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Effects of Combinations of Medetomidine-Diazepam-Ketamine and Medetomidine-Ketamine Anesthesia on Haematology and Serum Biochemistry in Dogs

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ABSTRACT

This study was aimed at evaluating the effects of combinations of medetomidine-diazepam-ketamine and medetomidine-ketamine anesthesia haematology and serum chemistry in dogs. Ten (10) apparently healthy local dogs comprising of five (5) females and five (5) males with Mean ± SD body weight of 20.4 ± 2.93 kg sought from Maiduguri and environs and randomly allocated into two groups A (n=5), B (n=5) were used to conduct the experiment for this study. Total Intravenous anesthesia (TIVA) was induced in all the dogs. Dogs in group A were premedicated with an intravenous injection of 0.005 mg/kg Medetomidine, followed by an intravenous injection of 0.25 mg/kg Diazepam and 4 mg/kg Ketamine combination 3 - 5 minutes later. Meanwhile group B dogs were given an intravenous injection of Medetomidine (0.005 mg/kg) and Ketamine (5 mg/kg) combination. Blood sample was collected for haematological analysis while serum was collected to assay Total protein (TP), Alanine Aminotransferase (ALT), Blood urea nitrogen (BUN) and Creatinine (Cr) using standard laboratory protocols. There was no significant difference ($p>0.05$) in the measured haematological indices, ALT, BUN, Cr and TP in both groups A and B throughout the study. However, there was non-significant decrease in ALT, BUN, Cr and TP in both groups up to 30 minutes post administration of the drug combinations but return to almost baseline values at 60 minutes post anaesthesia. The results of this study showed that intravenous administration of Medetomidine-Diazepam-Ketamine and Medetomidine-Ketamine combinations have no significant effects on haematological indices and serum chemistry profile of dogs. Therefore, these anaesthetic combinations can be prescribed for clinical use and procedures in dogs.

Key Words: Dog, Anaesthesia, ketamine, Medetomidine, Diazepam

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INTRODUCTION

Anaesthetics are often combined to achieve optimum anaesthesia and analgesia, to enhance muscle relaxation, prolong the duration of anesthetic effect and to prevent adverse side effect [1]. The combined effects of anaesthesia on renal and hepatic functions may occur through direct action of some anaesthetics that are nephrotoxic and hepatotoxic or indirectly through the effects on hemodynamic or neuroendocrine response with relatively non-toxic drug [2]. Generally, perioperative anesthesia-related variables have been shown to include anaemia and ischaemia reperfusion, haemodynamic fluctuation, and surgical stress response [3], [4]. Anaesthetic drugs have been associated with varying degree of dose dependent organ effects and such should always be put into consideration when formulating dose for anaesthetic combination protocol aside from cardiopulmonary effects [5]

Ketamine is known to be a potent N-methyl-D-aspartate (NMDA) receptor antagonist in the spinal cord and dissociative induction anesthetic agent [6]. However, use of ketamine has raised the concern for toxicity [7]. Various combination of ketamine, diazepam and medetomidine anaesthetics have been reported [8], [9], [10], [1]. However, there is paucity of information on the haemodynamics and hepatorenal effects of such combination. Therefore, this study was carried out to evaluate effects of combinations of medetomidine-diazepam-ketamine and medetomidine-ketamine anesthesia haematology and serum chemistry in dogs.

MATERIALS AND METHODS

Experimental Animals

Ten (10) apparently healthy local dogs comprising of 5 females and 5 males with average body weight of 20.4 ± 2.93 kg and aged 2-3 years were used for this study. The dogs were randomly allocated into 2 groups A (n=5), B (n=5) and they were housed in kennels of the Department of Veterinary Surgery and Radiology, University of Maiduguri to acclimatize for 2 weeks. They were fasted (feed not water) for 12 hours before anaesthesia.

Anaesthetic Protocol

Dogs in group A were premedicated with an intravenous injection of 0.005 mg/kg Medetomidine (Domitor® Pfizer, Finland) and allowed to be sedated (3-5 minutes) followed by an intravenous injection of 0.25 mg/kg Diazepam (Diazepam®, Juhel, Nigeria) and 4 mg/kg Ketamine (Pauco Ketamine Injection®, Pauco, Nigeria) combination. Meanwhile group B was given an intravenous injection of Medetomidine (0.005 mg/kg) and Ketamine (5 mg/kg) combination. The drugs were combined at the said dosages in a single syringe.

Determination of Blood Parameters

Blood sample was obtained from each dog before administration of anaesthetic drugs and at 10, 20- and 60-minutes post anaesthesia. Sampling was done through the cephalic and recurrent tarsal veins. Blood samples (2 ml each time) were collected using sterile hypodermic syringe and needle and emptied into plain sample bottles to harvest serum meant for biochemical analysis while 3 ml of blood was collected in sample bottle containing anticoagulant (EDTA) and used for determination of heamatological parameters as described by [11].

Determination of Serum Biochemistry

Alanine Amino Transferase (ALT)

Method used was described by [12] was adopted with the use of commercially available kit (Randox Laboratory LTD, Ardmore, U.K.).

Blood Urea Nitrogen

Diacetyl method of [13] was adopted with the use of commercially available test kit (Randox Laboratory LTD, Ardmore, U.K.).

Creatinine

Randox Laboratory Test kits and Procedures were used for this test. Red colour is produced by creatinine with alkaline solution of picric acid (Jaffe's reaction) as described by [14].

Total Protein

Biuret Reaction Method of [15] was adopted for this test.

Statistical Analyses

Independent student sample t test and One-Way Repeated Measures ANOVA with a Dunnett's Multiple Comparison Post Test were used to analyze data within groups and between groups. Analyses were considered as significant at $p<0.05$. Graphpad Prism Version 5.0 software was used for the data analysis.

RESULTS

Heamathological Indicies

There was no significant difference ($p>0.05$) in the hematological parameters of dogs in the group that were administered combined medetomidine-diazepam-ketamine (MDK) and medetomidine-ketamine (MK) anesthesia in dogs (Table I). There was a slight insignificant ($p>0.05$) decrease in the blood parameters measured from 10-20 minutes after administration of either MDK or MK which later returned close to baseline values at 60 minutes.

Alanine Amino Transferase (ALT)

There was no significant difference ($P>0.05$) in the ALT values for dogs in groups A and B throughout the anaesthesia when compared to the baseline values 14.0 ± 0.6U/L and 12.6 ± 0.8U/L respectively. In both groups A and B, there was no significant decrease of ALT ($P>0.05$) at 60 minutes post anaesthesia when compared with baseline values (Table II).

Table I: Effects of Medetomidine – Diazepam– Ketamine combination or Medetomidine – Ketamine combination anaesthesia on Hematological indices in dogs

Time interval	Baseline		10 mins		20 mins		60 mins PA	
Parameter	MDK	MK	MDK	MK	MDK	MK	MDK	MK
PCV (%)	42.2±2.3	38.6±2.0	34.4±4.7	37.8±2.2	34.2±4.2	37.0±4.0	40.0±3.6	41.4±4.5
Hb(g/dl)	14.5±0.6	12.9±1.1	12.8±0.6	12.1±1.2	12.4±1.4	12.3±1.9	12.9±1.9	14.1±2.2
RBC(x106/µl)	7.5±0.4	6.4±0.7	5.6±0.6	6.1±1.2	5.5±0.8	6.3±1.5	6.9±0.1	7.3±1.4
WBC(x10^3/µl)	12.8±1.0	10.3±1.3	8.7±1.3	10.2±2.2	8.4±1.6	10.1±2.0	11.0±2.2	12.8±2.4
NEU (x10^3/µl)	8.2±0.6	6.5±0.2	5.3±0.2	6.5±0.3	5.4±0.7	6.5±0.4	8.3±0.4	9.5±0.4
LYM(x10^3/µl)	3.5±0.5	3.1±0.4	2.6±0.2	2.7±0.4	2.3±0.4	3.0±0.3	1.8±0.7	2.4±0.4
MON(x10^3/µl)	0.6±0.2	0.3±0.1	0.3±0.1	0.4±0.1	0.4±0.1	0.3±0.1	0.3±0.2	0.4±0.1
EOS(x10^3/µl)	0.9±0.2	0.5±0.2	0.5±0.1	0.7±0.2	0.5±0.1	0.5±0.1	0.6±0.2	0.6±0.2

There was no significant difference between groups ($p>0.05$)
PA= post anaesthesia

Total Protein

There were no significant differences in Total Protein values within groups A and B when compared to the baseline values of 61.6± 4.8 g/L for group A and 61.0± 7.0 g/L for group B. There was a non-significant ($p>0.05$) decrease in total protein values in Group A during anaesthesia but increased to 62.0± 3.6 g/L above the baseline value at 60 minutes post anaesthesia. In group B however, there was a non-significant ($p>0.05$) decrease of total protein value to 59.6 ± 7.5 at 10 minutes but slightly increased reaching a value of 60.8± 5.9g/L at 60 minutes post anaesthesia (Table II).

Blood Urea Nitrogen

Blood Urea Nitrogen concentration of dogs in group A showed no significant change from the baseline value of 7.4 ± 1.3 mmol/L. There was no significant difference within the group reaching the lowest value of 7.0 ± 1.2mmol/L at 60 minutes post anaesthesia. In Group B, baseline value was 6.3 ± 0.6 mmol/L was recorded which decreased non-significantly at 10 and 20minutes post administration of anaesthetic agent. There was an increase in the value at 60 minutes post anaesthesia to 6.7 ± 0.9 mmol/L but do not differ significantly from the baseline value (Table III).

Creatinine

Baseline creatinine values for groups A and B were 70.8±5.8 umol/L and 68.6± 7.9 umol/L. In group A, there were decreases at 10, 20 and 30 minutes but do not differ significantly from the baseline value. There was a non-significant increase at 60 minutes post anaesthesia. In group B also, there was a non-significant increase at 20 and 30 minutes (70.2±7.26 umol/L and 70.9± 7.2 umol/L) and 60 minutes post anaesthesia (72.8± 7.0 umol/L). There were no significant differences within both groups (p>0.05; Table III).

Table II: Effects of Medetomidine– Diazepam– Ketamine combination or Medetomidine Ketamine combination anaesthesia on alanine amino transferase and total protein in dogs

Time Interval (minutes)	ALT (U/L)		TP (g/L)	
	MDK	MK	MDK	MK
Baseline	14.0 ± 0.6	12.6 ± 0.8	61.6 ± 4.8	61.0 ± 7.0
10	13.8 ± 0.7	12.4 ± 0.5	61.4 ± 4.7	59.6 ± 7.5
20	13.8 ± 0.7	12.6 ± 0.1	60.6 ± 4.8	57.6 ± 7.0
30	13.4 ±1.0	12.0 ± 0.7	60.4 ± 4.1	59.3 ± 7.6
60 *	13.6 ± 0.8	11.8 ± 0.8	62.0 ± 3.6	60.8 ± 5.8

No significant difference within columns (p>0.05), n=5/group

* Post anaesthesia

Table III: Effects of Medetomidine – Diazepam– Ketamine combination or Medetomidine – Ketamine combination anaesthesia on blood urea nitrogen and creatinine in dogs.

Time Interval (minutes)	BUN (mmol/L)		CRE (umol/L)	
	MDK	MK	MDK	MK
Baseline	7.38 ± 1.3	6.3 ± 0.6	70.8 ± 5.9	68.6 ± 7.9
10	7.7 ± 1.4	5.9 ± 0.5	69.4 ± 4.8	69.2 ± 7.7
20	7.7 ± 1.4	5.7 ± 0.6	69.2 ± 4.2	70.2 ± 7.3
30	7.6 ± 0.8	6.0 ± 0.6	68.0 ± 3.9	70.9 ± 7.2
60*	7.0 ± 1.2	6.7 ± 0.9	71.6 ± 3.8	72.8 ± 7.0

No significant difference within columns (p>0.05), n=5/group

* Post anaesthesia

DISCUSSION

The effects of MDK and MK combination on haematology and serum biochemistry were assessed in dogs in this study. It is known that BUN and serum creatinine levels, as well as kidney pathological changes, could reflect kidney damage while ALP and Total serum protein could provide evidence of liver damage. Similar to the study of [16], there was no significant difference in the hematological profile in both group that received Medetomidine– Diazepam– Ketamine combination or Medetomidine – Ketamine combination. This is an indication that the drug combination at the doses used in this study has no effect on negative the hematological parameters

The effects of MDK and MK on Alanine amino transferase (ALT), Blood Urea Nitrogen (BUN), Creatinine (Cre) levels and Total Protein (TP) values of the dogs in both groups were not significant within the groups. Non-significant changes observed indicated that the two combinations cause minimal changes on ALT up to 30 minutes during anaesthesia. At 60 minutes post anaesthesia creatinine however, increased significantly which is in line with the report of [17] showing significant increase in Creatinine levels of dogs at 60 minutes after injection of MK at 0.004 mg/Kg – 5 mg/kg. In group B, BUN value decreased non-significantly compared to baseline value. This may be due to short term effect of the drugs on renal function which also explains the return of the value close to the baseline at 60 minutes post anaesthesia. In another study to determine the effects of combination of ketamine-medetomidine anaesthesia on haematology and some serum chemistry parameters in dogs, [17] similarly reported no significant effect of the said combination on BUN and ALT.

Similar to the report of [18] where the difference amongst the treated groups was non-significant after treating dogs with medetomidine HCl at a dose rate of 15, 30, 45 and 60 μg/Kg body weight intravenously, combination MDK containing medetomidine produce no change in liver and kidney parameters after administration. Similar findings with use of medetomidine HCl anaesthetic were also recorded by [19]. This could be due to hepatorenal protective effects of medetomidine as earlier reported [20], [21], [22] [23], [24]. Despite several reports elucidating different patterns of renal and liver toxicity caused by ketamine [25] [26] [27] [28], the current study which uses combination of ketamine and other drugs did not show any significant effect on the kidney and the liver. Administration of diazepam in rats and cats has been shown to cause significant dose dependent increase in serum ALT due to its hepatotoxicity [29], [30]. Similarly, [31] observed that diazepam at 0.0046 mg/100 g body weight, 0.0036 mg/100 g body weight, 0.0026 mg/100 g body weight, and 0.0016 mg/100 g body weight respectively in wistar rats increased total serum protein significantly in a dose-dependent manner. Also, drug like diazepam has been shown to produce no significant changes on the marker of kidney functions such as Urea and creatinine [31]. The combination of diazepam with medetomidine and ketamine in this study did not produce any significant change in ALT, Total protein, BUN and Creatinine which is suggestive of non-toxic effects of this combinations at the dose used in this study.

[17] reported no significant effect of MK on BUN and ALT. The variation of the results with those of this present study could be due to difference in dosages where medetomidine was used at 00.4 mg/kg and ketamine at 5 mg/kg. The use of more females in this study may have also influenced the results [32]. As the ALT, BUN, Cre and TP gradually returns to pre-administration level, the possibility of pathological changes in the liver could be, therefore, ruled out. Preanalytical factors such as age and breed can have an impact on plasma creatinine (P-creatinine) concentration, while many intra-individual factors of variation have

little effect. Dehydration and drugs mainly affect P-creatinine concentration in dogs by decreasing GFR [33]. Reaction to the blood collection, causing some local muscle trauma, might also be responsible for the changes in these values. It is evident that the anaesthetic combinations had no significant effect on dog's liver and kidney function.

Conclusion

This study showed that intravenous administration of Medetomidine-Diazepam-Ketamine and Medetomidine-Ketamine combination have no significant effects on haematology and serum chemistry in dogs. The anaesthetic combinations can be evaluated for clinical procedures in dogs.

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