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Comparative evaluation of haemato biochemical changes after intravenous administration of dexmedetomidine-butorphanol and dexmedetomidine-midazolam as preanaesthetic with propofol anaesthesia in dog

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Abstract
A combination of drugs may be preferred over the use of a single agent to induce deep sedation and the synergistic interaction between the drugs reduces the dose requirements of the drugs thereby minimising the unwanted side effects associated with each drug and improving recovery with this aim the present study was conducted to compare and evaluate the intravenous dexmedetomidine-butorphanol and dexmedetomidine-midazolam as preanaesthetic with propofol anaesthesia in dog. The study was conducted on 14 (fourteen) clinical cases of dogs of either sex, irrespective of age presented to the T.V.C.C., COVAS, Parbhani for various surgical interventions randomly divided into two groups each consisting of seven dogs. All the dogs in study were administered with inj. Atropine sulphate @ 0.04 mg/kg body weight subcutaneously. In group A, after administration of inj. Atropine sulphate, 10 min later combination of Inj. Dexmedetomidine HCL (10 µg/kg body weight) and Inj. But orphanol tartarate (0.2 mg/kg body weight) mixed in single syringe was administered intravenously whereas in group B, combination of Inj. Dexmedetomidine HCL (10 µg/kg body weight) and Inj. Midazolam maleate (0.2 mg/kg body weight) mixed in single syringe was administered intravenously. Quality of sedation was assessed followed by inj. Propofol was administered till the effect to get the surgical stage of anaesthesia and required amount was calculated as induction dose. Intermittent doses of propofol were given for maintenance of anaesthesia when required. Overall significant difference haemoglobin (*P*<0.01) and non-significant difference in packed cell volume, total erythrocyte count, total leucocyte count, blood urea nitrogen, serum creatinine, blood glucose, alanine transaminase and aspartate transaminase values were observed between the groups during sedation and anaesthesia. From the present study it could be concluded that intravenous administration of dexmedetomidine-butorphanol and dexmedetomidine-midazolam with propofol anaesthesia could be indicated as per the clinical demand in dogs without alarming changes in important physiological and haemato biochemical parameters if so they are transient without affecting the normal physiology, liver and kidney functions.

Keywords: Premedicants, sedation, induction, dexmedetomidine, butorphanol, midazolam, propofol

1. Introduction
Anaesthesia is an indispensable pre-requisite for many surgical interventions with maximum technical efficiency and accuracy, so that surgeon can perform surgeries at ease. Propofol (2, 6-diisopropylphenol) is a newer generation injectable anaesthetic agent which was introduced in Veterinary medicine in the 1990's. The α_2-adrenergic receptor agonists are useful adjuncts to anaesthesia because of their sedative, anxiolytic and analgesic effects and their anaesthetic-sparing properties. Anticholinergic premedication has been recommended with α_2-agonists to prevent bradyarrhythmias and potential reduction in cardiac output produced by these agents. Dexmedetomidine was approved by the Food and Drug Administration at the end of 1999 for use in humans as a short-term medication (<24 hours) for analgesia and sedation in the intensive care unit (Gertler *et al.,* 2001) [6]. Butorphanol is a centrally acting analgesic with both agonist and antagonist properties. Midazolam is a benzodiazepine with a fused imidazole ring that accounts for water solubility of the drug, exert their main sedative effect through

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Depression of limbic system and their muscle relaxing properties through inhibition of the internuncial neurons at spinal levels (Lumb and Jones, 2007) [13]. Keeping in view the above, the present study was planned with an objective of comparative evaluation of haemato biochemical changes after intravenous administration of dexmedetomidine-butorphanol and dexmedetomidine-midazolam as preanaesthetic with propofol anaesthesia in dog.

Materials and Methods
Selection of Animals
The present clinical study was conducted on 14 (fourteen) clinical cases of canine of either sex, irrespective of age presented to the Teaching Veterinary Clinical Complex, College of Veterinary & Animal Sciences, Parbhani for various surgical interventions.

Preparation of Animals
In all the dogs under study food was withheld for twelve hours and water withheld for eight hours prior to surgery. Weighing of each dog was done for calculating the exact dose of the anaesthetic agent prior to administration. All the animals were subjected to clinical examination prior to surgery and physiological parameters were recorded as reference.

Anaesthetic protocol
All the 14 clinical cases were randomly divided into two groups each consisting of seven dogs. All the dogs in study administered with inj. Atropine sulphate1 @ 0.04 mg/kg body weight subcutaneously.
Group A: After administration of Atropine sulphate, 10 min later combination of Inj. Dexmedetomidine HCL (10 µg/kg body weight) and Inj. Butorphanol tartarate (0.2 mg/kg body weight) mixed in single syringe was administered intravenously.
Group B: After administration of Atropine sulphate, 10 min later combination of Inj. Dexmedetomidine HCL (10 µg/kg body weight) and Inj. Midazolam maleate (0.2 mg/kg body weight) mixed in single syringe was administered intravenously.
The sedation was assessed after the administration of drug combination (DB/DM) in both the groups. The grading of sedation was done depending upon the quality as described by Rauser and Lexmaulova, (2002) [17]. After the onset of sedation, inj. Propofol was given till the effect to get the surgical stage of anaesthesia and required amount was calculated as induction dose. Intermittent dosage for the maintenance of anaesthesia were given when required.

Haematological parameters
All the blood samples were collected from (cephalic/sephanous) veins aseptically. For haematological studies, blood samples collected in EDTA vials activator before anaesthesia (BP), during sedation (AP), during surgery (DS) and 24 hours after recovery (AR) from anaesthesia from both the groups. Haemoglobin (gram%), Packed cell volume (percentage), Total erythrocyte count (x10^6/cumm), Total leucocytic count (x10^3/cumm) were recorded.

Biochemical parameters
For biochemical estimation, the serum samples were separated from blood samples, collected in clot activator before preanaesthetic administration, after sedation, during surgery and after recovery from anaesthesia from animals of both the groups. Blood urea nitrogen (mg/dl), Blood creatinine (mg/dl), Blood glucose (mg/dl), Alanine transaminase (IU/dl), Aspartate transaminase (IU/dl) were recorded.

Statistical analysis
The data collected in the present study of different parameters were analyzed by conventional tools for data analysis (two-way ANOVA and 't' test) using WASP (Anonymus, 2018 WASP version 2.0 http://www.ccari.res.in/wasp2.0/index.php Last assessed on 4 August 2018).

Results
Haematological parameter
Haemoglobin
The mean $\pm$ SE values of haemoglobin in group A and group B are 13.18 ± 0.80 and 11.72 ± 0.91, respectively. As shown in Fig. 1, comparision within the group revealed that a non-significant decrease in Hb values was recorded in the animals of group A till sedation from baseline. However, the values were significantly decrease ($P<0.05$) during anaesthesia till recovery after 24 hours. In group B significant decrease ($P<0.05$) was observed during sedation and thereafter a non-significant decrease was observed till recovery after 24 hours.
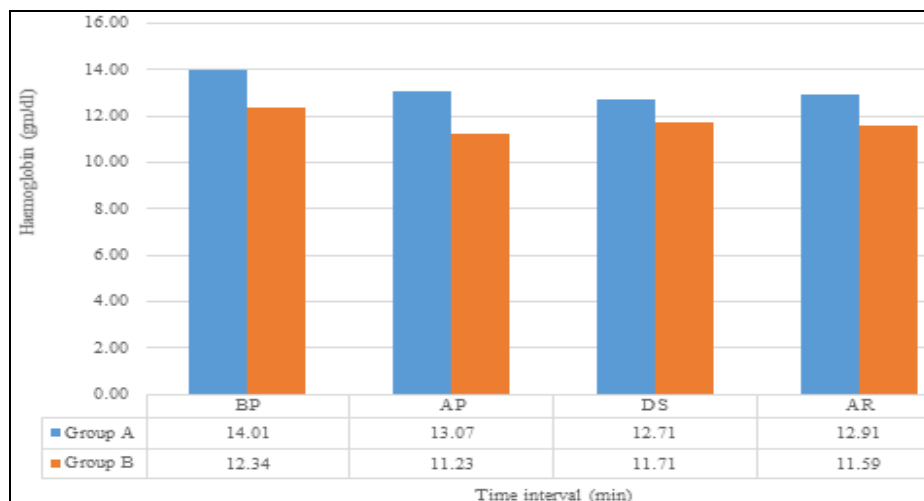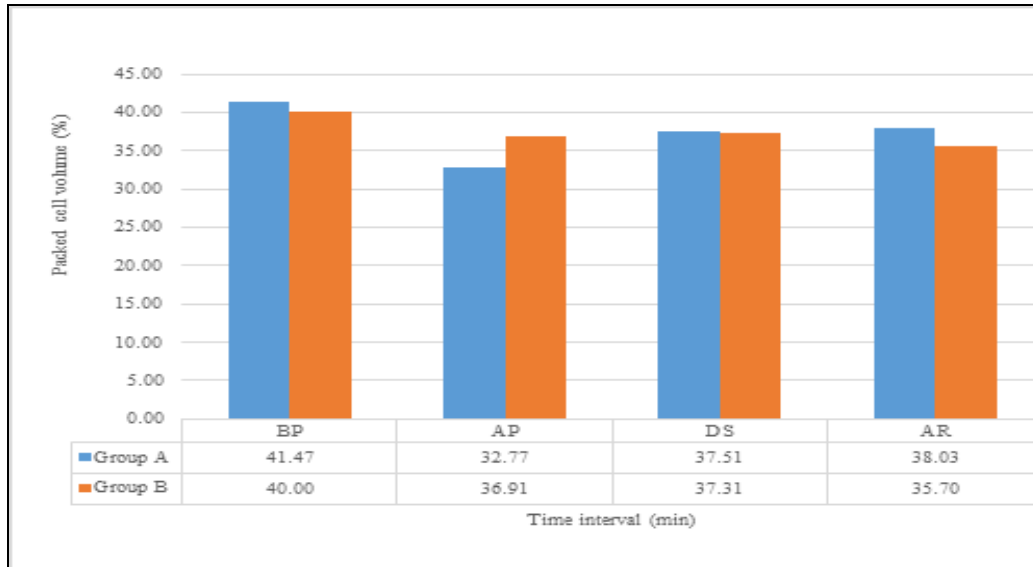

Fig 1: Mean $\pm$ SE scores of haemoglobin in group A and B

Comparison among both groups revealed significant difference ($P<0.01$) in Hb values at different interval before preanaesthetic, during sedation, during anaesthesia and at recovery 24 hours in both the groups. Overall significant difference ($P<0.01$) was observed between the groups.

Packed cell volume
The mean ± SE values of packed cell volume in group A and group B are 13.18 ± 0.80 and 11.72 ± 0.91, respectively. As

shown in Fig 2, comparision within the group revealed that a significant decrease ($P<0.01$) in PCV values was recorded in the animals of group A thereafter non-significant increase was observed during anaesthesia and 24 hour after recovery. In group B, a non-significant decrease was observed during sedation and thereafter it was non-significantly decreased till recovery after 24 hours.


	BP	AP	DS	AR
Group A	41.47	32.77	37.51	38.03
Group B	40.00	36.91	37.31	35.70

Fig 2: Mean ± SE scores of packed cell volume in group A and B

Comparison among both groups revealed non-significant difference in PCV values at different interval except during sedation where significant difference ($P<0.05$) was observed in both the groups. Overall nonsignificant difference was observed between the groups.

Total erythrocyte count
The mean ± SE values of total erythrocyte count in group A and group B are 6.65 ± 0.40 and 5.85 ± 0.46, respectively. As

shown in Fig 3, comparision within the groups revealed that a significant ($P<0.01$) decreased in TEC values in group A thereafter it was increased non-significantly was during anaesthesia and 24 hour after recovery. In group B, a non-significant decrease observed during sedation and thereafter non-significant decrease observed till recovery after 24 hours.

	BP	AP	DS	AR
Group A	6.84	6.69	6.56	6.53
Group B	6.14	5.60	5.84	5.81
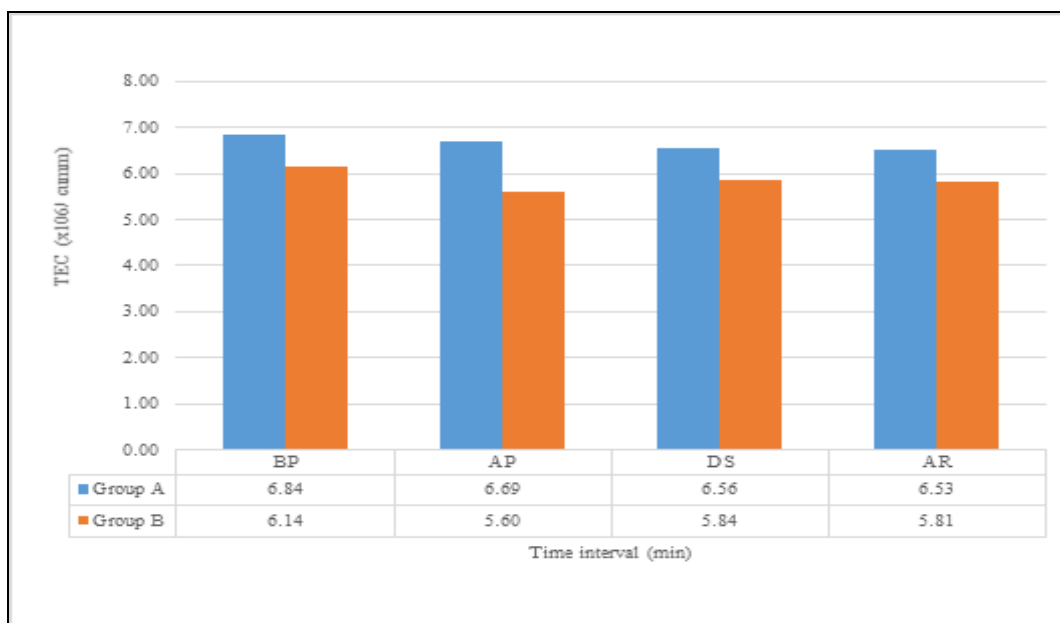
Fig 3: Mean ± SE scores of total erythrocyte count in group A and B.
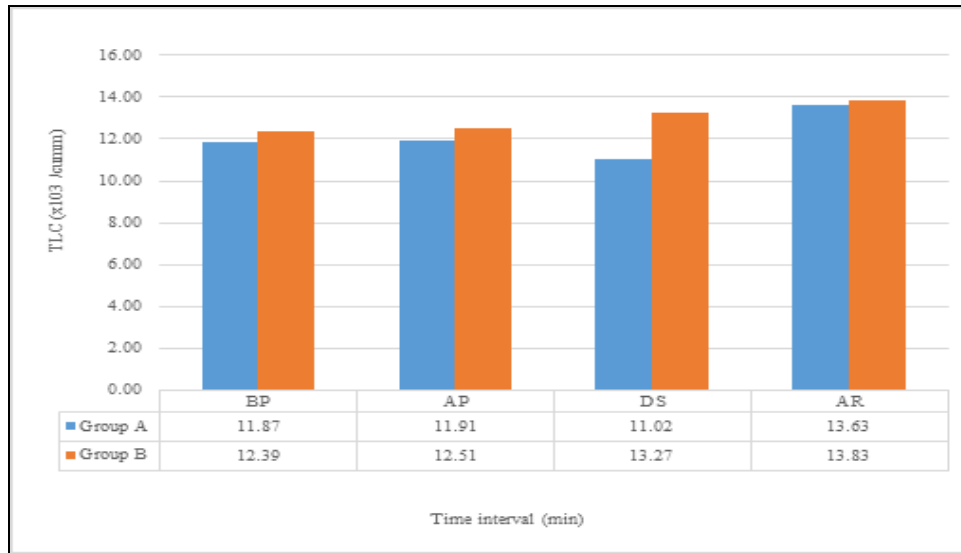
Comparison among both groups revealed non-significant difference in TEC values at different interval except during

sedation where significant difference ($P<0.05$) was observed in both the groups. Overall nonsignificant difference was observed between the groups.

Total leucocyte count
The mean ± SE values of total leucocyte count in group A and group B are 12.10 ± 1.62 and 13.00 ± 2.15, respectively. As shown in Fig 4, comparision within the groups revealed that a non-significant decrease in TLC values was observed in the group A from baseline till during anaesthesia thereafter non-significant increase observed at recovery after 24 hour. In group B, a non-significant decrease was observed during sedation from baseline and thereafter a non-significant increase was observed till recovery after 24 hours.

Fig 4: Mean ± SE scores of total leucocyte count in group A and B
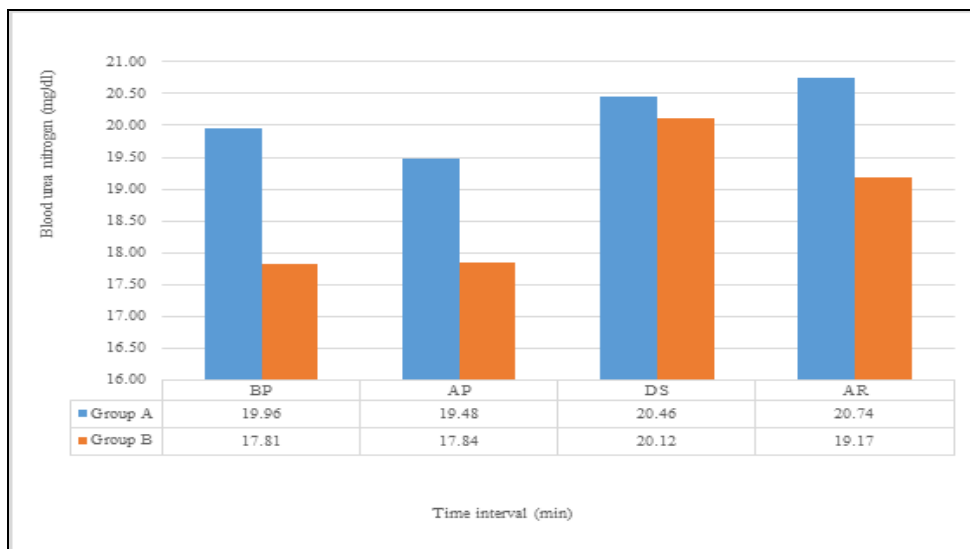
Comparison among both groups revealed non-significant difference in TLC values at different interval except during sedation where significant difference ($P<0.05$) was observed in both the groups. Overall no significant difference was observed between the groups.

Biochemical parameter
Blood urea nitrogen
The mean ± SE values of blood urea nitrogen in group A and group B are 20.17 ± 1.52 and 18.73 ± 1.14, respectively. As shown in Fig 5, comparision within the groups revealed that a non-significant decrease in BUN values was observed in group A during sedation thereafter it was non-significantly increased till recovery after 24 hour. In group B, a non-significant increase was observed during sedation from baseline, thereafter it was significantly increased ($P<0.05$) during anaesthesia and non-significantly decreased till recovery after 24 hours.

Fig 5: Mean ± SE scores of blood urea nitrogen in group A and B

Comparison among both groups revealed significant difference ($P<0.01$) in BUN values at the interval of before preanaesthetic and significant difference ($P<0.05$) during sedation level interval except thereafter a non-significant difference was observed in both the groups. Overall a
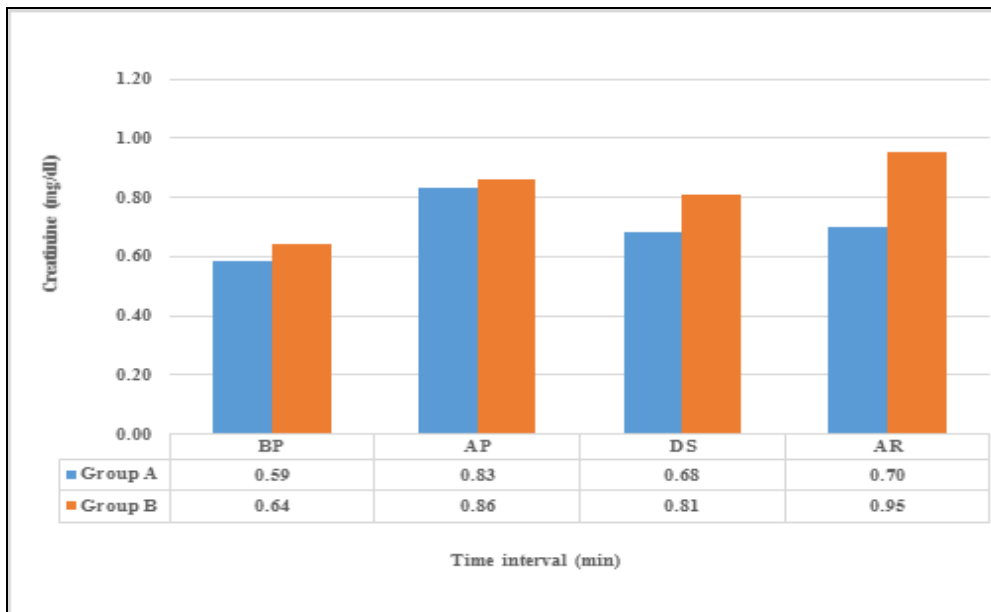
Nonsignificant difference was observed between the groups.

Blood creatinine
The mean ± SE values of blood creatinine in group A and group B are 0.70±0.09 and 0.81 ± 0.09, respectively. As

shown in Fig 6, comparision within the groups revealed that a significant increase ($P<0.01$) in blood creatinine values was observed in group A during sedation thereafter it was non-significantly decreased and then it was increased at the interval of during anaesthesia and 24 hours after recovery, respectively. In group B, a significant increase ($P<0.05$) was observed during sedation from baseline and thereafter it was non-significantly decreased and significantly increased ($P<0.01$) at the interval of during anaesthesia and recovery after 24 hours, respectively.

**Fig 6:** Mean ± SE scores of blood creatinine in group A and B

Comparison among both groups revealed non-significant difference in blood creatinine values at the interval of before preanaesthetic and during sedation whereas significant difference ($P<0.05$) during anaesthesia level and thereafter significant difference ($P<0.01$) at during recovery after 24 hours was observed in between groups. Overall a nonsignificant difference was observed between the groups.

Blood glucose
The mean ± SE values of blood glucose in group A and group B are 79.61 ± 6.77and 82.85 ± 12.18, respectively. As shown in Fig. 7, comparision within the groups revealed that a non-significant increase in blood glucose values was recorded in group A at the interval of before preanaesthetic and during sedation thereafter significant increase ($P<0.01$) at interval of during anaesthesia and non-significant decrease during recovery. In group B, similar findings was observed up to interval of during anaesthesia thereafter followed significant decrease ($P<0.05$) at recovery after 24 hours.

Comparison among both groups revealed non-significant difference in blood glucose values any interval in the animals of both groups. Overall a nonsignificant difference was observed between the groups.
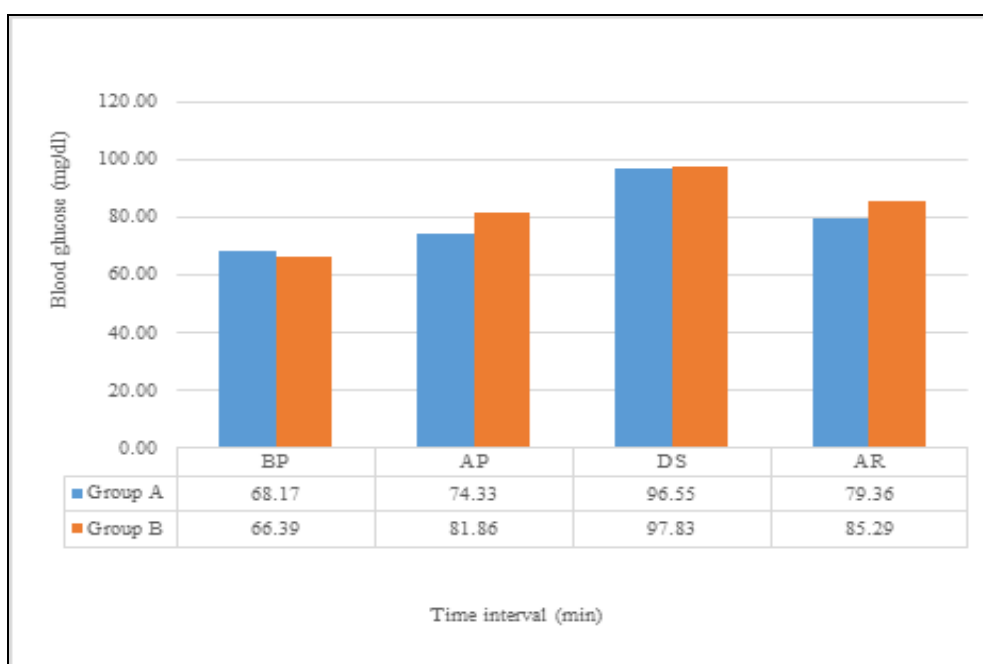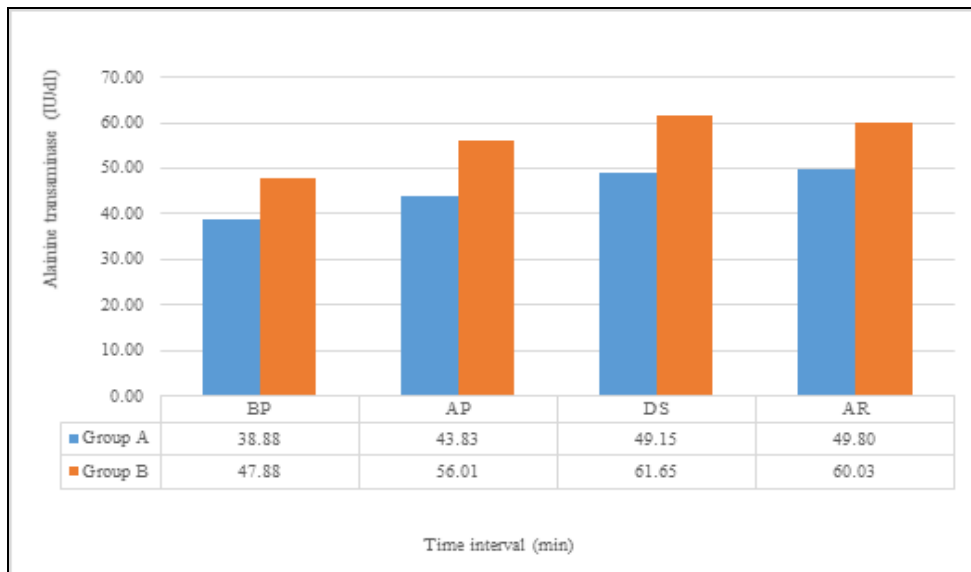

Fig 7: Mean ± SE scores of blood glucose in group A and B

Alanine transaminase
The mean ± SE values of alanine transaminase in group A and group B are 45.42 ± 5.70 and 56.39 ± 6.77, respectively. As shown in Fig 8, comparision within the groups revealed that a non-significant increase in ALT values was recorded in group A up to interval of during anaesthesia from baseline thereafter

significant increase ($P<0.05$) was observed 24 hours after recovery. In group B, a non-significant increase was observed up to during sedation from baseline and thereafter it was increased significantly ($P<0.01$) and decreased significantly at interval of during anaesthesia and at recovery after 24 hours, respectively.
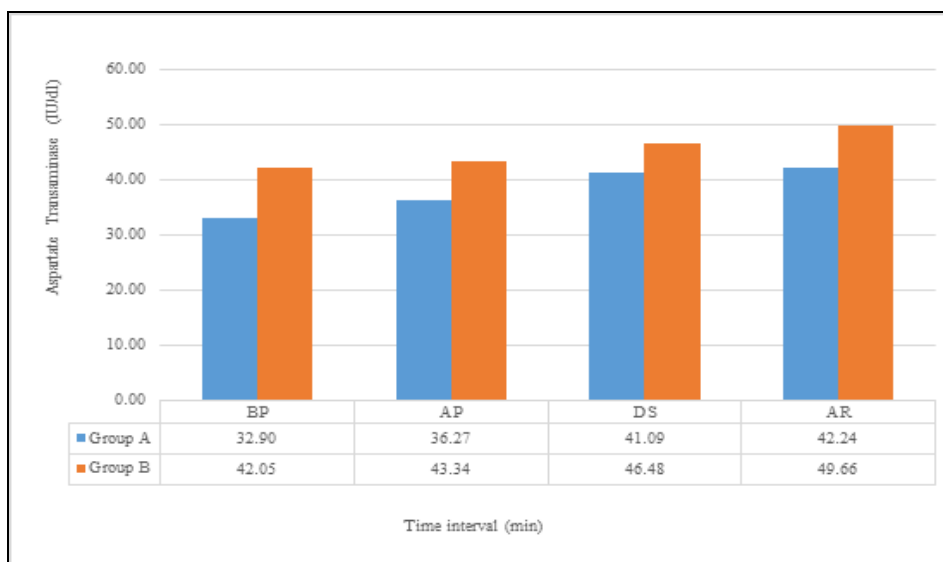

Fig 8: Mean ± SE scores of alanine transaminase in group A and B

Comparison among both groups revealed significant difference ($P<0.05$) in ALT values at the interval of before preanaesthetic and followed by significant difference ($P<0.01$) during sedation, during anaesthesia level and at during recovery after 24 hours was observed in between the animals of both groups. Overall a nonsignificant difference was observed between the groups.

Aspartate transaminase
The mean ± SE values of aspartate transaminase in group A and group B are 38.12 ± 5.62 and 45.39 ± 5.10, respectively. As shown in Fig 9, comparision within the groups revealed that a non-significant increase in AST values was observed in group A up to interval of during anaesthesia from baseline thereafter significant increase ($P<0.05$) was observed 24 hours after recovery. In group B, a non-significant increase was observed in all interval within the group.

Fig 9: Mean ± SE scores of aspartate transaminase in group A and B

Discussion
The decrease in haemoglobin levels might be due to due to shifting of fluids from the extravascular compartment to the intravascular compartment in order to maintain the cardiac output in the animals, haemodilution in response to fluid therapy and due to dexmedetomidine which has been shown to preserve blood flow to the most vital organs (brain, heart,

liver and kidne y) at the expense of organs like skin and pancreas. Similar findings observed by Jena *et al.* (2014) [9] and Sethi *et al.* (2017) [22].
The decrease in PCV levels might be due to due to shifting of fluids from the extravascular compartment to the intravascular compartment in order to maintain the cardiac output in the animals, haemodilution in response to fluid therapy and due to

dexmedetomidine which has been shown to preserve blood flow to the most vital organs (brain, heart, liver and kidney) at the expense of organs like skin and pancreas. Similar findings observed by Jena *et al.* (2014) [9] and Sethi *et al.* (2017) [22].

This might be due to pooling of red blood cells in the spleen during early stage of anaesthesia. Similar findings were observed by Ozaydin *et al.* (2001) [16], Jain *et al.* (2004) and Dewangan *et al.* (2016) [5] in dogs.

The administration of α_2-agonists suppresses the circulating catecholamines by exerting a modulating effect on leukocyte subpopulations (Kaname *et al.,* 2002) [10]. Similar findings observed by Jena *et al.* (2014) [9] and Sethi *et al.* (2017) [22].

The increase in urea nitrogen values might be attributed to the temporary inhibitory effects of anaesthetic drugs on the renal blood flow, which in turn might have caused a rise in plasma urea nitrogen level. The increased hepatic urea production from amino acid degradation could also account for the observed increase in blood urea values during the maximum depth of anaesthesia. However, it is difficult to describe this to possible renal damage, because all the reported values were within normal physiological limits. Similar findings was observed by Surbhi *et al.* (2010), Santosh *et al.* (2011) [19] and Jena *et al.* (2014) [9].

The increase in plasma creatinine values might be attributed to the temporary inhibitory effects of anaesthetic drugs on the renal blood flow as reported in goats (Kinjavdekar *et al.,* 2000) [12] and sheep (Monsang, 2011) [15].

The increase in blood glucose observed in the present study might be attributed to an α_2-adrenergic inhibition of insulin released from beta cells of pancreas and increased glucose production in the liver. Hyperglycemia might also be attributed to the traumatic stress or increased muscular activity and sympathetic stimulation caused by restraining the animals resulting into increased secretion of adrenocortical hormones (Mirakhur *et al.,* 1984). Similar findings was observed by Kinjavdekar *et al.* (2000) [12], Surbhi *et al.* (2010) and Jena *et al.* (2014) [9].

Most ALT activity in the opioid group might be attributed to the fact that the opioids are exclusively metabolized in liver hence causes no more changes at cellular level (Scott and Perry, 2000) [21]. Similar findings was observed by Kanto and Gepts, (1989) [11] and Chandrakala *et al.* (2017) [4].

Non-significant increase in AST level might be associated with increased cell membrane permeability in response to haemodynamic changes induced by anaesthetic agents in xylazine-butorphanol-propofol in dogs (Chandrakala *et al.*, 2017) [4].

Conclusion
From the present study it could be concluded that intravenous administration of dexmedetomidine-butorphanol and dexmedetomidine-midazolam both as preanaesthetic combinations with propofol anaesthesia could be indicated as per the clinical demand in dogs without alarming changes in important physiological and haematobiochemical parameters if so they are transient without affecting the normal physiology, liver and kidney functions.

References
1. Amarpal AM, Pawde GR, Singh K, Pratap N, Kumar. Clinical evaluation of medetomidine with or without pentazocine in atropinized dogs. Indian J Anim. Sci. 1996; 66(3):219-222.
2. Arunkumar S, Dilipkumar D, Shivaprakash BV. Clinical and physiological evaluation of dexmedetomidine, xylazine and triflupromazine as preanaesthetics with propofol-isoflurane anaesthesia for various surgeries in dogs. The Pharma Innovation Journal. 2017; 6(8):100-105.
3. Bol CJJG, Vogelaar JPW, Tang JP, Mandema JW. Quantification of pharmacodynamics interaction between dexmedetomidine and midazolam in the Rat. The Journal of Pharmacology and Experimental Therapeutics. 2000; 294(1):347-355.
4. Chandrakala K, Sharma AK, Kumari L, Prasad R, Singh KK, Praveen Kumar. Haemato-Biochemical Evaluation of Ketamine or Butorphanol as Analgesic in Xylazine and Propofol Anaesthesia in Canine Ovariohysterectomy. International Journal of Livestock Research. 2017; 7(7):2277-1964.
5. Dewangan R, Tiwari SK, Sharda R. Haemato-biochemical response to xylazine-propofol anaesthesia and its reversal by yohimbine and atipamezole in dogs. I.J.S.N. 2016; 7(3):647-650.
6. Gertler R, Brown HC, Mitchell DH, Silvius EN. Dexmedetomidine: a novel sedative-analgesic agent. BUMC proceedings. 2001; 14:13-21.
7. Jangam AK, Wadekar PN. Web Agri Stat Package 2.0 ICAR Research Complex, Goa, 2018. http://www.ccarl.res.in/wasp2.0/index.php.
8. Jain R. Clinical and haematological studies on propofol anesthesia using ether in dogs. M.V. Sc. thesis submitted to Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.), India, 2003.
9. Jena B, Das J, Nath A, Sardar KK, Sahoo A, Beura SS, et al. Clinical evaluation of total intravenous anaesthesia using Xylazine or/and Dexmedetomidine with Propofol in surgical management of canine patients. Vet. World. 2014; 7:671-680.
10. Kaname H, Mori Y, Sumida Y, Kojima K, Kubo C, Tashiro N. Changes in the leukocyte distribution and surface expression of adhesion molecules induced by hypothalamic stimulation in the cat. Brain, Behavior and Immunity. 2002; 16:351-367.
11. Kanto J, Gepts E. Pharmacokinetics implications for the clinical use of propofol. Clin. Pharmacokinet. 1989; 17:308-326.
12. Kinjavdekar P, Singh GR, Amarpal HP, Aithal, Pawade AM. Physiological and biochemical effects of subarchnoidally administered xylazine and medetomidine in goats. Small Rum. Res. 2000; 38:217-228.
13. Lumb, Jones. Ruminants. In: Veterinary Anaesthesia and Analgesia. 4th edn. Blackwell Publishing Ltd., Iowa, USA, 2007, 731-746.
14. Mirakhur KK, Sobti VK, Nigam JM. Effect of thiopentone anaesthesia in plasma cortisol in buffalo calves. Indian J Vet. Surg. 1984; 3:86.
15. Monsang SW. Comparison of medetomidine and dexmedetomidine with and without butorphanol and midazolam as preanaesthetics to propofol anaesthesia in sheep. Ph.D. Thesis, Submitted to Indian Veterinary Research Institute, Izatnagar (U.P.), India, 2011.
16. Ozaydin I, Atalan G, Uzun M, Kilic E, Cenesiz M. Assessment of anaesthetic properties and clinical, cardiovascular and respiratory effects of metomidine, propofol and ketamine combination in dogs. Kafkas. Uni. Veteriner. Fakultesi. Dergisi. 2001; 7(1):71-76.
17. Rauser P, Lexmaulova L. Clinical comparision of medetomidine-Butorphanol and Medetomidine-Buprenorphene combination for intravenous

premedication of general anaesthesia in the dog. ACTA VET. BRNO. 2002; 71:69-76.

18. Salmenpera MT, Szlam F, Hug CCJ. Anesthetic and hemodynamic interactions of dexmedetomidine and fentanyl in dogs. Anesthesiology. 1994; 80:837-846.

19. Santhosh KM. Evaluation of dexmedetomidine as preanaesthetic to ketamine anaesthesia in midazolam or midazolam-fentanyl premedicated dogs. M.V.Sc. Thesis submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar, (U.P.), India, 2011.

20. Salunke VM. Clinical evaluation of propofol as an intravenous general anaesthetic in dogs. Ph.D. Thesis submitted to College of Veterinary & Animal Sciences, Marathwada Agricultural University, Parbhani, 2016

21. Scott LJ, Perry CM. Tramadol: a review of its use in perioperative Pain. Drugs. 2000; 60(1):139-176.

22. Sethi S, Singh J, Nath I, Das RK, Nayak S, Sahu RK. Haemato-biochemical comparision of xylazine / dexmedetomidine in combination with butorphanol / pentazocine as preanesthetic to ketamine anaesthesia in canine pyometra patients. The Pharma Innovation Journal. 2017; 6(9):393-399.

23. Surbhi. Clinical studies on anaesthetic and analgesic management of canine orthopaedic patients. M.V. Sc. Thesis Deemed University, IVRI, Izatnagar (U.P.), India, 2008.

24. Trim CM. Cardiopulmonary effect of butorphanol tartarate in dogs. American Journal of Veterinary Research. 1983; 44:329-331.