpO_2)	I	94.11±1.44$^{b}_{A}$	97.22±0.28$^{a}_{A}$	98.00±0.24$^{a}_{A}$	98.33±0.17$^{a}_{A}$	98.56±0.24$^{a}_{A}$	98.78±0.15$^{a}_{A}$	0.0001
	II	94.00±1.09$^{b}_{A}$	97.44±0.63$^{a}_{A}$	98.11±0.20$^{a}_{A}$	98.11±0.11$^{a}_{A}$	98.67±0.17$^{a}_{A}$	98.89±0.11$^{a}_{A}$	0.0001
	p-value	0.95	0.74	0.72	0.72	0.10	0.55	
Systolic blood pressure (mm Hg)	I	133.00±2.57$^{bc}_{A}$	158.33±3.39$^{a}_{A}$	142.33±2.42$^{b}_{B}$	127.11±1.78$^{cd}_{B}$	120.44±1.82$^{d}_{B}$	109.56±1.98$^{e}_{B}$	0.0001
	II	135.78±4.86$^{cd}_{A}$	163.22±2.99$^{a}_{A}$	157.00±2.98$^{ab}_{A}$	147.00±3.09$^{bc}_{A}$	139.44±3.08$^{cd}_{A}$	127.11±3.49$^{d}_{A}$	0.0001
	p-value	0.62	0.29	0.0015	0.0001	0.0001	0.0005	
Diastolic blood pressure (mm Hg)	I	88.89±2.81$^{b}_{A}$	99.89±1.90$^{a}_{A}$	92.22±1.98$^{b}_{A}$	89.00±1.98$^{bc}_{A}$	85.67±1.08$^{bc}_{A}$	84.67±0.65$^{c}_{A}$	0.0001
	II	92.33±5.42$^{bc}_{A}$	98.67±1.31$^{a}_{A}$	94.89±0.92$^{ab}_{A}$	91.44±1.33$^{bcd}_{A}$	87.11±2.12$^{cd}_{A}$	86.00±1.09$^{d}_{A}$	0.0001
	p-value	0.90	0.60	0.23	0.32	0.55	0.30	
Mean arterial blood pressure (mm Hg)	I	105.56±2.09$^{bc}_{A}$	125.00±1.94$^{a}_{A}$	113.11±2.70$^{b}_{B}$	110.78±1.68$^{cd}_{B}$	102.56±0.71$^{d}_{B}$	99.89±0.39$^{e}_{B}$	0.0001
	II	105.78±2.17$^{c}_{A}$	131.56±2.39$^{a}_{A}$	126.22±1.88$^{ab}_{A}$	120.40±2.05$^{bc}_{A}$	117.56±1.61$^{cd}_{A}$	112.33±1.86$^{d}_{A}$	0.0001
	p-value	0.54	0.51	0.0002	0.0001	0.0001	0.0003	

Means bearing same subscripts between the groups differ non-significantly ($p > 0.05$)
Means bearing different subscripts between the groups differ highly-significantly ($p \leq 0.01$)
Means bearing different superscripts within the groups differ highly significantly ($p \leq 0.01$)

Table 2: Mean±SE values of haematological parameters at different time interval in cats

Parameters	Group	0 minute (Baseline)	Time interval (After administration of anaesthetic combination)		p-value
			20th minute	40th minute	
Haemoglobin (g/dl)	I	11.67±0.32^{a}A	10.33±0.39^{ab}A	9.44±0.54^{b}A	0.004
	II	11.50±0.76^{a}A	10.50±0.71^{a}A	9.56±0.69^{a}A	0.18
	p-value	0.84	0.83	0.90	
Packed Cell Volume (%)	I	35.00±0.97^{a}A	31.00±1.17^{ab}A	28.33±1.63^{b}A	0.004
	II	34.69±2.43^{a}A	31.50±2.12^{a}A	28.67±2.07^{a}A	0.18
	p-value	0.84	0.83	0.90	
Total Erythrocyte Count (million/cu.mm)	I	8.81±0.20^{a}A	7.77±0.23^{b}A	6.88±0.17^{c}A	0.0001
	II	8.61±0.26^{a}A	7.54±0.13^{b}A	7.17±0.09^{b}A	0.0001
	p-value	0.55	0.40	0.18	
Total Leukocyte Count (thousand/cu.mm)	I	17.86±0.64$^{a}_{A}$	15.05±0.60$^{b}_{B}$	13.12±0.39$^{b}_{B}$	0.0001
	II	17.66±1.20$^{a}_{A}$	17.27±1.24$^{a}_{A}$	16.43±0.69$^{a}_{A}$	0.10
	p-value	0.67	0.0013	0.0007	
Neutrophils (%)	I	42.00±0.83$^{a}_{A}$	40.44±0.73$^{a}_{A}$	37.67±0.58$^{b}_{A}$	0.001
	II	41.33±1.27$^{a}_{A}$	41.00±1.0$^{a}_{A}$	38.22±1.77$^{a}_{A}$	0.23
	p-value	0.66	0.65	0.76	
Lymphocytes (%)	I	53.78±1.09$^{a}_{A}$	54.00±1.05$^{a}_{A}$	54.44±1.68$^{a}_{A}$	0.93
	II	54.00±1.19$^{a}_{A}$	54.22±0.91$^{a}_{A}$	54.56±1.57$^{a}_{A}$	0.96
	p-value	0.89	0.87	0.63	
Monocytes (%)	I	3.11±0.54$^{a}_{A}$	2.67±0.17$^{a}_{A}$	2.22±0.36$^{a}_{A}$	0.28
	II	3.00±0.29$^{a}_{A}$	2.89±0.11$^{a}_{A}$	1.67±0.17$^{b}_{A}$	0.0005
	p-value	0.85	0.26	0.18	
Eosinophils (%)	I	1.78±0.28$^{b}_{A}$	3.33±0.24$^{a}_{A}$	4.00±0.33$^{a}_{A}$	0.0001
	II	1.67±0.37$^{c}_{A}$	3.22±0.40$^{b}_{A}$	4.67±0.33$^{a}_{A}$	0.0001
	p-value	0.81	0.81	0.23	

Means bearing same superscripts within the groups and subscripts between the groups differ non-significantly ($p > 0.05$)
Means bearing different subscripts between the groups differ highly-significantly ($p \leq 0.01$)
Means bearing different superscripts within the groups differ highly significantly ($p \leq 0.01$)

Table 3: Mean±SE values of biochemical parameters at different time interval in cats

Parameters	Group	0 minute (Baseline)	Time interval (After administration of anaesthetic combination)		p-value
			20th minute	40th minute	
Blood Glucose (mg/dl)	I	73.67±1.76$^{c}_{A}$	100.67±1.13$^{a}_{A}$	91.67±1.56$^{b}_{A}$	0.0001
	II	75.78±1.69$^{c}_{A}$	97.22±0.52$^{a}_{B}$	92.67±0.62$^{b}_{A}$	0.0001
	p-value	0.39	0.013	0.56	
Total Protein (g/dl)	I	6.19±0.05$^{a}_{A}$	6.03±0.08$^{b}_{A}$	5.96±0.07$^{c}_{A}$	0.0001
	II	6.11±0.06$^{b}_{A}$	5.99±0.08$^{ab}_{A}$	5.84±0.06$^{a}_{A}$	0.011
	p-value	0.36	0.71	0.26	
Alanine Amino Transferase (IU/L)	I	51.86±1.77$^{c}_{A}$	49.31±1.23$^{a}_{A}$	45.92±1.22$^{b}_{A}$	0.004
	II	53.33±1.44$^{c}_{A}$	46.94±2.82$^{a}_{A}$	46.43±0.89$^{b}_{A}$	0.003
	p-value	0.52	0.07	0.74	
Blood Urea Nitrogen (mg/dl)	I	15.43±0.08$^{c}_{A}$	15.93±0.13$^{a}_{A}$	16.11±0.27$^{b}_{A}$	0.001
	II	15.36±0.10$^{c}_{A}$	15.70±0.08$^{a}_{A}$	15.96±0.19$^{b}_{A}$	0.009
	p-value	0.55	0.051	0.64	
Creatinine (mg/dl)	I	1.24±0.01$^{c}_{A}$	1.31±0.03$^{b}_{A}$	1.39±0.04$^{a}_{A}$	0.036
	II	1.25±0.02$^{c}_{A}$	1.29±0.01$^{b}_{A}$	1.38±0.05$^{a}_{A}$	0.035
	p-value	0.62	0.48	0.81	

Means bearing same subscripts between the groups differ non-significantly ($p > 0.05$)
Means bearing different subscripts between the groups differ significantly ($p \leq 0.05$)
Means bearing different superscripts within the groups differ highly significantly ($p \leq 0.01$)
Means bearing different superscripts within the groups differ significantly ($p \leq 0.05$)

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