Research Article

Comparative analysis of some hematobiochemical and electrocardiographic changes following administration of medetomidine alone and combination with selected opioid agonists in Goats

Maryam Salarpoor[a], Amir Saeed Samimi[a *], Ehsanollah Sakhaee[a], Omid Azari[a], Zahra Saminzadeh[a]

[a]Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

*Corresponding author: Dr. Amir Saeed Samimi

email address: Samimi@uk.ac.ir

Keywords: α_2-adrenergic agonists, Opioids, Hematobiochemical parameters, Electrocardiogram, Goats

Abstract

Medetomidine is effective for sedation in small ruminants and is often combined with opioids to tailor depth and mitigate adverse effects. We conducted a randomized Latin-square crossover study in goats (n=10) comparing five intravenous regimens: medetomidine 20 µg/kg alone (MED) or combined with methadone 0.5 mg/kg (MME), morphine 0.5 mg/kg (MMO), tramadol 5 mg/kg (MTR), or pethidine 1 mg/kg (MPE). Drugs were administered via the left jugular vein over 2 min. Blood was collected at 0, 120 min, and 24 h for WBC, PCV, cardiac troponin I (cTnI), homocysteine (Hcy), and ALT/AST; bipolar-limb ECGs were recorded at 0, 5, and 120 min. Baseline hematologic, biochemical, and ECG values did not differ across periods. cTnI remained within assay reference limits with no within-regimen change, although between-regimen differences appeared at 120 min and 24 h, with MED lower than methadone/tramadol at 120 min and lower than pethidine/tramadol at 24 h. Hcy showed no intergroup differences but increased modestly only with MED at 24 h. Hematologic effects were regimen-specific: WBC decreased with MMO at 2 h ($p<0.05$), and PCV decreased with MTR at 2–24 h ($p<0.05$). Hepatic enzymes showed no significant treatment or time effects. On ECG, PR and QT intervals lengthened over

time (notably with MED and MMO), RR intervals generally prolonged consistent with bradycardia, and MPE increased T-wave amplitude ($p<0.05$). Overall, medetomidine–opioid combinations produced distinct hematologic and ECG signatures without detectable within-regimen cTnI elevation. These data support individualized opioid selection with routine ECG monitoring when using medetomidine in goats.

List of Abbreviations

Medetomidine (MED), medetomidine/methadone (MME), medetomidine/morphine (MMO), medetomidine/ tramadol (MTR), medetomidine/pethidine (MPE) , alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), glomerular filtration rate (GFR), Homocysteine (Hcy), Cardiac troponin I (cTn I), Kilo Dalton (kDa), white blood count (WBC), packed cell volume (PCV), Kolmogorov-Smirnov (K-S), Chi-squared (χ^2), electrocardiogram (ECG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), atrioventricular (AV), heart rate (HR), respiratory rate (RR), rectal temperature (RT), capillary refill time (CRT), ruminal motility (RM), Ethylenediaminetetraacetic acid (EDTA), enzyme-linked immunosorbent assay (ELISA), Analysis of Variance (ANOVA), Institutional Animal Care and Use Committee (IACUC)

Introduction

Anesthetic and sedative agents are essential in both surgical and non-surgical procedures for humans and animals alike [1, 2]. Alpha-$_2$ adrenoceptor agonists' medications (such as medetomidine and xylazine) are commonly performed in veterinary medicine to induce sedation, provide analgesia, and facilitate anesthesia [3]. Ruminants have α2 adrenergic receptors, which makes them sensitive to their sedative effects [4].

Opioids such as tramadol, methadone, pethidine and morphine are routinely used to provide sedation and analgesia during surgery and also to control severe arthritis pain, as well [5]. However, the compounds are associated with several adverse side effects, including notable respiratory depression, constipation, urinary retention, and bradycardia [6].

Medetomidine has high α_2 receptor selectivity and shows fewer significant side effects compared to xylazine, detomidine and romifidine [7]. The combination of medetomidine with selected opioids has been shown to reduce adverse effects while providing effective somatic analgesia and rapid sedation onset, accompanied by minimal cardiorespiratory complications [8, 9].

There are several studies documenting changes in hematologic and biochemical parameters following anesthetic treatments in ruminants [10]. Biochemical parameters related to cardiovascular and hepatic function are the most important diagnostic tests, can be an effective step in determining the effect of drugs on the body of animals.

Hepatic enzyme evaluation remains a common component of biochemical monitoring during sedative and anesthetic protocols in veterinary medicine. Although alanine aminotransferase (ALT) is a highly specific indicator of hepatocellular injury in small animals, its diagnostic value in ruminants is limited because hepatic ALT activity is low and extrahepatic tissues contribute minimally to serum levels. Nevertheless, ALT measurement can still provide supplementary information when interpreted alongside other markers. In contrast, aspartate aminotransferase (AST) is more widely distributed in ruminant liver, cardiac, and skeletal muscle, and thus serves as a sensitive but non-specific index of hepatocellular and myocellular injury. The combined interpretation of AST and ALT can therefore offer a broad overview of hepatic and muscular enzyme activity during pharmacologic interventions. In the absence of liver-specific enzymes such as sorbitol dehydrogenase (SDH) or glutamate dehydrogenase (GLDH), simultaneous measurement of AST and ALT remains a practical approach for evaluating general hepatocellular integrity in ruminants under experimental or clinical conditions [11-14].

Homocysteine (Hcy) is a sulfur-containing amino acid and is a byproduct of methionine metabolism. It has been reported that high level of serum Hcy can be employed as a risk factor for a number of disorders including vascular and neurodegenerative and renal diseases [15]. Cardiac troponin I (cTn I) is a 23.8 kDa cardiac protein, acting to inhibit muscle contraction in the absence of calcium [16]. It is a highly specific and sensitive biomarker for a spectrum of cardiovascular disease such as acute myocardial infarction, coronary heart disease, and heart failure [17]. The

effects of various anesthetics on cardiovascular and hepatic functions are not yet well demonstrated in goats.

Analysis of electrocardiographic variables is essential for evaluating health, nutritional status, disease differential diagnosis, and drug pharmacodynamics in animals [18, 19]. Electrocardiography is a noninvasive, affordable, and valuable diagnostic tool for assessing cardiac electrical activity [20]. The significance of electrocardiographic parameters following the administration of adrenergic α2-agonists in specific animal species has been highlighted [21, 22].

Our hypothesis proposed that administering medetomidine, alone and in combination with intravenous opioids, could induce changes in electrocardiographic and biochemical parameters related to cardiac and hepatic functions in goats. This would enable us to identify the most effective sedative combination for livestock.

To the best of our knowledge, this is the first report that combines medetomidine, medetomidine with opioids (tramadol, methadone, pethidine, and morphine) and compares changing in hepatorenal, cardiovascular and electrocardiographic parameters in goats.

Results

Descriptive parameters for the WBC variable across the studied groups are presented in Fig 1. A significant difference was observed in the morphine group ($p < 0.05$). Furthermore, the paired samples t-test revealed that the WBC variable in the morphine group demonstrated a significant decrease at 2 hours after treatment compared to the pre-treatment time (time 0) ($p < 0.05$).

The PCV showed a significant time effect only in the tramadol group ($p < 0.05$), whereas no temporal changes were detected in the other groups ($p \geq 0.05$). A statistically significant decrease in the PCV variable level in the tramadol group 24 hours after treatment compared to the time before treatment. ($p < 0.05$) (Fig 2).

The results showed significant differences in the mean WBC variable among the treatment groups at the time points ($p < 0.05$). The mean WBC variable at 0 time was significantly higher in the methadone and tramadol treatments compared to the other treatments ($p < 0.05$). The mean

WBC variable at 2 hours after treatment in the tramadol group was significantly higher than in both the morphine and pethidine groups ($p < 0.05$). At 24 hours after treatment, the mean WBC variable in the tramadol treatment was significantly higher than in the morphine, medetomidine, and pethidine treatments ($p < 0.05$). However, no significant differences were noted between the tramadol and methadone treatments, nor among the morphine, pethidine, methadone, and medetomidine treatments ($p > 0.05$). Additionally, no significant differences in the mean PCV variable among the studied treatments at the studied time points.

Baseline mean cTnI values were similar among all groups ($p > 0.05$), indicating comparable pre-dose conditions. Repeated-measures ANOVA showed no significant time-dependent changes in cTnI within any treatment ($p > 0.05$ for all). Mean concentrations remained within assay-appropriate reference limits throughout the 24-hour period.

However, between-group comparisons revealed significant differences in cTnI at 120 minutes ($F_{4,45} = 3.81$, $p < 0.05$) and 24 hours ($F_{4,45} = 4.20$, $p < 0.05$) post-injection. Post hoc Tukey testing demonstrated that cTnI levels at 120 minutes were significantly lower in the medetomidine group compared with the methadone and tramadol groups ($p < 0.05$). Similarly, at 24 hours, cTnI was significantly lower in medetomidine-treated animals than in those receiving pethidine or tramadol ($p < 0.05$). No other between-group differences were observed. Overall, cTnI values across all regimens remained within physiological limits, suggesting no biochemical evidence of myocardial injury (Fig 3).

A significant time effect of Hcy only in the medetomidine group ($F_{2,18} = 4.58$, $p = 0.025$, $\eta^2 = 0.34$, power = 0.70). Paired-sample t-test analysis indicated that Hcy in this group increased significantly at 24 hours compared with baseline by 0.61 μmol/L (95% CI: 0.09–1.13; $p = 0.01$). In contrast, changes in Hcy over time were not statistically significant in the morphine, methadone, tramadol, or pethidine groups ($p > 0.05$).

One-way ANOVA demonstrated no significant intergroup differences in Hcy concentrations at baseline, 120 minutes, or 24 hours (all $p > 0.05$). Thus, the transient increase observed with medetomidine likely reflects a mild, regimen-specific metabolic response rather than a pathologic effect (Fig 4).

The changes in the studied liver biomarkers among the treatment groups at the time points are illustrated in the following figures (Fig 5 and 6).

Results of the friedman test showed no significant effect of sampling times on the mean P amplitude component in the medetomidine (χ^2=0.333, p=0.846), methadone (χ^2=3.938, p=0.140), and pethidine (χ^2=1.727, p=0.422) groups. However, significant temporal differences were observed in the morphine (χ^2=8.720, p=0.013) and tramadol (χ^2=7.00, p=0.030) groups. Results of the Wilcoxon signed-rank tests indicated a significant decrease in mean P amplitude at 120 minutes post-injection compared to baseline in both morphine and tramadol groups ($p<0.05$) (Fig 7). For QRS amplitude, no significant time effect was found in morphine, methadone, tramadol, and pethidine groups, but medetomidine showed a significant increase at 120 minutes post-injection compared to 5 minutes post-injection (χ^2=6.414, p=0.040; Wilcoxon $p<0.05$) (Fig 8). Regarding T amplitude, significant changes over time were only noted in the pethidine group (χ^2=13.130, p=0.001), with increases at 5 and 120 minutes post-injection compared to baseline (Wilcoxon $p<0.05$) (Fig 9).

Results of the Kruskal-Wallis test revealed no significant differences among groups in mean P amplitude before injection (p=0.372) or at 120 minutes post-injection (p=0.349), but a significant difference at 5 minutes post-injection (p=0.024). Pairwise Mann-Whitney tests showed medetomidine differed significantly from morphine, tramadol, and methadone at this time point. For QRS amplitude, no group differences were found before injection or at 5 minutes, but significant differences emerged at 120 minutes post-injection (p=0.0090, with medetomidine differing from pethidine, tramadol, and methadone. T amplitude showed no baseline differences, but significant group differences were found at 5 and 120 minutes post-injection($p<0.05$). Pairwise comparisons indicated significant differences involving medetomidine, pethidine, tramadol, and morphine groups at these times.

No significant temporal changes in P duration were observed in medetomidine, morphine, methadone, or tramadol groups, but pethidine showed a significant increase at 120 minutes post-injection (p=0.009) (Fig 10). QRS duration showed significant time effects in morphine (p=0.048) and pethidine (p=0.028) groups, with morphine showing a decrease at 120 minutes compared to 5 minutes, and pethidine showing decreases at 5 and 120 minutes compared to baseline (Fig 11). T

duration was significantly altered only in the morphine group (p=0.025), with an increase at 120 minutes compared to 5 minutes post-injection (Fig 12).

No significant differences among groups were found for P duration at any time point. Similarly, QRS duration and T duration showed no significant group differences before or after injection.

PR interval showed no significant time effect in methadone, tramadol, or pethidine groups, but significant increases over time were found in medetomidine and morphine groups (p=0.001) (Fig 13). Wilcoxon tests confirmed significant increases at 5 and 120 minutes post-injection compared to baseline. QT interval showed significant temporal changes in all groups ($p<0.05$). Wilcoxon tests indicated significant increases at 5 and 120 minutes post-injection compared to baseline in morphine, methadone, tramadol, and pethidine groups; medetomidine showed a significant increase at 5 minutes (Fig 14). RR interval was not significantly changed over time in tramadol but showed significant increases in medetomidine, morphine, methadone, and pethidine groups at various post-injection times (Fig 15).

Discussion:

Various α_2 agonist drugs and opioids are widely used to provide sedation, analgesia, and restraint in small ruminants [3, 23].

There was a synergistic effect in combining α2 agonist drugs with opioids. Alpha-$_2$ agonist drugs act by binding to α_2 receptors in the brain and spinal cord. In the present study, the doses of medetomidine, tramadol, morphine, methadone, and pethidine used were based on previous studies on small ruminants [24, 25].

Alpha-$_2$ agonist drugs can cause hypoxemia in healthy ruminants and serious complications in animals with pre-existing hypoxia. Therefore, it is important to check PCV before anesthetic drugs because animals with a PCV of less than 25% can be at risk of complications during anesthesia [26].

Our results demonstrated that the mean PCV variable in the medetomidine, morphine, methadone, and pethidine groups did not show statistically significant differences at studied time

points which indicating overall stability in red blood cell volume. But tramadol caused a significant reduction of PCV, 24 hours after treatment that was similar to that reported with the use of dexmedetomidine and xylazine. A previous study showed that dexmedetomidine and xylazine cause a severe drop in PCV in sheep [10]. This finding was not consistent with the report of Bani Ismail *et al.* In their study, they investigated the effect of diazepam, xylazine, and ketamine anesthesia on clinical pathological factors in small ruminants. Their results showed that PCV was significantly increased after 24 h in sheep and 2 h in goats [27].Clinically, all PCV values, remained within caprine reference ranges, indicating preserved oxygen-carrying capacity; the drop likely reflects transient hemodilution/splenic sequestration and merits observation only.

In ruminants, hypoxemia with α2-agonists mainly stems from effects in the lungs: they quickly disturb ventilation; perfusion balance and make the air–blood barrier leaky, a response amplified by resident intravascular macrophages. The accompanying fall in PCV most likely reflects temporary pooling of red cells in the spleen and mild dilution of the blood, rather than true anemia, supporting straightforward measures such as oxygen supplementation and routine hemodynamic monitoring during α2-based sedation [28, 29].

Our results indicated that the mean WBC variable for the medetomidine, methadone, tramadol, and pethidine groups did not exhibit statistically significant differences at the time points. These findings were in agreement with the previous studies after xylazine-ketamine and ketamine alone administration in sheep [30, 31].

In the present study, a significant reduction in WBC was observed in morphine group, these changes can be attributed to the stress of moving the animal and the pain caused by the jugular intravenous injection during anesthesia [32]. Because a differential was not performed, the observed WBC change is compatible with (but not diagnostic of) a stress response. Future work should include manual/automated differentials (neutrophils, lymphocytes, eosinophils, eosinophils, monocytes) and adjunct measures to confirm mechanism.

Previous studies have shown that anesthetic drugs can modulate immune responses, potentially leading to changes in WBC. Clinically, all WBC values stayed within caprine reference ranges. The morphine-associated drop is most consistent with a transient stress response, not clinically significant leukopenia.

In addition, the reason for the decrease in PCV and WBC could be due to the decrease in the accumulation of circulating blood cells in the spleen due to the decrease in sympathetic activity. Also, the decrease in PCV during the anesthesia period may be attributed to the transfer of fluid from the extravascular to the intravascular compartment of the animals to maintain normal cardiac output [33].

The importance of analyzing cardiovascular biomarkers during sedation by α_2 adrenergic agonists has been emphasized for specific animal species [34]. Hcy and cTn I are known as cardiovascular biomarkers [35].

In our crossover study, baseline levels of cTnI and homocysteine (Hcy) were comparable across treatment groups, supporting reliable within-subject analyses. Throughout the 24-hour period, cTnI levels remained stable within each regimen and stayed within assay-specific reference ranges. Minor inter-regimen differences; where certain opioid combinations produced slightly higher values than medetomidine, are more likely due to pharmacodynamic effects rather than myocardial injury. This observation aligns with existing data on healthy goats, which typically show minimal cTnI levels [37], though it contrasts with reports in other species (e.g., canines),[38] where α_2-agonists or α_2–opioid combinations have been associated with slight post-sedation increases. These findings highlight the importance of species- and protocol-specific interpretations. Homocysteine showed a modest increase only in the medetomidine group, with stable levels in others. Given the lack of standardized diagnostic thresholds for Hcy in goats and its known sensitivity to metabolic and stress-related variables, this isolated elevation carries uncertain clinical weight. Overall, the biomarker pattern (cTnI remaining within normal limits and a regimen-specific but limited Hcy change) suggests no evidence of myocardial injury over 24 hours. These results support clinical monitoring over intervention in healthy animals, though isolated biomarker fluctuations should not be overinterpreted without corresponding clinical signs, echocardiography, or extended follow-up.

Liver biomarker concentrations (ALT, AST) are changed by conditions such as hepatocytes, fibrosis and cirrhosis, hepatomegaly, and inflammatory conditions such as hepatitis. The use of sedatives and narcotics (inappropriately or in high doses) can cause hepatotoxicity [39, 40]. The results of our study indicated that hepatic biomarkers did not show statistically significant

differences 24 hours after administration, which is consistent with a previous study [34]. Other previous studies were not consistent with the results of the present study and reported that liver enzyme levels changed significantly 24 hours after administration [42]. Another study reported that tramadol use increased the level of the liver enzyme ALT [2]. Clinically, ALT/AST stayed within reference limits at 24 h, indicating no hepatocellular injury. Given ALT's limited utility in ruminants, the unchanged AST profile is particularly reassuring.

Because ALT is not a reliable marker of hepatocellular injury in ruminants, normal ALT values do not exclude liver damage. In large animals, SDH and GLDH are preferred for detecting hepatocellular leakage, while AST lacks specificity and GGT reflects cholestasis; future studies should include these analytes for a definitive hepatic assessment.

This study systematically evaluated the temporal effects of various analgesic and sedative agents-medetomidine, morphine, methadone, tramadol, and pethidine on electrocardiographic (ECG) amplitude, duration, and interval parameters. The findings reveal distinct drug-specific patterns of cardiac electrophysiological response, with implications for both clinical and research settings.

The significant reduction in P wave amplitude observed at 120 minutes post-injection in the morphine and tramadol groups suggests a potential depressant effect on atrial depolarization. Previous studies have demonstrated that opioids, particularly morphine, can modulate autonomic tone and reduce sympathetic activity, which may underlie these changes [43]. In contrast, medetomidine, an α2-adrenergic agonist, induced a significant increase in QRS amplitude at 120 minutes, consistent with its known effects on cardiac conduction and contractility through enhanced vagal tone and decreased sympathetic outflow [44, 45]. The significant increases in T wave amplitude in the pethidine group at both 5 and 120 minutes post-injection may reflect alterations in ventricular repolarization, possibly mediated by direct myocardial effects or changes in electrolyte balance, as previously reported for synthetic opioids [41].

Duration parameters were less consistently affected. Notably, Pethidine significantly prolonged P wave duration at 120 minutes, while morphine and Pethidine both affected QRS duration, albeit in opposite directions. The shortening of QRS duration with morphine and pethidine may have different effects on ventricular conduction velocity and could be mediated by their different receptors and intrinsic cardiac functions [43]. The increase in T wave duration in

the morphine group aligns with prior reports of opioid-induced changes in ventricular repolarization [46].

The PR interval was significantly prolonged in both Medetomidine and morphine groups, consistent with enhanced atrioventricular (AV) nodal delay. This is in agreement with the known bradycardic and AV-nodal slowing effects of α2-agonists and opioids [44, 45]. The QT interval was significantly increased in all groups, a finding that warrants clinical attention due to the risk of proarrhythmic effects, particularly torsade's de pointes, associated with QT prolongation [47]. The RR interval was also prolonged in most groups, reflecting drug-induced bradycardia, especially with medetomidine and morphine, which is well-documented in the literature [44].

Intergroup analyses revealed that medetomidine and morphine most consistently induced significant changes in ECG parameters compared to other agents, particularly at early (5 min) and late (120 min) post-injection time points. These findings are clinically relevant, as they underscore the need for careful cardiac monitoring during and after administration of these drugs, especially in patients with pre-existing cardiac conditions.

The observed drug-specific ECG changes highlight the importance of individualized drug selection and monitoring in both veterinary and human clinical practice. While the study provides valuable insights, limitations include the lack of direct measurement of serum drug concentrations and the absence of long-term follow-up for arrhythmic events. Future studies should integrate continuous ECG monitoring and explore the underlying molecular mechanisms.

Overall, none of the opioids eliminated the characteristic cardiovascular effects of medetomidine, although the risk profile differed by agent. Morphine with medetomidine produced more marked atrioventricular nodal delay and bradycardia (PR and RR prolongation) and a transient fall in total WBC, making it less suitable in animals with conduction disease or high vagal tone. Tramadol with medetomidine caused a significant reduction in PCV at 24 hours (still within reference limits), consistent with relative hemodilution or splenic sequestration and less desirable when anemia or peri-procedural blood loss is a concern. Pethidine with medetomidine increased T-wave amplitude at early and late times, warranting caution in patients with repolarization abnormalities or concurrent QT-prolonging therapies. In contrast, methadone with medetomidine showed a neutral hematologic profile and did not exacerbate conduction delay, offering a more balanced option when an opioid adjunct is needed. Across regimens, cardiac troponin I remained

within reference limits without within-regimen rises, and homocysteine was stable except for a modest 24-hour increase with medetomidine alone, supporting monitoring rather than intervention in healthy animals. Although some between-group differences reached statistical significance, their absolute magnitudes were small and largely within caprine reference intervals, reinforcing individualized opioid selection based on comorbidities (conduction disease, anemia risk, repolarization concerns) and the routine use of electrocardiographic and oxygenation monitoring.

Conclusion

In summary, among medetomidine and opioid combinations in healthy goats, methadone with medetomidine showed the most neutral overall profile, with no hematologic disturbance and no atrioventricular nodal delay beyond medetomidine's class effect. By comparison, morphine with medetomidine accentuated atrioventricular nodal delay and bradycardia, tramadol with medetomidine produced a reduction in PCV at 24 hours, and pethidine with medetomidine increased T wave amplitude. Cardiac troponin I remained within reference limits without rises within each regimen, and homocysteine rose modestly only with medetomidine alone. Clinically, opioid choice should be individualized: avoid morphine in animals with conduction disease, avoid tramadol where anemia risk or hemodilution is a concern, use pethidine cautiously in repolarization abnormalities, and consider methadone when a balanced adjunct is desired, recognizing that observed effects were modest and largely within species reference ranges under the conditions of this study.

Materials and methods

Animals

In accordance with international legal frameworks, the present article ensures the protection and respect for animal rights. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study involving ten goats was conducted with the approval of the Animal Welfare Committee at the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman (IR.UK.VETMED.REC.1399.023). The selected goats had a mean age of 12±4 months and an average weight of 22±3 kg. All goats were female.

These animals were chosen using a simple randomization method, specifically a sample lottery approach, ensuring unbiased selection. All goats were housed in a single group pen where they experienced uniform environmental, nutritional, and management conditions.

The goats were provided with a consistent diet that included a mix of roughages, primarily alfalfa hay and wheat straw, and concentrates, consisting of barley grain, soybean meal, and wheat bran. This diet was formulated to meet their physiological maintenance needs, maintaining a forage-to-concentrate ratio of 90:10. Additionally, the goats had access to sufficient vitamins, minerals, and fresh water to support their overall health. To prevent potential internal and external parasitic infestations, all animals were treated with broad-spectrum antiparasitic medications two months prior to the experiment.

Routine health assessments were conducted to monitor the well-being of the goats. These assessments included clinical examinations that measured heart rate (HR), respiratory rate (RR), rectal temperature (RT), capillary refill time (CRT), and ruminal motility (RM). Furthermore, paraclinical evaluations were performed, which involved hematological tests white blood count (WBC) and packed cell volume (PCV) and fecal analysis for parasites.

Two days before the study began, the animals underwent comprehensive clinical and paraclinical evaluations to confirm their normal health status. This step is essential to ensure that any effects observed during the experiment are attributable to the treatments rather than pre-existing conditions.

To standardize physiological measurements, food was withheld from the animals for 12 hours and water for 6 hours prior to the experiment. This fasting period helps reduce variability related to digestion and hydration. This controlled setting minimizes external stressors that could impact animal behavior and physiological responses.

The animals were weighted prior to each treatment to determine the appropriate drug dosages. At any given time, two animals were studied each time separately, and they were kept from seeing one another. The animals were carefully restrained on a specialized bed, which was placed on a soft mattress, in a calm, enclosed area measuring 5×6 m^2. They rested for 20 minutes prior the initial measurements of clinical and para-clinical variables were taken.

The area over the left jugular vein was surgical prepared for intravenous administration. Clinical and para-clinical assessments were conducted again 48 hours post-experiment to assess the animal's health condition.

Treatments

The study involving goats utilized a randomized crossover Latin square design to evaluate the effects of five intravenous treatments. Each goat was assigned to receive one of the treatments at a time, with a minimum washout period of 8 days between each treatment to ensure that any effects from the previous treatment has dissipated before the next one was administered [21,22].

The study used a randomized crossover Latin-square design with five intravenous regimens:

MED: medetomidine (DorbeneVet; N-Vet AB, Sweden) 20 µg/kg IV, a dose widely used/recommended for small ruminants and supported by recent goat/sheep studies. (BioMed Central)[48].

MME: medetomidine (20 µg/kg) + methadone (Faran Shimi Pharmaceutical Company, Iran) 0.5 mg/kg IV, within the reported 0.05–0.5 mg/kg range for sheep/goats and used in goat studies combining α2-agonists with opioids [25].

MMO: medetomidine (20 µg/kg) + morphine (Dimorf; Cristália Produtos Químicos e Farmacêuticos Ltda) 0.5 mg/kg IV, consistent with small-ruminant recommendations (0.2–0.5 mg/kg), and within pharmacokinetic dose ranges evaluated in goats [49].

MTR: medetomidine (20 µg/kg) + tramadol (Tehran Chime Pharmaceutical Company, Iran) 5 mg/kg IV, a dose supported by sheep antinociception data (4–6 mg/kg IV) and used in recent goat work with α2-opioid combinations [25].

MPE: medetomidine (20 µg/kg) + pethidine (Caspian Tamin Pharmaceutical Company, Iran) 1 mg/kg IV; a conservative IV dose selected to limit histamine-mediated effects recognized for meperidine, while remaining within reported efficacious IV ranges in sheep (antincioception at 5 mg/kg IV) [25]

For all treatments, drugs were prepared in a single syringe and diluted with 0.9% saline to a final volume of 5 mL, then administered via the left jugular vein over 2 minutes using an 18-gauge needle, with animals standing, to standardize injection rate/volume across regimens.

Blood sample collection

Blood samples were taken from the jugular vein of the goats at the beginning of the study, 120 min and 24 hours after the start of the study.

Hematology: PCV and WBC measurement

Hematology in this study was limited to serial PCV and total WBC by design; RBC indices, platelet counts, and leukocyte differentials were not obtained.

Jugular venous blood (2 mL) was collected into K_2-EDTA tubes, gently inverted 8–10 times, and analyzed within 2 hours of collection; hemolyzed or clotted specimens were excluded.

Packed cell volume (PCV): Whole blood was drawn into heparinized microcapillary tubes, sealed with clay, and centrifuged in a microhematocrit centrifuge (Sigma-Aldrich, Model 5254, Germany) for 5 minutes. The hematocrit was read with a microhematocrit card-reader and recorded as a percentage. Each sample was run in duplicate and the mean reported.

White blood cell count (WBC), WBC was determined by hemocytometer using Natt–Herrick diluent. Briefly, well-mixed EDTA blood was diluted 1:200 in Natt–Herrick solution, mixed for 2–3 minutes, and the improved Neubauer chamber was charged. After a short settling period, leukocytes were counted in the four large corner squares on each grid (both sides), and the average count was used for calculation of WBC.

Biochemical parametrs

Blood samples from all animals were collected via the jugular vein and stored in tubes without anticoagulants. Subsequently, after clotting, serum was separated using a centrifuge (3000 rpm) for 15 minutes. The serum concentrations of hepatic biomarkers such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as serum cardiac biomarkers including cardiac troponin I (cTn I) enzyme and homocysteine (Hcy) were measured using a specific commercial kit with the ELISA method.

Electrocardiogram

Heart rate monitoring was performed on all animals using the bipolar limb lead derivation method and the Cardiomax FX-2111 electrocardiograph machine (Fukuda, Japan) at 0, 5, and 120 minutes. The paper speed on the electrocardiograph machine was set at 25 millimeters per second, and the voltage setting was considered as 1 millivolt equivalent to 10 millimeters. The heart rate recording was done after stabilizing the animal in a seated position on a soft mat in a quiet and soundless environment with electrodes attached to the skin. The positive electrode was placed at the apex of the heart (fifth intercostal space on the left side, on the chest wall, behind the elbow),

the negative electrode at the black ridge of the neck at 1/3 lower left side, and the neutral electrode in the body junction area of the animal. The electrodes were installed on the skin after using methyl alcohol spray. The heart rate was calculated by measuring the average time interval between 6 RR intervals. The QRS complex was described as the first negative deflection below Q, the first positive deflection as R, and the negative deflection after R as S. A comparison was made between different periods of electrocardiography in terms of heart rate, the potential presence of cardiac arrhythmias, the shape and size of waves, and the measurement of intervals on the electrocardiogram

Statistical analysis

The statistical analysis of the data obtained was conducted using IBM SPSS software, version 27. Initially, descriptive findings of the studied variables were calculated and reported, including indicators such as mean, standard deviation, standard error, etc. First, the normality of the data was assessed using the Kolmogorov-Smirnov test. In cases where the significance level from the K-S test was below 0.05, statistical methods were employed to normalize the data distribution. Subsequently, research hypotheses were tested using parametric tests. In the next phase, for data with a normal distribution, the effect of measurement times on the mean indices examined before and after drug injection was analyzed using repeated measures ANOVA. For data that did not meet normal distribution criteria and for non-parametric data, the Friedman test was utilized. If significant differences were found, to determine differences between measurement times in parametric data, the student’s t-test for dependent groups was applied, while for non-parametric data, the Wilcoxon signed-rank test was used. Additionally, to compare parametric data with normal distribution between treatment groups at identical measurement times, one-way ANOVA and Tukey's post-hoc test were employed. For comparing non-normally distributed residuals and non-parametric data, the Kruskal-Wallis test and Mann-Whitney post-hoc test were utilized. It is noteworthy that throughout all stages of analysis, a significance level of 5% was considered for rejecting the null hypothesis.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

.1 Samimi, A.S., E. Sakhaee, and F. Iranmanesh, *Evaluation of sedative, analgesic, physiological, and laboratory effects of two doses off medetomidine and xylazine in dromedary calves.* J Vet Pharmacol Ther, 2019. **42**(4): p. 411-419.

.2 Atiba, A., et al., *Evaluation of Analgesic Effect of Caudal Epidural Tramadol, Tramadol-Lidocaine, and Lidocaine in Water Buffalo Calves (Bubalus bubalis).* Veterinary Medicine International, 2015. **2015**(1): p. 575101.

.3 Borges, L.P., et al., *Behavioral and cardiopulmonary effects of dexmedetomidine alone and in combination with butorphanol, methadone, morphine or tramadol in conscious sheep.* Veterinary Anaesthesia and Analgesia, 2016. **43**(5): p. 549-560.

.4 Clark, D.S.T., *Selectivity of atipamezole, yohimbine and tolazoline for alpha-2 adrenergic receptor subtypes: implications for clinical reversal of alpha-2 adrenergic receptor mediated sedation in sheep.* J. Vet. Pharmacol. Ther, 1998. **21**: p. 342-347.

.5 Odom-Forren, J., *Responsible social networking.* Journal of PeriAnesthesia Nursing, 2012. **27**(1): p. 61-63.

.6 White, D.M., A.R. Mair, and F. Martinez-Taboada, *Opioid-free anaesthesia in three dogs.* Open Veterinary Journal, 2017. **7**(2): p. 104-110.

.7 Dorfman, A.H. and R. Valliant, *Commentary on: Monson KL, Smith ED, Peters EM .Repeatability and reproducibility of comparison decisions by firearms examiners. J Forensic Sci. 2023; 68 (5): 1721–40. https://doi. org/10.1111/1556-4029.15318.* Journal of forensic sciences, 2023. **68**.(6)

.8 Mulier, J.P., *Perioperative opioids aggravate obstructive breathing in sleep apnea syndrome: mechanisms and alternative anesthesia strategies.* Current Opinion in Anesthesiology, 2016. **29**(1): p. 129-133.

.9 Hubbell, J.A. and W.W. Muir, *Monitoring anesthesia*, in *Equine anesthesia*. 2009, Elsevier. p. 1-49 .170

.10 Debiage, R.R., et al., *Dexmedetomidine and xylazine in sheep: A study of cardiopulmonary, hematological, and gastrointestinal effects.* Small Ruminant Research, 2023. **218**: p. 106863.

.11 Kaneko, J.J., J.W. Harvey, and M.L. Bruss, *Clinical biochemistry of domestic animals*. 2008: Academic press.

.12 Divers, T.J. and S.F. Peek, *Rebhun's diseases of dairy cattle*. 2007: Elsevier Health Sciences.

.13 Stockham, S.L. and M.A. Scott, *Fundamentals of veterinary clinical pathology*. 2024: John Wiley & Sons.

.14 Wilson, R.A., *Evaluation of Carcass Performance and Biomarkers Associated with Liver Abscess.* 2023.

.15 Zhang, Z.-F., et al., *Association between preoperative serum homocysteine and delayed neurocognitive recovery after non-cardiac surgery in elderly patients: a prospective observational study.* Perioperative Medicine, 2021. **10**(1): p. 37.

.16 Zethelius, B.r., N. Johnston, and P. Venge, *Troponin I as a predictor of coronary heart disease and mortality in 70-year-old men: a community-based cohort study* .Circulation, 2006. **113**(8): p. 1071-1078.

.17 Devereaux, P., et al., *High-sensitivity troponin I after cardiac surgery and 30-day mortality.* New England journal of medicine, 2022. **386**(9): p. 827-836.
.18 Constable, P.D., et al., *Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats*. 2016: Elsevier Health Sciences.
.19 Samimi, A.S., *Electrocardiographic and clinico-paraclinical evaluation of a dromedary camel suffered from theileriosis.* Comparative Clinical Pathology, 20 :(5)27 .18p. 1409-1415.
.20 Samımı, A.S., et al., *Electrocardiographic analysis of ST-segment duration and morphology in sheep and goats: effect of species, breed, age and sex.* İstanbul Üniversitesi Veteriner Fakültesi Dergisi, 2017. **43**(1): p. 39-43.
.21 Azari, O., et al., *Haematological and biochemical alterations caused by epidural and intramuscular administration of xylazine hydrochloride in dromedary camels (Camelus dromedarius).* Veterinaria Italiana, 2012. **48**(3): p. 313.
.22 Samimi, A.S. and O. Azari, *The effect of detomidine on clinical signs, serum electrolytes, electrocardiographic indices, and cardiac arrhythmias in Camelus dromedarius.* Comparative Clinical Pathology, 2017. **26**(3): p. 707-712.
.23 de Carvalho, L.L., et al., *Sedative and cardiopulmonary effects of xylazine alone or in combination with methadone, morphine or tramadol in sheep.* Veterinary anaesthesia and analgesia, 2016. **43**(2): p. 179-188.
.24 Pawde, A., A.G. Singh, and N. Kumar, *Clinicophysiological effects of medetomidine in female goats.* Small Ruminant Research, 1996. **20**(1): p. 95-98.
.25 Salarpour, M., et al., *Comparative evaluation of the sedative and physiological effects of medetomidine alone and in combination with pethidine, morphine, tramadol, and methadone in goats.* Veterinary Medicine and Science, 2022. **8**(4): p. 1664-1670.
.26 Caulkett, N., *Anesthesia of ruminants.* 2003.
.27 Bani Ismail, Z., K. Jawasreh, and A. Al-Majali, *Effects of xylazine–ketamine–diazepam anesthesia on blood cell counts and plasma biochemical values in sheep and goats.* Comparative clinical pathology, 2010. **19**: p. 571-574.
.28 Abouelfetouh, M.M., et al., *Application of α(2) -adrenergic agonists combined with anesthetics and their implication in pulmonary intravascular macrophages-insulted pulmonary edema and hypoxemia in ruminants.* J Vet Pharmacol Ther, 2021. **44**(4): p. 478-502.
.29 Kästner, S.B., *A2-agonists in sheep: a review.* Vet Anaesth Analg, 2006. **33**(2): p. 79-96.
.30 Rahman, M.S., et al., *Clinico-hemato-biochemical evaluation of general anesthesia with combination of Xylazine and Ketamine and Ketamine alone in sheep (Ovis aries).* Bangladesh Veterinary Journal, 2021. **55**(1-4): p. 8-15.
.31 Ramaswamy, V., et al., *Studies on the efficacy of ketamine hydrochloride as general anaesthetic in combination with xylazine, diazepam and promazine in canines (quantitative changes).* 1991.
.32 Yakubu, A., et al., *Hematological and cardiopulmonary effects of propofol anesthesia in red Sokoto goat of Nigeria.* Animal Science Reporter, 2020. **13**.(2)
.33 Ukwueze, C ,.C. Eze, and K. Ogbanya, *Haematologic and clinical change assessment in propofol anaesthesia in combination with xylazine and ketamine in West African Dwarf goat.* Comparative Clinical Pathology, 2015. **24**: p. 841-846.
.34 Samimi, A.S., E. Sakhaee, and F. Iranmanesh, *Evaluation of sedative, analgesic, physiological, and laboratory effects of two doses off medetomidine and xylazine in dromedary calves.* Journal of veterinary pharmacology and therapeutics, 2019. **42**(4): p. 411-419.
.35 Chalmeh, A., et al., *Alterations in electrocardiographic parameters and serum cardiac biomarkers in an ovine experimental endotoxemia model.* İstanbul Üniversitesi Veteriner Fakültesi Dergisi, 2014. **40**(2): p. 211-219.

.36 Samimi, A.S., et al., *Comparative evaluation of xylazine, detomidine, and medetomidine on cardiovascular biomarkers in dromedary camels.* Comparative Clinical Pathology, 2020. **29**: p. 337-340.
.37 Sullivan, A., et al., *Twenty-Four-Hour Electrocardiographic Monitoring for Assessment of Cardiac Arrhythmias in Healthy and Hospitalized Goats.* J Vet Intern Med, 2025. **39**(4): p. e70160.
.38 Singletary, G.E., et al., *Cardiac troponin I concentrations following medetomidine-butorphanol sedation in dogs.* Vet Anaesth Analg, 2010. **37**(4): p. 342-6.
.39 Barbosa, J., et al., *Repeated administration of clinically relevant doses of the prescription opioids tramadol and tapentadol causes lung, cardiac, and brain toxicity in wistar rats.* Pharmaceuticals, 2021. **14**(2): p. 97.
.40 Ali, H.A., et al., *Neurotoxic, hepatotoxic and nephrotoxic effects of tramadol administration in rats.* Journal of Molecular Neuroscience, 2020. **70**: p. 1934-1942.
.41 Constable, P.D., et al., *A textbook of the diseases of cattle, horses, sheep, pigs, and goats.* Veterinary medicine, 2017. **11**: p. 412-18.
.42 Barbosa, J., et al., *Repeated administration of clinical doses of tramadol and tapentadol causes hepato-and nephrotoxic effects in Wistar rats.* Pharmaceuticals, 2020. **13**(7): p. 149.
.43 Zhang, X., et al., *Efficacy and safety of dexmedetomidine as an adjuvant in epidural analgesia and anesthesia: a systematic review and meta-analysis of randomized controlled trials.* Clinical drug investigation, 2017. **37**(4): p. 343-354.
.44 Sinclair, M.D., *A review of the physiological effects of α2-agonists related to the clinical use of medetomidine in small animal practice.* The Canadian veterinary journal, 2003. **44**(11): p. 885.
.45 Dugdale, A.H., et al., *Comparison of two thiopental infusion rates for the induction of anaesthesia in dogs.* Veterinary Anaesthesia and Analgesia :(6)32 .2005 ,p. 360-366.
.46 Wedam, E.F. and M.C. Haigney, *The impact of opioids on cardiac electrophysiology.* Current cardiology reviews, 2016. **12**(1): p. 27-36.
.47 Roden, D.M., *Drug-induced prolongation of the QT interval.* New England Journal of Medicine, 2004. **350**(10): p. 1013-1022.
.48 Aghamiri, S.M., et al., *Effect of xylazine, detomidine, medetomidine and dexmedetomidine during laparoscopic SCNT embryo transfer on pregnancy rate and some physiological variables in goats.* BMC Vet Res, 2022. **18** :(1)p. 98.
.49 Nahvi, A., et al., *Evaluation of the sedative and physiological effects of xylazine, detomidine, medetomidine and dexmedetomidine in goats.* Vet Med Sci, 2022. **8**(3): p. 1205-1210.

Figure 1. The changes in white blood cells (WBC) in various treatment groups at different time points . Different letters indicate statistically significant differences ($p < 0.05$)

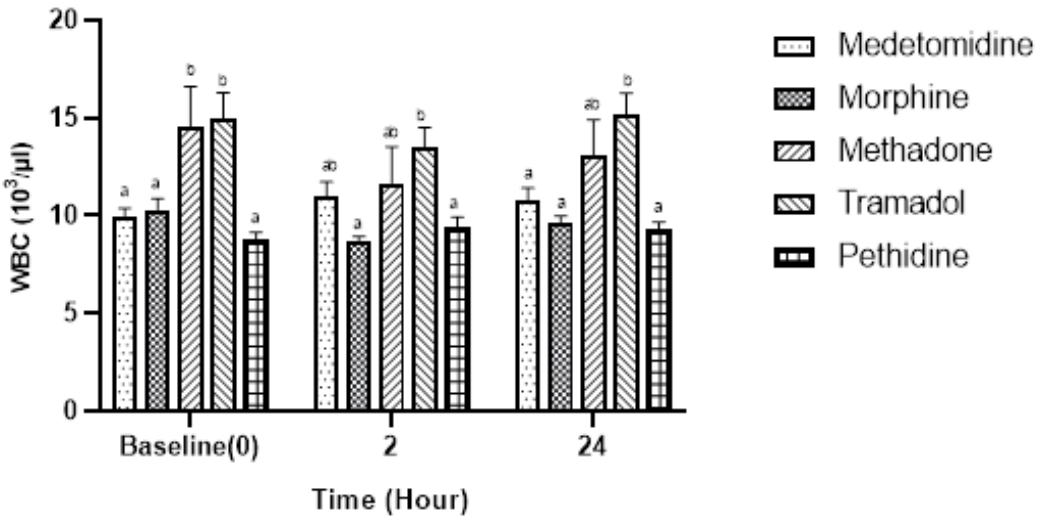

Figure 2. The changes in packed cell volume (PCV) in various treatment groups at different time points.

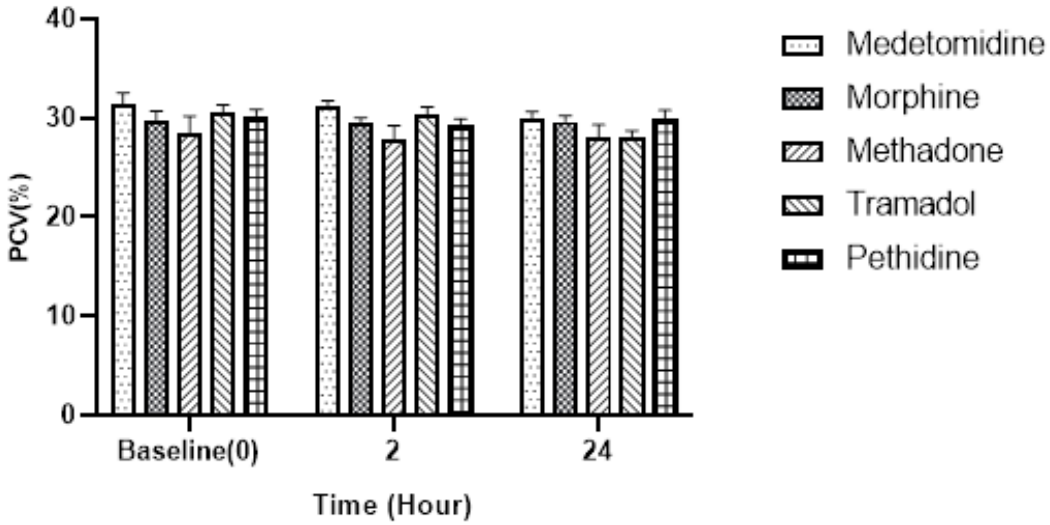

Figure 3. The changes in cardiac troponin I (cTn I) in various treatment groups at different time points . Different letters indicate statistically significant differences ($p < 0.05$)

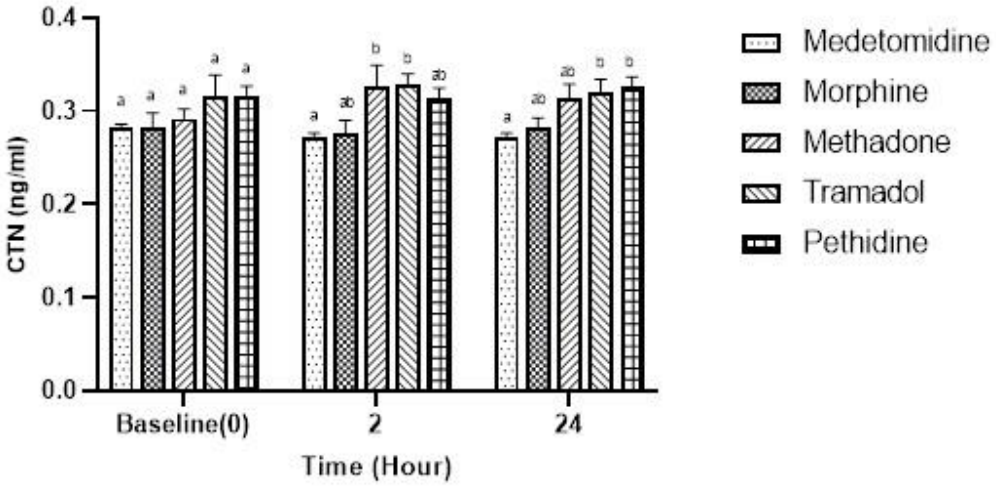

Figure 4. The changes in homocystein (Hcy) in various treatment groups at different time points . Different letters indicate statistically significant differences ($p < 0.05$)

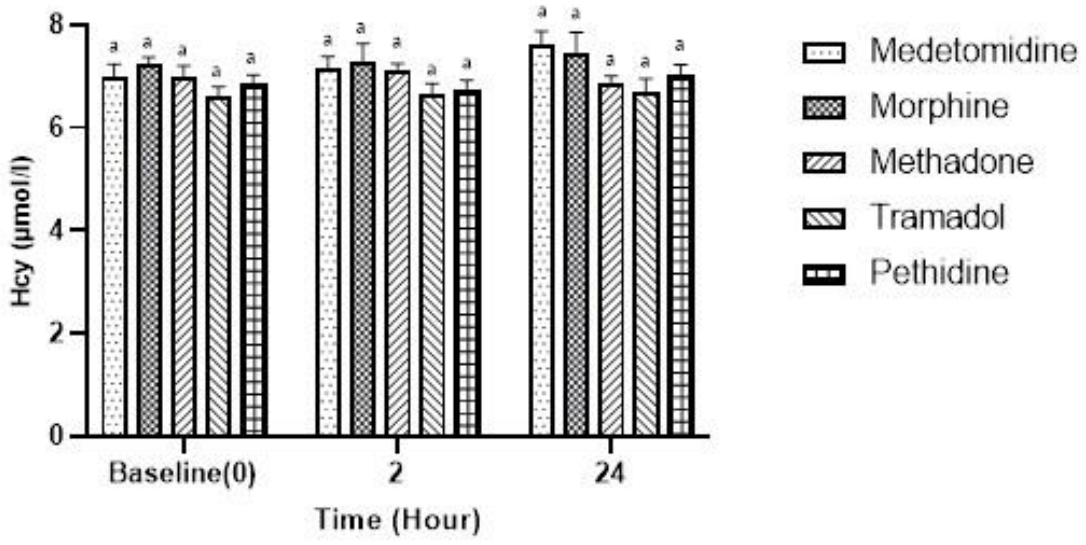

Figure 5. The changes in aspartate aminotransferase (AST) in various treatment groups at different time points.

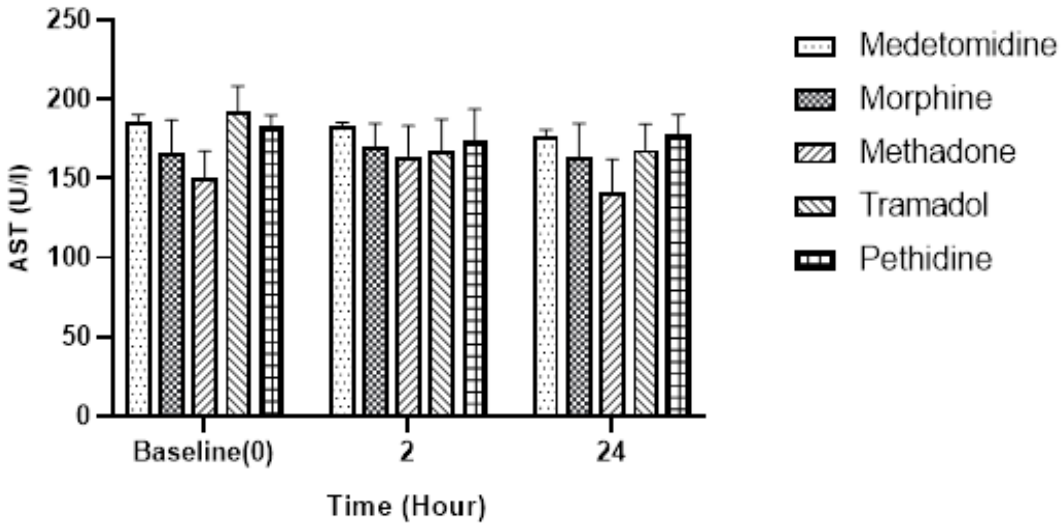

Figure 6. The changes in alanine aminotransferase (ALT) in various treatment groups at different time points.

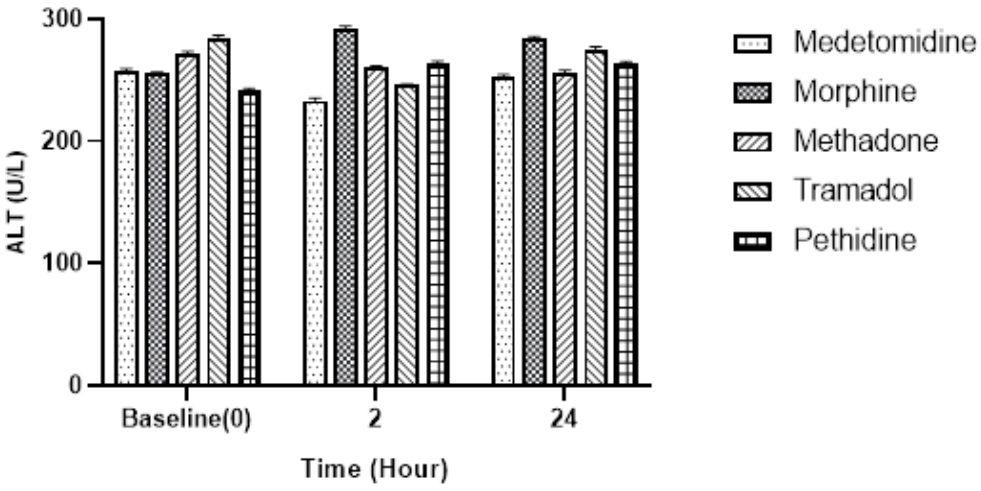

Figure 7: Trend of changes (mean ± standard error) in the P Amplitude component across the studied groups before and after drug administration. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

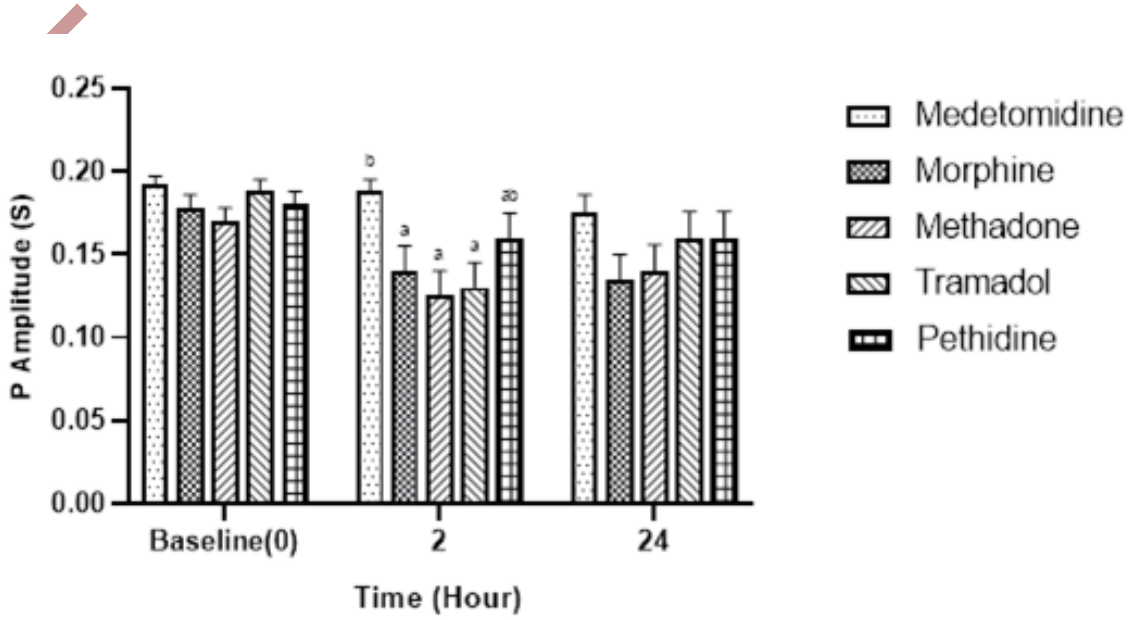

Figure 8: Trend of changes (mean ± standard error) in the QRS Amplitude component across the studied groups before and after drug administration. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

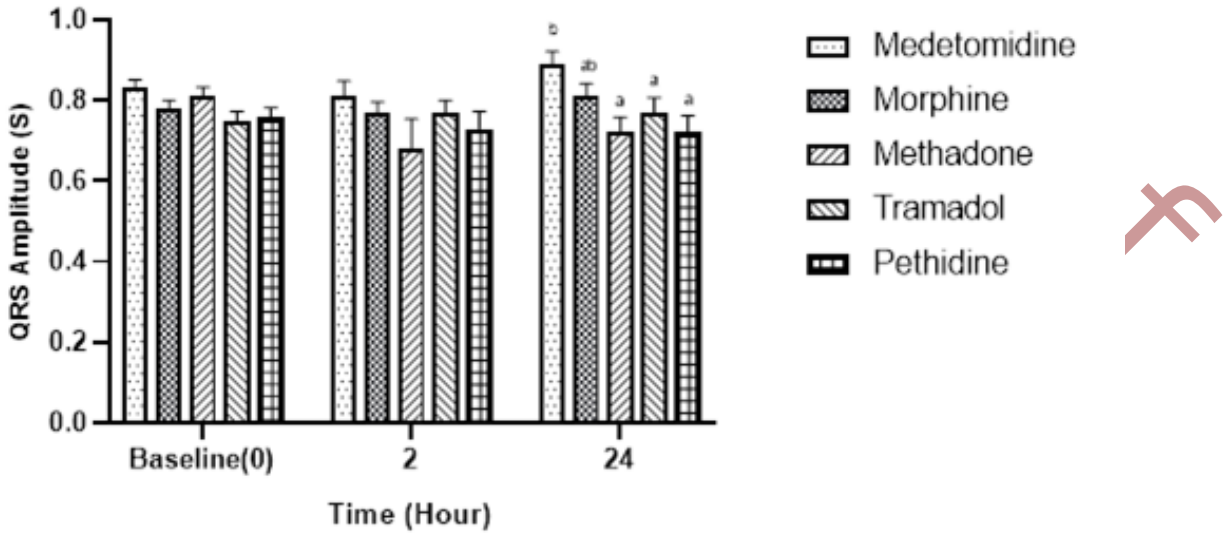

Figure 9: Trend of changes (mean ± standard error) in the T Amplitude component across the studied groups. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

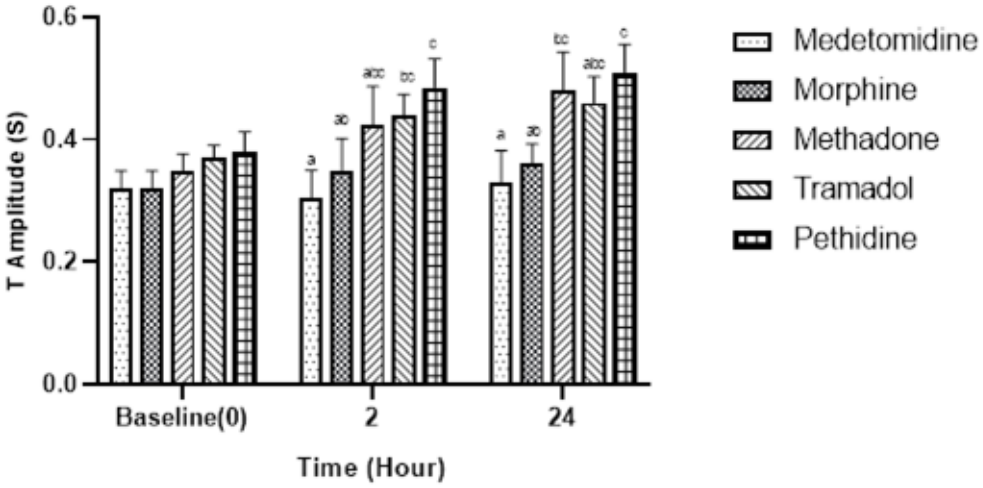

Figure 10: Trend of changes (mean ± standard error) in the P Duration component across the studied groups. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence l

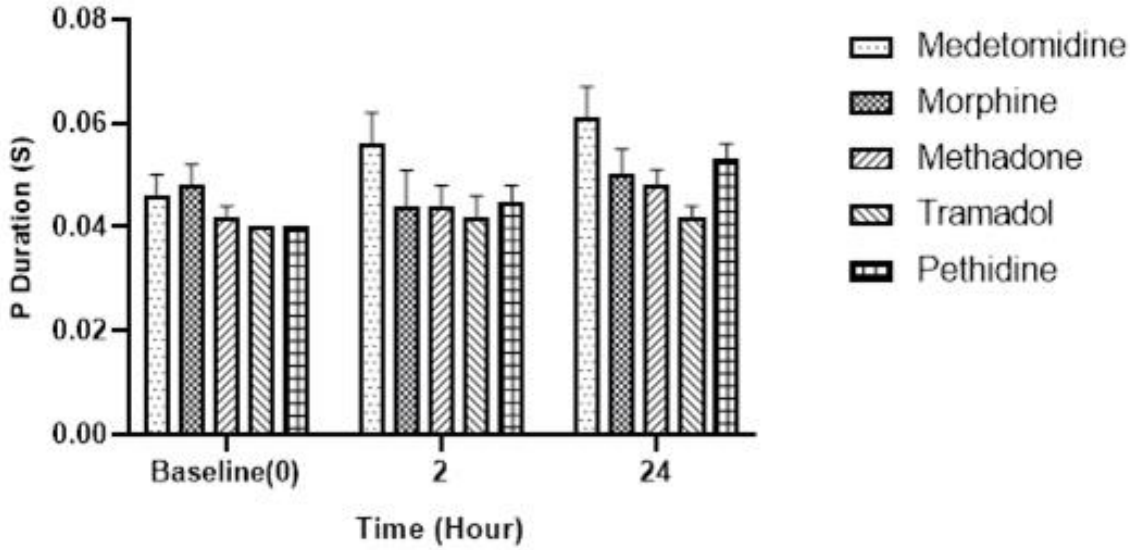

Figure 11: Trend of changes (mean ± standard error) in the QRS Duration component across the studied groups before and after drug administration. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

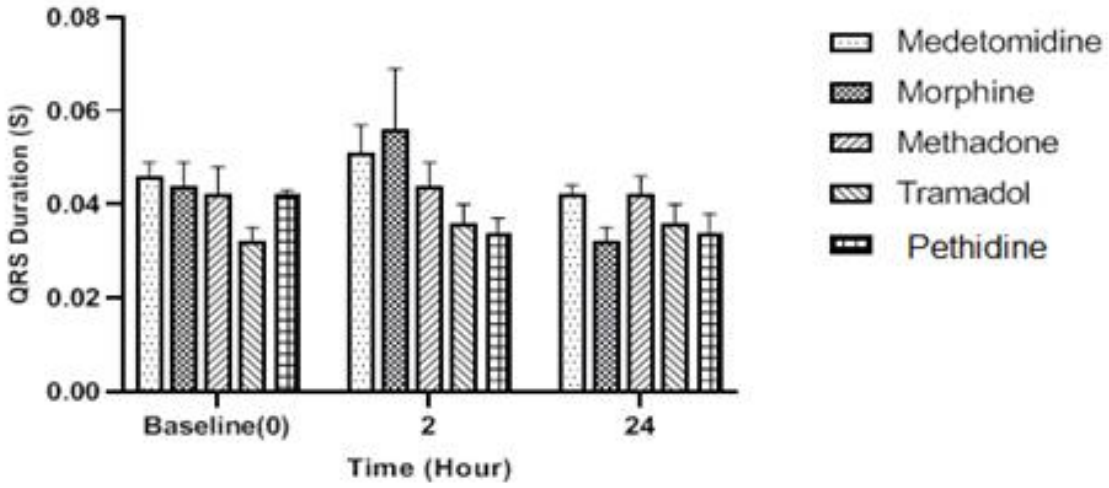

Figure 12: Trend of changes (mean ± standard error) in the T Duration component across the studied groups before and after drug administration. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

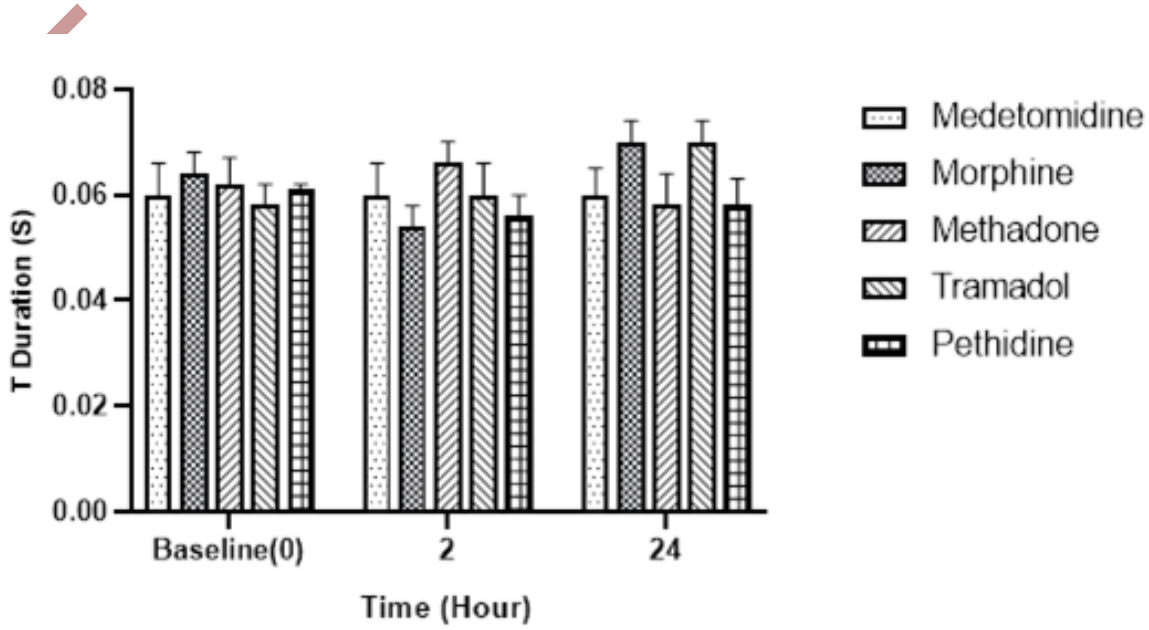

Figure 13: Trend of changes (mean ± standard error) in the PR Interval component across the studied groups. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

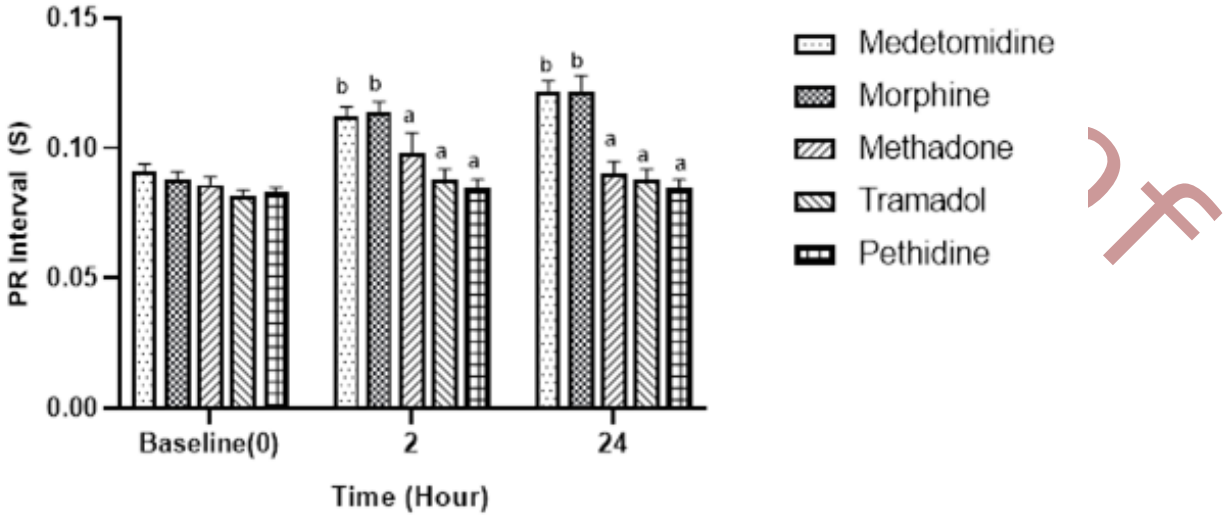

Figure 14: Trend of changes (mean ± standard error) in the QT Interval component across the studied groups before and after drug administration. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

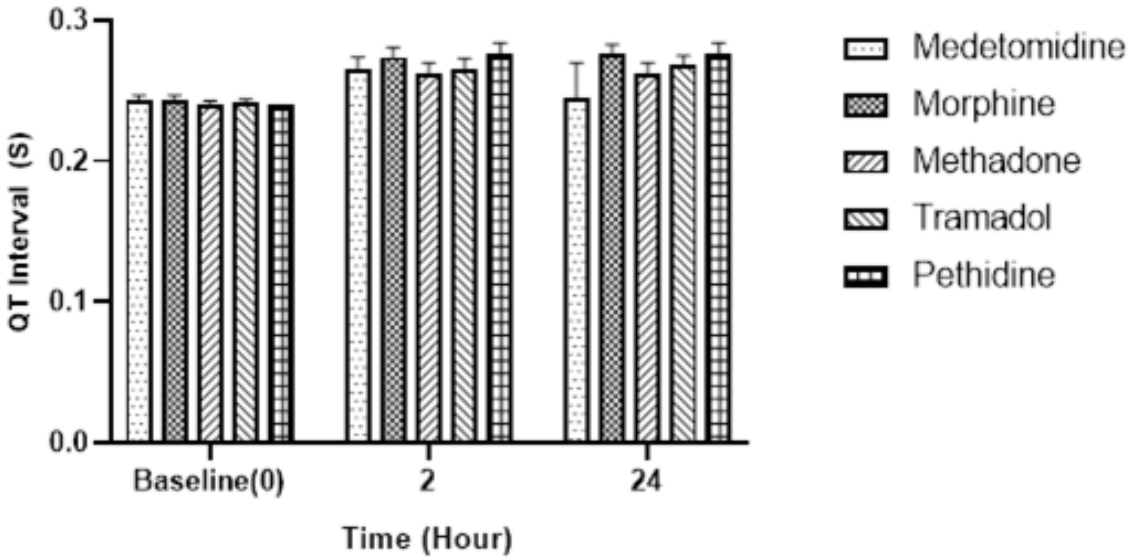

Figure 15: Trend of changes (mean ± standard error) in the RR Interval component across the studied groups. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level