

## PAPER DETAILS

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## Comparative Clinical and Serum Biochemical Evaluation of Two Intramuscular Anaesthetic Combinations (Diazepam/Ketamine and Diazepam/Fentanyl/Ketamine) in Rabbits

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### ABSTRACT

The research has been conducted to find the clinical efficacy and safety of preanaesthetics and induction agents based on clinical and serum biochemical indicators in rabbits. Eight healthy rabbits (3.0-3.5kg) of either sex were arbitrarily allocated into two groups: Group DK (diazepam-ketamine) and Group DFK (diazepam-fentanyl-ketamine). Rabbits were received intramuscular diazepam (5mg/kg), fentanyl (0.02 mg/kg) and ketamine (35 mg/kg). Data on clinical parameters (rectal temperature, heart rate, and respiratory rate) and reflexes (righting reflex, palpebral reflex, and pedal reflex) were evaluated before and at 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 minutes after injection of anaesthetics. Blood samples were aspirated before anaesthesia and 30 minutes following induction. Serum Albumin, Globulin, Cholesterol, Triglyceride, Total Protein, High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Creatinine, Calcium, Phosphorus, Sodium, Potassium, and Chlorine were evaluated through a semiautomatic biochemistry analyzer. In this study, no significant changes in rectal temperature were observed in the animals of group DK during anaesthesia. During anaesthetic cascade, heart rates and respiration rates in both the groups were reduced significantly ( $P < 0.05$ ). In DFK-injected rabbits, the return of righting and palpebral reflexes were delayed. The DFK groups had considerably ( $P < 0.05$ ) longer surgical anaesthesia than the DK groups. In terms of serum biochemistry, DK-treated rabbits had significantly lower total protein, globulin, creatinine, HDL, sodium, and potassium concentrations ( $P < 0.05$ ). Total protein, cholesterol, HDL, and potassium levels decreased significantly ( $P < 0.05$ ) after DFK treatment. The DFK combination offered appropriate anaesthesia for rabbits, as evidenced by a prolonged anaesthetic time, acceptable cardiopulmonary and other clinical indices, and moderate changes in serum biochemical profiles.

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### INTRODUCTION

Rabbits are popular companion animals that are frequently employed in experimental surgery and scientific research (Kihç 2004; Brodbelt 2009). Because of their biological homology to humans, rabbits are frequently used to develop various sorts of human disease model for wide angle research (Oguntoye and Oke 2014). The susceptibility of the rabbit respiratory center to the depressive effects of anaesthetic regimens has been blamed for the high rate of death during rabbit anaesthesia. As a result, in rabbits, achieving a safe and suitable depth of anaesthesia, as well as a

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sufficient length of anaesthesia, remains difficult. There are a variety of injectable anesthetic drugs available for rabbit anaesthesia (Grint and Murison 2008).

Diazepam and ketamine are commonly used for anaesthesia in rabbits (Oguntoye and Oke 2014). When ketamine hydrochloride is operated as a single anesthetic agent, hypertonus, inadequate muscular relaxation, tenacious pain responses, and vicious recovery from anaesthesia occur (Chen 2015), necessitating the inclusion of preanaesthetic drugs. When used in conjunction with xylazine or diazepam to produce surgical anaesthesia, ketamine is remarkably effective. Diazepam generates good sedation but has no analgesic effect and causes very minor haemodynamic and respiratory alterations (Yanmaz et al. 2016). Although it can induce severe respiratory depression, fentanyl citrate causes very minor alterations in circulatory variables. The beginning of the activity after intramuscular administration is seven to eight minutes, and the activity will last one to two hours. The respiratory depressive effect of fentanyl, like that of longer-lasting opioid analgesics, could remain longer than the analgesic effect (Dupras et al. 2001). Based on these facts, the research has been conducted to evaluate the cardiopulmonary and other clinical changes and to assess the serum biochemical alterations following anaesthesia with diazepam-ketamine and diazepam-fentanyl-ketamine combinations in the rabbit.

## **MATERIALS AND METHOD**

The Study has been conducted under the guidelines provided by Animal Welfare, Experimentation and Ethics Committee (AWEEC) of the Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh [Permission number (AWEEC/BAU/2021 (45))].

### **Experimental Animals**

Eight clinically healthy White New Zealand rabbits of either sex, weighing 3.0-3.5 kg and aged between 8 and 10 months were used in this investigation. The rabbits were given a 7-day acclimatization period before the commencement of the experiment. They were housed in individual cages. Seasonal fresh grass, fresh vegetables, commercial rabbit feed, and ad libitum water were supplied to the animals. Food, but not drink, was put on hold for 12 hours before the initiation of the experiment.

### **Experimental Design**

The experimental animals were indiscriminately allocated into 2 groups consisting of 4 rabbits in each group. Drugs were administered as follows: Group DK: Diazepam- Ketamine, Group DFK: Diazepam- Fentanyl- Ketamine.

#### **Group DK**

The animals were anesthetized with Diazepam (Easium®, Opsonin Pharmaceuticals, Bangladesh) and Ketamine hydrochloride (Ketalar®, Popular Pharmaceuticals, Tongi, Bangladesh). Diazepam was administered intramuscularly at 5 mg/kg body weight. After 15 minutes, Ketamine was injected at 35 mg/kg body weight intramuscularly. This anaesthetic protocol was adopted from published paper reported by Khan et al (2019).

#### **Group DFK**

The animals of this group were anesthetized with Diazepam (Easium®, Opsonin Pharmaceuticals, Bangladesh), Fentanyl (Fentanyl Citrate®, Martindale Pharmaceuticals, Romford, UK), and Ketamine hydrochloride (Ketalar®, Popular Pharmaceuticals, Tongi, Bangladesh). Diazepam was administered intramuscularly at a dosage rate of 5 mg/kg BW. Fentanyl was delivered intramuscularly at a dosage rate of 0.02 mg/kg BW after 15 minutes (Henke et al., 2005). After 15 minutes of fentanyl administration, ketamine was given intramuscularly at a dosage rate of 35 mg/kg BW.

### **Anesthetic Procedure**

The animal was placed on the operating table before anaesthesia. The animal was then placed in a dorsal position. The anesthetic drugs were then administered intramuscularly with disposable plastic syringes containing 1 ml and 3 ml. Puncture of the needle and observation of various reflexes were used to investigate and confirm induction.

### **Clinical Evaluation**

Before the injection (0 minutes) and at 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 minutes after the administration of the anesthetic agent ketamine, the heart rate, respiration rate, and body temperature were recorded. Following the injection, the level of anaesthesia was measured using the righting reflex, palpebral reflex, and pedal reflexes every 10 minutes until the anaesthesia was terminated in both groups. The time between the administration of anaesthetics and the disappearance of the righting reflex was used to calculate the induction time. The capacity of the animal to reestablish the righting reflex was used to assess recovery from anaesthetic.

### Clinical Examination of Temperature, Respiratory Rate, and Heart Rate

A stethoscope was placed on the lower left lateral thoracic wall to assess the heart rate. The body temperature was recorded using a thermometer and the respiratory rate was determined using a stethoscope by measuring the chest movement/excursion of the thoraco-abdomen.

### Clinical Examination of Reflexes

The rabbit's righting reflex was assessed by timing how long it took it to move from dorsal to sternal recumbency. When no response was elicited by stroking the dorsal eyelid with a cotton-tip applicator, the palpebral reflex was reported as missing. The pedal reflexes were checked by pinching the hind limb with a needle (right and left).

### Collection of Blood Sample for Biochemical Examinations

Each experimental animal had three ml of blood drawn with a 5 ml disposable syringe, which was immediately transferred to a vacutainer (clot activator tube) for serum separation. For biochemical analysis, the supernatant serum was collected in an Eppendorf tube using a micropipette. Total Protein (TP), Albumin, Globulin, Cholesterol, Triglyceride (TG), Calcium (Ca), Phosphorus (P), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Creatinine, Sodium, Potassium, and Chloride were all measured in the serum samples. The serum biochemistry was measured using a semiautomatic biochemistry analyzer.

### Statistical Analysis

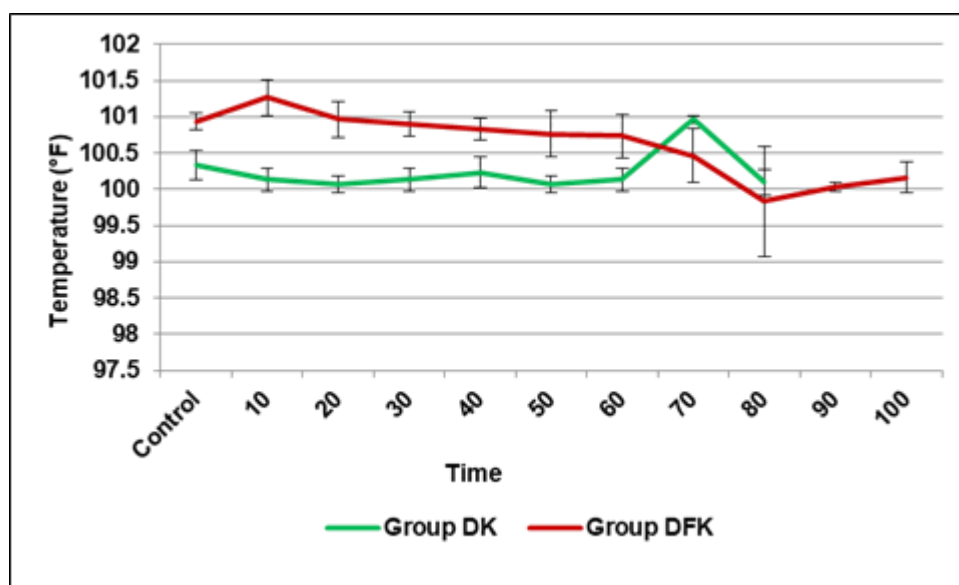
All the data were expressed as Mean  $\pm$  SEM (Standard Error of Mean). To compare data within and between the groups, one-way ANOVA (Analysis of Variance) was done using Statistical Package for the Social Sciences (SPSS) version 20.0. Probability  $P < 0.05$  or less was regarded as statistically significant.

## RESULTS

### Effect of Different Anaesthetic Combinations on Clinical Parameters in Rabbit

#### Effect on Rectal Temperature (RT)

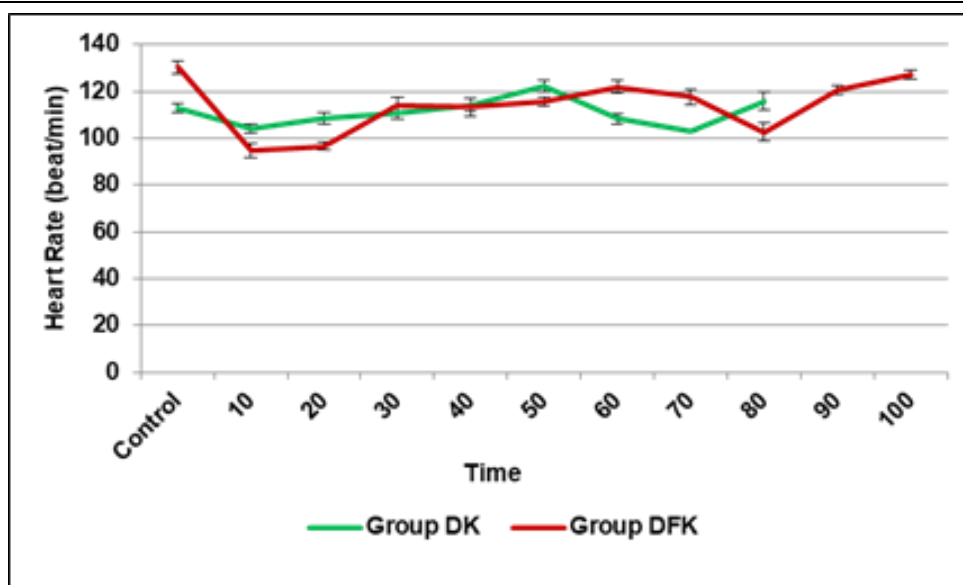
Throughout the experiment, no significant deviations in rectal temperature in the animals in group DK were notified. When compared to the preanaesthetic control value, the rectal temperature in group DFK was considerably lower at 80 minutes during the anaesthetic period (Figure 1).



**Figure 1.** Effects of different anaesthetic combinations on body temperature

#### Effect on Heart Rate (HR)

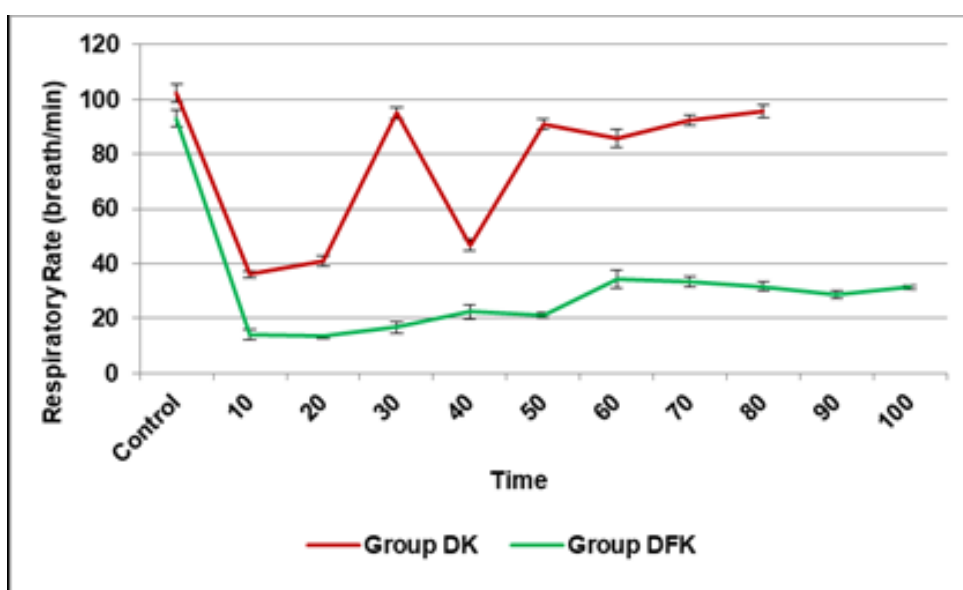
We found significant ( $P < 0.05$ ) changes in the animals of group DFK at various time intervals during the anaesthetic period when compared to the preanaesthetic control value. HR in the animals of group DK was considerably ( $P < 0.05$ ) reduced during the anaesthesia period at 10 and 70 minutes (Figure 2).



**Figure 2.** Effects of different anaesthetic combinations on heart rate

#### *Effect on Respiratory Rate (RR)*

The respiration rate was considerably lower in the animals of group DFK at 10-100 minutes when compared to preanaesthetic control values. When compared to preanaesthetic control values, the RR in group DK animals was considerably lower at 10 minutes (Figure 3).



**Figure 3.** Effects of different anaesthetic combinations on respiratory rate

#### **Comparison of Different Anaesthetic Regimens on the Reflex Responses in Rabbits**

Table 1 shows the effect of different anaesthetic combinations on certain reflexes in rabbits. Animals of both groups lost their righting reflex within 1 minute of induction. The animals in group DFK have the faster disappearance of righting reflex. In comparison to group DFK, the duration of the loss of the palpebral reflex was longer in group DK. In the animals of group DK, the pedal reflexes were never fully lost. The animals in group DK had a faster recovery of the righting reflex. In the DFK group, the withdrawal time of the palpebral reflex was found to be as long as  $114.5 \pm 0.707$  minutes.

**Table 1.** Effect of different anaesthetic combinations on the reflex responses following anaesthesia in rabbits

Group	Loss of Righting Reflex (sec)	Return of Righting Reflex(min)	Loss of Palpebral Reflex (sec)	Return of Palpebral Reflex (min)	Loss of Pedal Reflex (min)	Return of Pedal Reflex (min)
<b>DK</b>	19.5±0.707	88± 2.828	24± 2.828	76.5± 2.121	-	-
<b>DFK</b>	14± 0	116.5±2.121	16.5± 0.707	114.5±0.707	25.5± 0.707	24±2.828

### Comparison of Different Anaesthetic Regimens on the Onset of Induction and Duration of Anaesthesia in Rabbits

Table 2 displays the influence of various anaesthetic combinations on the beginning of induction and duration of anaesthesia. In both the groups, the average onset of the induction period was 19.5± 0.707 sec and 14± 0 sec, respectively. The animals in group DFK had a substantially longer anaesthetic duration (2.255± 0.049h).

**Table 2.** Effect of different anaesthetic combinations in rabbits on the onset of induction and duration of anaesthesia

Anaesthetic Combinations	Onset of Induction Time (sec)	Duration of Anaesthesia (hr)
<b>DK</b>	19.5± 0.707 <sup>a</sup>	1.135±0.035 <sup>a</sup>
<b>DFK</b>	14± 0 <sup>b</sup>	2.255±0.049 <sup>b</sup>

Values with different superscript letters in the same column differ significantly at 5% level of significance. ±: SE

### Effect of Different Anaesthetic Combinations on Biochemical Parameters in Rabbit

When compared to the preanaesthetic control value, the TP values of groups DK and DFK were significantly ( $P < 0.05$ ) lower at 30 minutes during anaesthesia (Table 3). At 30 minutes after anaesthesia, there were significant differences in the serum total protein levels between the groups DK and DFK. At 30 minutes' post-induction, we found no significant ( $P > 0.05$ ) changes in serum albumin levels in the animals of groups DK and DFK. While at 30 minutes after anaesthesia, we noticed a substantial difference in the albumin levels between the groups DK and DFK. The serum creatinine level in group DK animals was significantly ( $P < 0.05$ ) lower at 30 minutes of the onset of anaesthesia. In the group DFK, however, there were no significant changes in creatinine levels were observed at this point. The differences in creatinine values between the groups DK and DFK were determined to be statistically significant. In this study, the serum cholesterol level in the animals of group DK was substantially higher at 30 minutes contrasted to its preanaesthetic control values. When compared to preanaesthetic control values, blood cholesterol levels in group DFK animals were significantly ( $P < 0.05$ ) lower after 30 minutes following induction of anaesthesia. When the groups DK and DFK were compared 30 minutes after the anesthetic, we noted a significant difference in cholesterol levels. At 30 minutes, the triglyceride level in the animals in group DK was substantially ( $P < 0.05$ ) higher than the preanaesthetic control values. The animals in group DFK showed no significant change after 30 minutes. At 30 minutes after anaesthesia, there were significant differences in the triglyceride levels between the groups DK and DFK. When compared to preanaesthetic control values, the value of HDL in the animals of groups DK and DFK was significantly ( $P < 0.05$ ) lower 30 minutes following induction. The value of LDL was significantly ( $P < 0.05$ ) elevated in the animals of groups DK than those of DFK after 30 minutes of induction. At 30 minutes after induction, it was observed that the difference in HDL and LDL values between the DK and DFK groups was statistically significant.

The value of sodium in the animals of group DK was found to be significantly ( $P < 0.05$ ) lower but in the animals of group DFK was found to be significantly ( $P < 0.05$ ) higher at 30 minutes after induction. At 30 minutes following anaesthesia, we noticed a substantial difference in sodium levels between the groups DK and DFK. When compared to the preanaesthetic control values, the potassium level in the animals of groups DK and DFK was significantly ( $P < 0.05$ ) lower at 30 minutes after induction. At 30 minutes after anaesthesia, the difference in potassium levels between the groups DK and DFK was determined to be statistically significant. Calcium and phosphorus levels, on the hand, in the animals of groups DK and DFK were significantly ( $P < 0.05$ ) higher at 30 minutes in both the groups compared to their initial control values. At 30 minutes after induction, the difference in calcium and phosphorus between the groups DK and DFK was found to be statistically significant. At 30 minutes after induction, the value of chloride was significantly ( $P < 0.05$ ) greater in the animals of group DFK. In contrast to preanaesthetic control values, we noticed no significant

change in the animals of group DK at 30 minutes after induction of anaesthesia. At 30 minutes' post-induction, we found significant ( $P>0.05$ ) changes in serum chloride level between the group DK and DFK.

**Table 3.** Effects of different anaesthetic combinations on certain serum biochemical parameters

Parameter	Group	Preanaesthetic Control Value	30 min after induction
Total protein (gm/dl)	DK	6.54± 0.36 <sup>ax</sup>	5.454± 0.028 <sup>bx</sup>
	DFK	6.52± 0.212 <sup>ax</sup>	5.567 ± 0.057 <sup>by</sup>
Albumin (gm/dl)	DK	3.350± 0.212 <sup>ax</sup>	3.127± 0.028 <sup>ax</sup>
	DFK	3.54± 0.30 <sup>ax</sup>	3.237± 0.012 <sup>ay</sup>
Creatinine (mg/dl)	DK	1.37±0.124 <sup>ax</sup>	0.55± 0.05 <sup>bx</sup>
	DFK	1.44±0.09 <sup>ax</sup>	1.584± 0.040 <sup>ay</sup>
Cholesterol (gm/dl)	DK	94.33±0.38 <sup>ax</sup>	97.71± 0.130 <sup>bx</sup>
	DFK	94.69±0.414 <sup>ax</sup>	82.287± 0.689 <sup>by</sup>
Triglyceride (gm/dl)	DK	85.13±2.39 <sup>ax</sup>	117.747 ± 0.143 <sup>bx</sup>
	DFK	83.83±1.514 <sup>ax</sup>	86.457± 0.487 <sup>by</sup>
HDL (gm/dl)	DK	41.54±0.250 <sup>ax</sup>	35.594± 0.020 <sup>bx</sup>
	DFK	44.33±0.455 <sup>ax</sup>	24.634± 0.058 <sup>by</sup>
LDL (gm/dl)	DK	33.44±0.405 <sup>ax</sup>	38.917 ± 0.035 <sup>bx</sup>
	DFK	33.46±0.22 <sup>ax</sup>	40.714± 0.032 <sup>by</sup>
Sodium (mmol/l)	DK	154.14±0.33 <sup>ax</sup>	145.4± 0.556 <sup>bx</sup>
	DFK	154.1±0.53 <sup>ax</sup>	195.534± 0.378 <sup>by</sup>
Potassium (mmol/l)	DK	4.51±0.286 <sup>ax</sup>	3.547± 0.030 <sup>bx</sup>
	DFK	4.46±0.121 <sup>ax</sup>	3.437± 0.015 <sup>by</sup>
Calcium (mg/dl)	DK	9.36±0.35 <sup>ax</sup>	13.3± 0.2 <sup>bx</sup>
	DFK	9.36±0.36 <sup>ax</sup>	11.34± 0.102 <sup>by</sup>
Phosphorus (mg/dl)	DK	2.61±0.209 <sup>ax</sup>	4.184± 0.078 <sup>bx</sup>
	DFK	2.79±0.22 <sup>ax</sup>	3.76± 0.04 <sup>by</sup>
Chloride (mmol/l)	DK	106.9±0.47 <sup>ax</sup>	106.434 ± 0.450 <sup>ax</sup>
	DFK	107.81±0.549 <sup>ax</sup>	130.4± 0.3 <sup>by</sup>

Values with different superscript letters in the same row (a, b) and the same column (x, y) differ significantly at 5% level of significance. ±: Standard Error

## DISCUSSION

Throughout the anaesthetic period, there were no significant changes in rectal temperature in groups DK and DFK in this study. Similar findings have been reported by others (Gonzalez et al. 2003; Oguntayo and Oke 2014). The heart rate of the animals in group DK was considerably higher at 50 minutes than it was before anaesthesia. In the animals of group DFK, the heart rate was dramatically reduced at 10- 20 minutes, then gradually increased throughout the anaesthetic period before returning to preanaesthetic control values at 100 minutes. Similar findings have been reported by others (Gil et al. 2004; Chen 2015). They found that the sympathetic provocation of ketamine and the limited cardiovascular influence of diazepam raised heart rate. In another study, the heart rate was marginally affected by hypertension and cardio-stimulant consequences of dissociative anaesthetic agents (Dupras et al. 2001).

In this investigation, the RR was first reduced from its preanaesthetic control values, then fluctuated up to recovery in both groups of animals. In group DFK, there was much higher respiratory depression. Thurmon (2007) found that diazepam-ketamine combination reduced respiratory rate in rats, which matched our findings. Ketamine, on the other hand, decreased respiratory rate due to CNS depression and a reduction in the respiratory center's sensitivity to carbon dioxide (Dubois et al. 2004).

Several investigations have identified the loss of the righting reflex, palpebral reflex, and pedal withdrawal reflex as the onset of anaesthesia, while recovery has been described as the reappearance of all of these reflexes (Henke et al. 2005; Karasu et al. 2018). Because no surgical intervention was conducted in this investigation, the surgical anaesthetic duration was measured using the righting reflex, palpebral reflex, and pedal withdrawal reflex. When compared to the other groups, group DFK had the longest duration of anaesthesia and the shortest period of loss of righting and palpebral reflex. Karasu et al. (2018) and Bienert et al. (2014) described that in adults, healthy rabbits' reflex loss and



return timings vary according to the dose of anaesthetic regimes, with higher doses resulting in longer sedation durations and longer pedal withdrawal reflex recovery times. The administration of a combination of diazepam, fentanyl, and ketamine may have produced the difference in the periods of loss and recovery of the righting and palpebral reflexes in this investigation.

In this study, we found that after 30 minutes, the values of TP and albumin in both groups of rabbits were lower than their preanaesthetized control values. The decrease in TP and albumin values under intramuscular anaesthesia in rabbits was reported by Akter et al. (2020) in sheep, which is consistent with this finding. The decrease in TP and albumin in this study could be related to anaesthetic drug haemodilution and haemodynamic alterations in cell membrane permeability. Because haemodilution lowers the haematocrit, the serum protein content may drop Orr et al. (2005). At 30 minutes after induction, animals in the DFK group had higher values of total protein and albumin than those in the DK group. It might be because DFK combinations have a long-lasting analgesic effect. When compared to preanaesthetic control values, serum creatinine levels in group DK were lower at 30 minutes. Rahman et al. (2021) found an increase in serum creatinine 5 minutes following xylazine-ketamine treatment in sheep, which contradicts our findings. In this experiment, the DFK group's serum creatinine levels at 30 minutes after induction were higher than those of the animals in the DK group. The anaesthetic mixture (DFK) may cause an increase in serum creatinine due to a decrease in glomerular filtration rate, hypotension, and hypoxaemia (Dubois et al. 2004), which is agreed to this finding. However, in this investigation, lower creatinine concentrations in DK group could be linked to the anaesthetics' short-term effects on renal function.

The liver and the stress response have a direct impact on serum cholesterol levels. At 30 minutes after induction, blood cholesterol levels were considerably lower in group DK and significantly higher in group DFK in this investigation. Similar findings have been reported by others (Gil et al. 2004). At 30 minutes after anesthesia, the DFK group's animals had lower serum cholesterol values than the DK group's animals. The increase in serum cholesterol levels following diazepam-ketamine may be related to diazepam, which is a hepatotoxic medication. Lipolysis caused an increase in serum cholesterol levels. Under the stimulus of catecholamines, lipolysis increases, and corticosteroids aid fat mobilization. The most frequent form of lipid storage is triglycerides, which are an important source of energy. This conclusion is in line with the findings of Gil et al. (2004).

Some authors have reported an increase in plasma triglyceride levels after diazepam-ketamine injection (Hedenqvist et al. 2001), which is consistent with our findings in groups DK and DFK. In both groups, the HDL value was considerably lower at 30 minutes compared to preanaesthetic control values. However, the LDL value was considerably higher at 30 minutes compared to preanaesthetic control values in the animals of both groups. Similar findings have been reported by others (Venkatesan et al. 2006; Perumal et al. 2007). As opposed to the animals in the DK group, the DFK group's animals had lower HDL values and higher LDL values at 30 minutes' post-induction. Because ketamine promotes sympathetic nerve activity, ketamine anaesthesia decreased serum HDL levels while increasing serum LDL levels. The sympathetic nervous system increases the concentration of free fatty acids in plasma by promoting lipolysis in adipose tissue. In this investigation, the value of sodium was lower in group DK, contrary to Gil et al. (2004) findings. The value of sodium, on the other hand, was increased in group DFK, which corresponded with the findings of the previous study (Gil et al. 2004). As a result, the elevated serum sodium concentrations are probably linked to a decrease in renal blood flow. Potassium levels in all groups of animals were lower than preanaesthetic control levels. After the injection of fentanyl, which appears to modify the function of anaesthetic regimens, hypokalemia may arise in the DFK group.

In this investigation, the calcium value of the animals in groups DK and DFK was raised at 30 minutes when compared to preanaesthetic control values. Similar findings have been reported by others (Gil et al. 2004; Grint and Murison 2008). All groups' serum phosphorus concentrations were higher at 30 minutes than preanaesthetic control values, which is consistent with the findings of Khalaf et al. (2014) and Gallego (2017). During renal failure, the level of phosphorus rises. Increased phosphorus concentrations after DK treatment may be linked to a decrease in renal blood flow. Benzodiazepines diminish glomerular filtration rates, and ketamine-diazepam alters the renal anatomy of rabbits, according to other researchers (Zahir et al. 1995).

From preanaesthetic control levels, serum chloride concentration fell in group DK but increased in group DFK. Gil et al. (2004) found a considerable increase in serum chloride levels after DK treatment, which corroborated this finding. Because the kidney is the principal site of excretion of this electrolyte, decreased renal blood flow owing to diazepam could be the source of the higher plasma chloride levels (Bienert et al. 2014).



## CONCLUSIONS

Based on the findings of the clinical serum biochemical profile, it is suggested that diazepam-fentanyl-ketamine produced sufficient depth and duration of anaesthesia compared to diazepam-ketamine. Both combinations produced similar changes in serum biochemistry. Thus, for delicate and time-consuming surgery, diazepam-fentanyl-ketamine combination may be useful.

## ACKNOWLEDGEMENTS

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## ETHICAL STATEMENT

During the writing process of the study titled "Comparative Clinical and Serum Biochemical Evaluation of Two Intramuscular Anaesthetic Combinations (Diazepam/Ketamine and Diazepam/Fentanyl/Ketamine) in Rabbits", scientific rules, ethical and citation rules were followed; No falsification has been made on the collected data and this study has not been sent to any other academic media for evaluation. The study has been conducted under the guidelines provided by Animal Welfare, Experimentation and Ethics Committee (AWEEC) of the Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh [Permission number (AWEEC/BAU/2021 (45))].

## CONFLICT OF INTERESTS

The authors declared no conflict of interest.

## AUTHORS CONTRIBUTION

Akter MA, Yesmin N: Conducting experiment, acquisition of data. Talukder MBA: Conducting experiment, writing manuscript. Alam MM. Overall supervision, editing manuscript and final approval.

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