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Effect of different anesthetic mixtures—ketamine-xylazine, ketamine-acepromazine and tiletamine-zolazepam—on the physiological and blood biochemistry parameters of male rhesus monkeys (*Macaca mulatta*) at different ages

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Abstract

Background: Anesthetic agents are commonly utilized in the handling of non-human primates for prevent the stress caused in physical exploration or physical restrain. For this reason, the objective of this work was to describe the effect of age and dissociative anesthetics (ketamine and tiletamine), and their combinations with acepromazine, xylazine and zolazepam, on the physiological and blood biochemical parameters in *Macaca mulatta*.

Methods: Eighty male *Macaca mulatta* were divided into four experimental groups depending on the anesthetic mixture applied. Each group of 20 males was divided into five sub-groups according to age. Physiological parameters were recorded every five minutes during a 30-minute period. A blood sample was drawn to analyze blood biochemistry.

Results: Statistical analyses revealed significant differences in the physiological parameters between the ketamine-acepromazine and ketamine-xylazine groups compared to the control group. The analysis of blood biochemistry found significant differences by age and by anesthetic mixture among all groups.

Conclusion: These findings contribute to standardizing this animal model in biological research.

KEYWORDS

acepromazine, anesthesia, blood biochemistry, ketamine, *Macaca mulatta*, physiologic parameters, tiletamine, xylazine, zolazepam

1 | INTRODUCTION

Practices involved in the care and handling of non-human primates (NHPs) have changed significantly over time, due largely to advances in medical, diagnostic, and instrumental technology. Thus, there is an ongoing need to present normative data that includes refining the handling and diagnostic techniques used with NHPs, and to establish normative parameters for each research laboratory that consider several key factors, including age, sex, type of alimentation, type of confinement, and geographic location, among others.[1-3] Changes in many hematological and biochemical parameters have been reported during normal development and maturation in various NHP species. As is well-known, basal reference values are not only important for monitoring the care and maintenance of NHPs used in biomedical research, but are also critical for evaluating normal physiological states and the effects of treatments and experimental tests.[4] Indeed, reference values play a vital role in selecting healthy subjects for such research and in evaluating non-clinical findings.[5]

Performing physical and/or diagnostic examinations often requires administration of drugs to allow manipulation of animals or to restrain them while taking samples. Chemical restriction or subjection, in particular, reduces stress in animals while increasing safety and improving the quality of the samples obtained.[3] Many studies have reported the clinical effects of anesthesia on the physiological and biochemical parameters of NHPs.[1-15] Research has shown that anesthetic drugs, and combinations thereof, produce secondary effects that can modify physiological responses by altering an animal's homeostasis, and cause such deleterious effects as hypotension, hypoxia, and hypothermia, among other disturbances, in anesthetized animals, and this can affect the results of research.[8,12] The main anesthetic agents used with NHPs include phencyclidine and its derivatives, ketamine and tiletamine, normally used as induction agents and combined with α2 adrenergic agonists or benzodiazepines.[16,17] Standardized physiological parameters are essential prerequisites for diverse fields of scientific research, but because administering anesthesia can alter normal biochemical parameters and physiological responses, the objective of this work was to describe the effects of the anesthetics and tranquilizers used most frequently with NHPs, specifically *Macaca mulatta*, at different ages.

2 | METHODS

This experiment was conducted in accordance with the Official Mexican Norm, NOM-062-ZOO-1999, the *Guide for the Care and Use of Experimental Animals* (National Research Council (US) Institute for Laboratory Animal Research 1996) and the American Society of Primatologists (*Principles for the Ethical Treatment of Non-Humans Primates*). It was approved by the Internal Committee for the Care and Use of Laboratory Animals (ICCULA) and the Research Ethics Commissions of INBIOMA S.A.S de C.V.

The 80 male *Macaca mulatta* monkeys used were held in captivity at the Non-human Primate Unit at INBIOMA S.A.S de C.V. For this study, four experimental groups were formed according to the mixture of anesthetics applied: Group A, ketamine + acepromazine (KA; 4 mg/kg + 0.5-1.0 mg/kg IM)[18]; Group B, ketamine + xylazine (KX; 4 mg/kg + 0.5-1.0 mg/kg IM)[19]; Group C, tiletamine + zolazepam (TZ; 4 mg/kg IM)[20-22]; and Group D (evaluated as a control), ketamine only (K; 4 mg/kg IM)[19]. Each experimental group included 20 male rhesus monkeys, divided into five sub-groups, each with four animals, according to age (classification from Ibáñez-Contreras et al[20] and Hernández-Godínez et al[22]), as follows: Group 1, infants (0-1 year); Group 2, infants (1-3 years); Group 3, juveniles (3-5 years); Group 4, adults (5-15 years); and Group 5, senile monkeys (15 years and above). All monkeys were fed with Purina Monkey Chow 5045®, which contains 25% protein (Monkey Diet 5038, PMI Nutrition International, St Louis, MO, USA), five times per day, with water ad libitum.

The animals were isolated from the group in which they lived 72 hours before blood sampling by placing them in individual stainless-steel cages especially made for NHPs in a controlled climate room at a temperature of 23°C. This procedure was performed to reduce the stress generated by the capture of the animals. Subsequently they underwent an 8-hour fasting period prior to blood sampling and recording of physiological parameters.

2.1 | Physiological parameters

The following measurements were recorded every 5 minutes during one 30-minute period: heart rate (HR, bpm), respiratory rate (RR, rpm), temperature (*T*, °C), oxygen saturation (SpO_2, %), and systolic

TABLE 1 Biochemical parameters

Abbreviation	Parameter	Unit
GLU	Glucose	mg/dL
BUN	Ureic nitrogen	mg/dL
CREAT	Creatinine	mg/dL
TB	Total bilirubin	mg/dL
TP	Total protein	g/dL
ALB	Albumin	g/dL
GLO	Globulin	g/dL
ALT	Alanine aminotransferase	IU/L
AST	Aspartate aminotransferase	IU/L
LDH	Lactic dehydrogenase	IU/L
ALKP	Alkaline phosphatase	IU/L
GGT	Gamaglutamyltransferase	IU/L
LAC	Lactate	mmol/L
CK	Creatininase	IU/L
Na	Sodium	mEq/L
K	Potassium	mEq/L
CL	Chloride	mEq/L
Ca	Calcium	mEq/L
P	Phosphorus	mEq/L

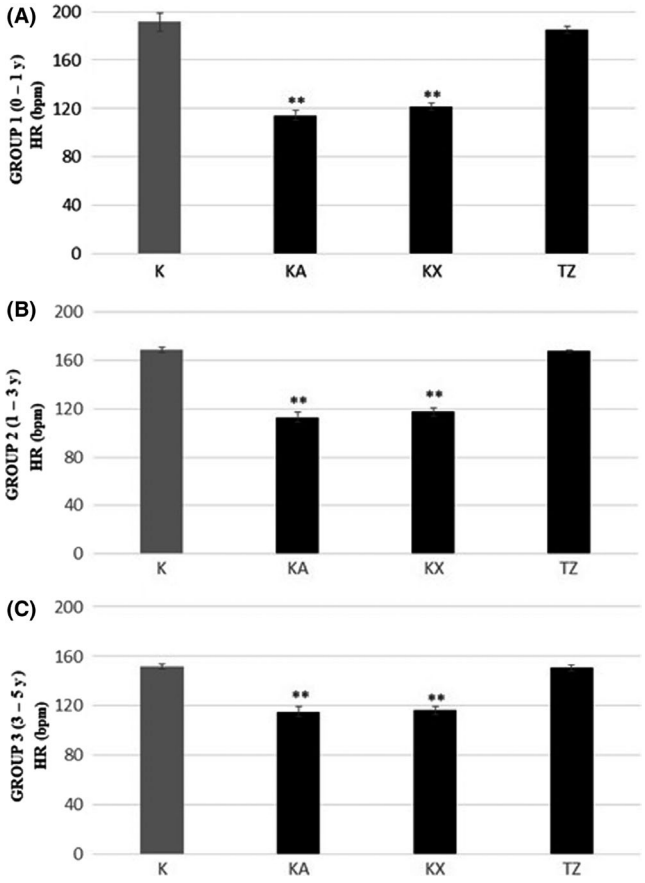

FIGURE 1 Effect of anesthetic mixtures on heart rate (bpm) in male rhesus monkeys (*Macaca mulatta*). A, Group 1; B, Group 2; C, Group 3. Statistical analysis of ANOVA reveals significant differences between anesthetic mixtures against the control group (K), using Bonferroni and Games Howell post hoc tests. $^{**}P = 0.0001$

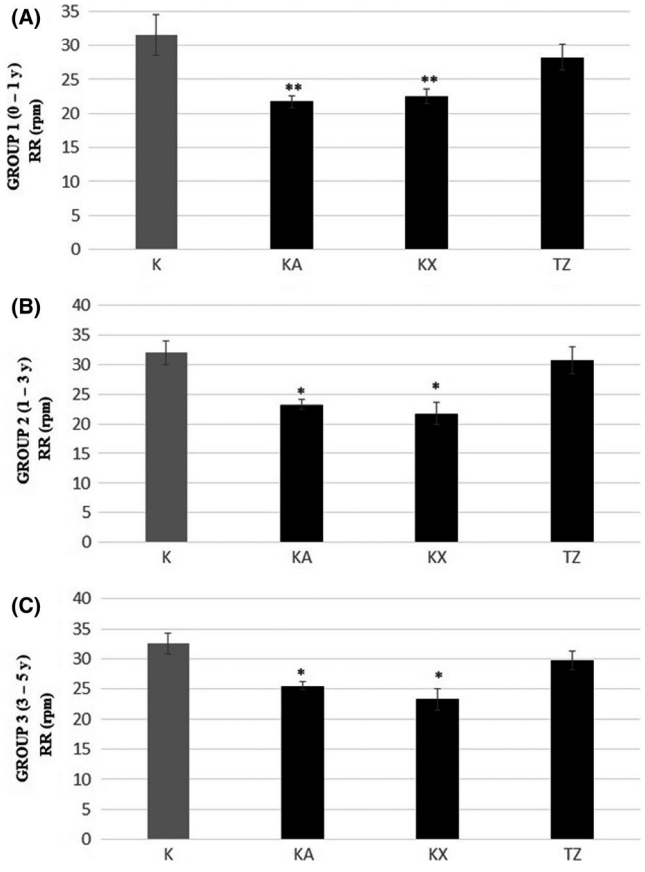

FIGURE 2 Effect of anesthetic mixtures on respiratory rate (rpm) in male rhesus monkeys (*Macaca mulatta*). A, Group 1; B, Group 2; C, Group 3. Statistical analysis of ANOVA reveals significant differences between anesthetic mixtures against the control group (K), using Bonferroni and Games Howell post hoc tests. $^{*}P = 0.001$, $^{**}P = 0.0001$

and diastolic pressure (SP and DP, respectively, in mm Hg). For this purpose, surface electrodes (electrical conductors) were attached to the skin on the palms of the monkeys' hands and the bottom of their left foot. Temperature was taken rectally. The study utilized Datex-Ohmeda Cardiocap/5® equipment for physiological constants (Datex-Ohmeda, Inc., USA).

2.2 | Blood biochemistry

Blood samples were drawn in the morning 10 minutes after the administration of anesthesia, through a puncture in the saphenous vein and collected in 23-caliber BD Vacutainer® tubes without anti-coagulant. They were analyzed in a Cobas 6000® auto-analyzer (Roche Diagnostics, USA). Table 1 shows the analytes assessed, and the abbreviations and units used.

2.3 | Statistical analyses

All statistical analyses were done with the SPSS statistical software package, version 19, for Windows (SPSS, Chicago, IL, USA). The following central tendency measures were obtained: means, typical deviation and typical error for each age group during both monitoring of the physiological constants and evaluation of blood biochemistry. The Shapiro-Wilk's test was applied and showed normality for all age groups in each treatment. Later, the homogeneity of variances test was conducted using Levene's test.

Two sets of statistical analyses were performed, the first dependent on the factor of age, the second on the factor of the anesthetic mixture administered. The former contrasted the age groups for each anesthetic mixture employed, using a one-factor ANOVA test with Welch correction, followed by post hoc Tukey and Dunnett T3 tests. The latter contrasted the anesthetic applied for each age group, with the ketamine-only group as a control, using one-factor ANOVA with Welch correction, followed by post hoc Bonferroni and Games-Howell tests. A $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Physiological parameters

Based on the analysis comparing the anesthetic mixtures to controls (K) in each age group, in all cases, results showed that KA and

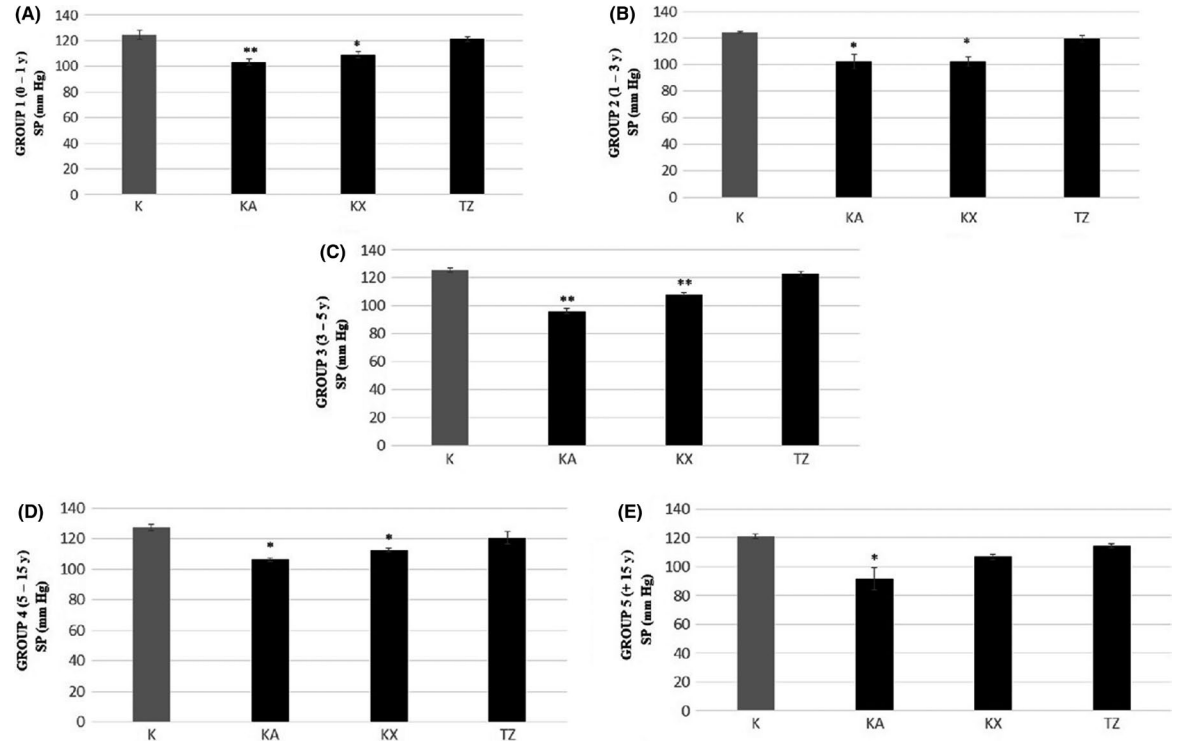

FIGURE 3 Effect of anesthetic mixtures on systolic pressure (mm Hg) in male rhesus monkeys (*Macaca mulatta*). A, Group 1; B, Group 2; C, Group 3; D, Group 4; E, Group 5. Statistical analysis of ANOVA reveals significant differences between anesthetic mixtures against the control group (K), using Bonferroni and Games Howell post hoc tests. **P* = 0.001, ***P* = 0.0001

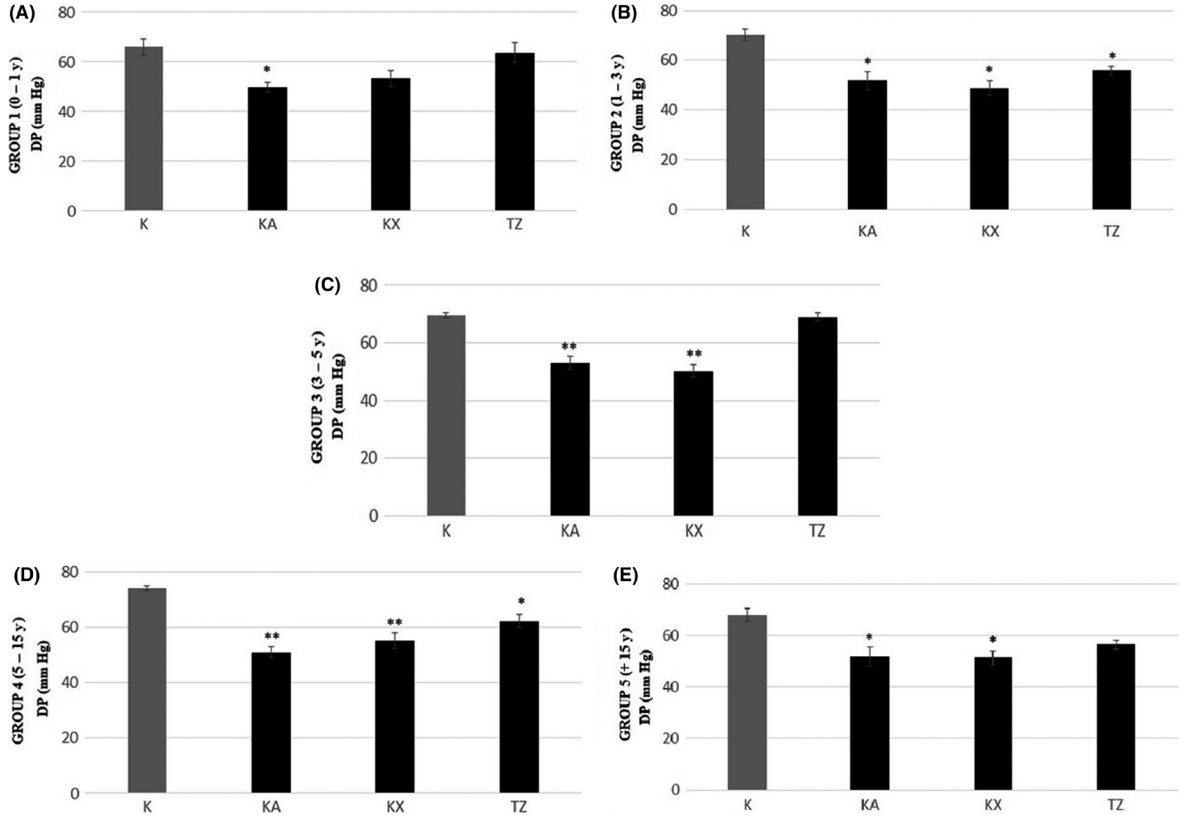

FIGURE 4 Effect of anesthetic mixtures on diastolic pressure (mm Hg) in male rhesus monkeys (*Macaca mulatta*). A, Group 1, B, Group 2, C, Group 3, D, Group 4, E, Group 5. Statistical analysis of ANOVA reveals significant differences between anesthetic mixtures against the control group (K), using Bonferroni and Games Howell post hoc tests. **P* = 0.001, ***P* = 0.0001

KX were statistically different compared to the control group (K). For HR, it was observed that Groups 1 ($F_{3,12}$ = 72.299, P = 0.0001), 2 ($F_{3,12}$ = 104.338, P = 0.0001) and 3 ($F_{3,12}$ = 49.288, P = 0.0001) presented significant differences, showing a decrease in comparison to the control group (Figure 1). Significant differences were also observed in RR, showing a decrease in KA and KX in Groups 1 ($F_{3,12}$ = 6.117, P = 0.009), 2 ($F_{3,12}$ = 7.955, P = 0.003) and 3 ($F_{3,12}$ = 7.580, P = 0.004) (Figure 2). In the case of T, in Group 3 only, KX was statistically different from the control group ($F_{3,12}$ = 6.170, P = 0.009). In the case of SpO_2, in Group 5 only, KX was significantly different ($F_{3,12}$ = 49.288, P = 0.0001) from the control group. Finally, in all age groups evaluated there were statistical differences in SP and DP, showing in all cases a decrease in anesthetic mixtures compared to the use of ketamine alone (Figures 3 and 4).

Based on the statistical analysis, contrasting the age groups in each treatment group showed some statistically significant differences. In the case of KX, there were no statistical differences between the age groups. In the evaluation of TZ, there were significant differences in HR ($F_{4,15}$ = 121.483, P = 0.0001) in Groups 1, 2 and 3 versus Groups 4 and 5 (P < 0.05). Finally, in the case of K, there were differences in HR ($F_{4,15}$ = 58.166, P = 0.0001) in Groups 1, 2 and 3 versus Groups 4 and 5 (P < 0.05). In both cases, the HR in the youngest animals was increased compared to the oldest animals.

3.2 | Blood biochemistry

3.2.1 | Age-dependent statistical analyses

The results of the statistical analysis that contrasted the age groups in each treatment group revealed statistically significant differences for KA in GLU between Group 1 and Groups 3 (P = 0.002) and 4 (P = 0.005), and in ALKP between Group 1 and Groups 2 (P = 0.013), 4 (P = 0.011) and 5 (P = 0.006) (Table 2).

In the case of KX, statistical differences in ALT were found between Groups 1 and 5 (P = 0.017), and between Groups 2 and 5 (P = 0.025); in AST between Group 1 and Groups 4 (P = 0.002) and 5 (P = 0.001), between Group 2 and Groups 3 (P = 0.017) and 5 (P = 0.047), and between Group 3 and Groups 4 (P = 0.0001) and 5 (P = 0.0001); in LDH between Group 1 and Groups 3 (P = 0.009) and 4 (P = 0.0001), between Group 2 and Groups 3 (P = 0.038) and 4 (P = 0.001), and between Groups 4 and 5 (P = 0.002); in ALKP, between Group 1 and Groups 3 (P = 0.030), 4 (P = 0.0001) and 5 (P = 0.001), and between Group 2 and Groups 3 (P = 0.007), 4 (P = 0.0001) and 5 (P = 0.0001); in CK, between Groups 1 and 5 (P = 0.009), and between Groups 2 and 5 (P = 0.033);

TABLE 2 Blood biochemistry values of ketamine-acepromazine-treated (Group A; KA) male rhesus monkeys (*Macaca mulatta)* of different ages (Groups 1-5)

	1		2		3		4		5	
Age group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GLU (mg/dL)*	103.75	9.88	91.25	10.24	75.75	6.85	78.75	4.11	91.25	8.77
BUN (mg/dL)	24.78	5.37	26.03	1.63	29.10	2.52	29.70	5.07	22.93	4.96
CREAT (mg/dL)	0.62	0.06	0.65	0.09	0.67	0.08	1.23	0.34	1.06	0.31
TB (mg/dL)	0.29	0.08	0.25	0.09	0.29	0.06	0.21	0.04	0.20	0.08
TP (mg/dL)	6.23	0.25	6.23	0.36	6.70	0.14	7.18	0.45	6.23	1.05
ALB (mg/dL)	3.13	0.59	3.13	0.46	3.38	0.17	3.10	0.37	3.19	0.41
GLO (mg/dL)	3.15	0.47	3.10	0.41	3.38	0.21	3.58	0.21	3.11	1.02
ALT (U/L)	36.50	4.43	40.00	9.76	40.25	15.41	41.50	13.13	31.75	5.32
AST (U/L)	53.00	9.63	49.50	10.08	60.75	18.41	55.25	9.81	50.00	16.57
LHD (U/L)	523.50	103.23	463.00	167.70	503.00	205.84	368.75	53.13	505.75	91.08
ALKP (U/L)*	384.50	64.32	227.00	21.43	274.25	76.29	223.75	59.11	211.00	57.56
GGT (U/L)	73.50	33.48	84.25	20.27	90.25	15.20	85.00	8.60	103.00	9.90
LAC	1.20	0.26	1.40	0.34	1.48	1.21	0.98	0.52	0.88	0.25
CK (U/L)	340.00	63.25	300.25	95.11	241.25	72.33	196.00	72.82	161.75	71.98
Na (mEq/L)	145.00	2.16	144.75	1.26	143.00	1.41	145.25	1.71	146.00	4.08
K (mEq/L)	4.20	0.88	4.15	0.29	4.24	0.30	3.40	0.72	3.68	0.51
CL (mEq/L)	106.25	4.03	106.00	2.16	105.25	1.71	110.00	8.52	108.00	4.55
Ca (mEq/L)	9.65	0.39	9.60	0.37	9.68	0.42	9.93	0.82	10.33	0.97
P (mEq/L)	3.68	1.52	5.40	0.29	4.18	0.58	5.13	1.72	4.83	1.41

Data are presented as means ± 1 standard deviation (SD).

Statistical analysis revealed significant differences (*P < 0.05) between the different age groups in GLU ($F_{4,7.19}$ = 6.364, P = 0.017) and ALKP ($F_{4,6.86}$ = 4.605, P = 0.040).

TABLE 3 Blood biochemistry values of ketamine-xylacine-treated (Group B; KX) male rhesus monkeys (*Macaca mulatta)* of different ages (Groups 1-5)

	1		2		3		4		5	
Age group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GLU (mg/dL)	83.50	10.25	74.75	5.12	72.25	3.30	71.25	5.74	75.00	4.32
BUN (mg/dL)	27.60	2.33	30.83	7.16	28.78	7.49	31.14	2.31	33.70	1.89
CREAT (mg/dL)	0.89	0.26	0.80	0.35	0.81	0.20	0.99	0.16	0.93	0.15
TB (mg/dL)	0.27	0.05	0.27	0.05	0.30	0.03	0.25	0.07	0.20	0.11
TP (mg/dL)	6.48	0.43	6.09	0.14	6.63	0.83	6.10	0.28	6.20	0.37
ALB (mg/dL)	3.06	0.37	3.01	0.13	3.15	0.89	2.76	0.24	2.58	0.30
GLO (mg/dL)	3.06	0.37	3.08	0.16	3.48	0.57	3.68	0.43	3.53	0.17
ALT (U/L)*	29.63	5.22	30.50	3.42	32.88	6.76	35.00	7.39	44.50	5.07
AST (U/L)*	63.50	1.29	56.00	5.48	69.50	5.07	45.75	8.54	44.50	2.38
LDH (U/L)*	644.75	36.45	609.00	68.01	456.25	60.62	367.00	22.54	589.25	110.47
ALKP (U/L)*	495.00	89.00	537.75	44.57	309.75	102.33	177.75	57.70	201.50	82.45
GGT (U/L)	84.13	14.64	86.75	9.14	80.75	34.63	59.25	11.35	65.88	13.75
LAC	1.78	0.74	1.68	0.38	1.98	0.61	1.45	0.48	1.53	0.41
CK (U/L)*	326.50	75.12	292.75	74.15	236.25	73.88	207.75	78.99	133.50	17.86
Na (mEq/L)	152.25	4.03	149.25	3.40	147.50	2.38	148.50	2.65	145.50	2.65
K (mEq/L)	3.60	0.45	3.23	0.43	3.18	0.45	3.63	0.48	3.68	0.21
CL (mEq/L)*	107.75	0.96	107.50	1.29	106.00	2.83	109.25	2.06	102.50	2.08
Ca (mEq/L)	9.30	0.89	10.03	0.48	9.13	0.43	9.63	0.31	9.20	0.28
P (mEq/L)*	5.93	0.98	7.03	0.69	6.15	1.03	4.30	0.86	4.93	0.79

Data are presented as means ± 1 standard deviation (SD).
Statistical analysis revealed significant differences (*$P < 0.05$) between the different age groups in ALT ($F_{4,7.33} = 4.776$, $P = 0.033$), AST ($F_{4,6.80} = 43.405$, $P = 0.0001$), LDH ($F_{4,7.01} = 38.045$, $P = 0.0001$), ALKP ($F_{4,7.30} = 24.842$, $P = 0.0001$), CK ($F_{4,6.52} = 8.982$, $P = 0.008$), CL ($F_{4,7.22} = 5.208$, $P = 0.027$) and P ($F_{4,7.45} = 5.941$, $P = 0.018$).

in CL between Groups 1 and 5 ($P = 0.013$), between Groups 2 and 5 ($P = 0.018$), and between Groups 4 and 5 ($P = 0.002$); and, finally, in P, between Group 2 and Groups 4 ($P = 0.004$) and 5 ($P = 0.029$) (Table 3).

The analysis of TZ showed statistical differences in LDH between Group 1 and Groups 3 ($P = 0.001$), 4 ($P = 0.0001$) and 5 ($P = 0.001$), as well as between Group 2 and Groups 4 ($P = 0.014$) and 5 ($P = 0.021$); in GGT, between Group 1 and Groups 3 ($P = 0.003$), 4 ($P = 0.005$) and 5 ($P = 0.004$), and between Group 2 and Groups 3 ($P = 0.001$), 4 ($P = 0.001$) and 5 ($P = 0.001$); in CK, between Group 1 and Groups 3 ($P = 0.019$), 4 ($P = 0.035$) and 5 ($P = 0.002$); and in P, between Group 1 and Groups 4 ($P = 0.041$) and 5 ($P = 0.025$), and between Groups 2 and 4 ($P = 0.039$) (Table 4).

Finally, for K, statistical differences in AST occurred between Groups 1 and 4 ($P = 0.030$); in Ca, between Groups 1 and 2 (0.025); and in P, between Groups 1 and 5 ($P = 0.006$), between Groups 2 and 5 ($P = 0.009$), and between Groups 3 and 5 ($P = 0.011$) (Table 5).

3.2.2 | Anesthetic mixture-dependent statistical analyses

The statistical analysis of blood biochemistry results contrasting the anesthetic mixture groups in each age group showed statistical differences in Group 1 for GLU ($F_{3,5.54} = 8.754$, $P = 0.016$), BUN ($F_{3,6.19} = 27.654$, $P = 0.001$), AST ($F_{3,5.93} = 104.768$, $P = 0.0001$), LDH ($F_{3,5.95} = 36.579$, $P = 0.0001$), GGT ($F_{3,6.08} = 4.969$, $P = 0.045$), and LAC ($F_{3,6.03} = 5.232$, $P = 0.041$); and for Ca ($F_{3,5.84} = 7.639$, $P = 0.019$) (Figure 5). In Group 2, statistical differences were found for BUN ($F_{3,6.17} = 43.396$, $P = 0.0001$), TP ($F_{3,6.12} = 16.705$, $P = 0.002$), ALB ($F_{3,6.20} = 12.362$, $P = 0.005$), AST ($F_{3,6.04} = 8.120$, $P = 0.015$), ALKP ($F_{3,5.90} = 43.745$, $P = 0.0001$), and P ($F_{3,6.26} = 8.092$, $P = 0.014$) (Figure 6). In Group 3, statistical differences were determined for BUN ($F_{3,6.37} = 13.341$, $P = 0.004$), TP ($F_{3,5.75} = 5.158$, $P = 0.045$), ALB ($F_{3,6.17} = 11.076$, $P = 0.007$), AST ($F_{3,6.40} = 17.894$, $P = 0.002$), GGT ($F_{3,5.97} = 52.873$, $P = 0.0001$), Na ($F_{3,6.43} = 7.630$, $P = 0.016$), K ($F_{3,6.61} = 11.106$, $P = 0.006$), CL ($F_{3,6.41} = 8.159$, $P = 0.013$) and Ca ($F_{3,6.21} = 7.305$, $P = 0.019$) (Figure 7). In Group 4 significant differences were seen for BUN ($F_{3,6.14} = 37.231$, $P = 0.0001$), ALB ($F_{3,6.34} = 8.339$, $P = 0.013$), AST ($F_{3,6.23} = 5.858$, $P = 0.031$), LDH ($F_{3,5.89} = 10.455$, $P = 0.009$) and GGT ($F_{3,5.98} = 13.614$, $P = 0.004$) (Figure 8). Finally, in Group 5 statistical differences were only found for BUN ($F_{3,6.09} = 11.167$, $P = 0.007$), LDH ($F_{3,5.21} = 72.068$, $P = 0.0001$), GGT ($F_{3,6.62} = 13.528$, $P = 0.003$), and LAC ($F_{3,6.55} = 8.827$, $P = 0.010$) (Figure 9). As these findings show, statistical differences occurred principally for NU, TP, ALB, AST, GGT, LDH and certain electrolytes.

TABLE 4 Blood biochemistry values of tiletamine-zolacepam-treated (Group C; TZ) male rhesus monkeys (*Macaca mulatta)* of different ages (Groups 1-5)

	1		2		3		4		5	
Age group	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
GLU (mg/dL)	76.25	1.71	71.50	2.65	72.00	2.94	71.75	5.06	75.50	3.70
BUN (mg/dL)	34.73	2.79	34.10	3.24	33.23	1.93	32.70	2.62	30.25	3.84
CREAT (mg/dL)	0.75	0.24	0.93	0.07	1.10	0.18	1.27	0.33	1.00	0.38
TB (mg/dL)	0.30	0.08	0.33	0.08	0.28	0.06	0.38	0.11	0.27	0.10
TP (mg/dL)	5.43	1.52	7.08	0.54	6.83	0.92	7.08	0.69	6.48	0.73
ALB (mg/dL)	2.93	0.96	3.38	0.17	3.83	0.33	3.60	0.78	3.48	0.90
GLO (mg/dL)	2.75	0.58	3.05	0.82	3.53	0.51	3.48	0.25	3.50	0.47
ALT (U/L)	36.50	6.24	32.25	6.08	42.25	7.50	42.13	6.22	41.25	6.55
AST (U/L)	57.00	8.25	42.00	1.83	59.25	7.41	51.50	6.61	48.50	4.65
LDH (U/L)*	681.50	60.63	521.00	103.27	261.75	64.78	168.25	66.31	121.00	11.69
ALKP (U/L)	484.75	25.83	358.25	83.99	319.50	35.12	283.00	55.12	180.00	89.14
GGT (U/L)*	87.40	5.58	92.50	20.92	52.00	3.37	53.50	4.20	52.75	11.30
LAC	2.15	0.60	1.75	1.04	1.70	0.68	1.75	0.13	1.35	0.26
CK (U/L)*	369.50	78.05	283.75	31.53	216.25	55.24	229.75	76.37	171.50	46.15
Na (mEq/L)	150.25	2.99	150.75	2.99	145.00	3.16	145.75	3.20	145.75	4.86
K (mEq/L)	3.85	0.19	3.51	0.41	3.10	0.32	3.53	0.48	3.80	0.32
CL (mEq/L)	110.00	2.94	109.75	3.86	112.25	4.50	105.75	4.35	104.00	2.83
Ca (mEq/L)	10.05	0.25	10.35	0.31	10.25	0.17	10.15	0.24	10.03	0.33
P (mEq/L)*	6.81	0.11	6.89	0.76	4.40	1.14	4.69	0.69	5.69	0.34

Data are presented as means ± 1 standard deviation (SD).

Statistical analysis revealed significant differences (*$P < 0.05$) between the different age groups in LDH ($F_{4,6.30} = 75.109$, $P = 0.0001$), GGT ($F_{4,7.17} = 27.204$, $P = 0.0001$), CK ($F_{4,7.26} = 5.351$, $P = 0.025$) and P ($F_{4,6.37} = 17.031$, $P = 0.002$).

4 | DISCUSSION

Anesthetic agents are commonly applied to laboratory animals to prevent the pain or stress caused by experimental procedures or to facilitate zootechnical handling by means of chemical restraint.[23] The drugs employed to anesthetize animals can be divided into two groups: those that produce unconsciousness or hypnosis, and those that produce sedation, tranquilization, analgesia and muscular relaxation. Dissociative agents have been widely used to produce hypnosis in animals. They may be used alone or in combinations to obtain and/or prolong the desired effect.[24,25]

Ketamine and tiletamine are chemically related, dissociative anesthetics that are among those most often used in veterinary medicine.[26] They achieve their effect by interrupting ascendant transmission from the unconscious to the conscious part of the brain (thalamic-cortical and reticular activating systems), rather than through the generalized depression of all brain centers.[25,27] Studies show that ketamine and tiletamine produce distinct effects depending on dosage, while observations indicate that anesthetized animals present a cataleptic state with eyes open and slight nystagmus, accompanied by hypertonicity and muscular rigidity, but without inhibition of the motor reflexes; also, the swallowing and pharyngeal reflexes persist.[25,27,28] While this achieves a wide margin of clinical safety, these effects can be mitigated by combining other sedating and/or tranquilizing agents. Indeed, numerous combinations of drugs have been employed to sedate or anesthetize NHPs, as is well-documented in the scientific literature.[1-5,7,10-12,14,28-31] Studies such as these underline the importance of monitoring key physiological parameters in order to predict and prevent potential complications during anesthesia. However, we know that different anesthetic drugs depress or alter vital functions in distinct degrees. So it necessary to administer more of a drug in combination, called balanced anesthesia, since this reduces the risks of using anesthesia.[17] It was in this light that the present study assessed the effects of distinct combinations of anesthetics on physiological constants and blood biochemistry, using ketamine alone (K), ketamine + acepromazine (KA), ketamine + xylazine (KX) and tiletamine + zolazepam (TZ).

The results obtained from measuring the physiological constants in each treatment group showed that there is an increase in HR in younger age groups, diminishing in all cases in the senile group. However, in the statistical analysis comparing the age groups in each treatment group only in the case of TZ and K, there were statistical differences. This phenomenon is widely described in the literature, and is attributed mainly to a sensory immaturity of the vagus nerve related to the baroreflex control of heart rate in the case of young animals, together with faster metabolic processes, than in the case

TABLE 5 Blood biochemistry values of ketamine-treated (Group D; K) male rhesus monkeys (*Macaca mulatta)* of different ages (Groups 1-5)

	1		2		3		4		5	
Age group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GLU (mg/dL)	79.50	4.43	72.75	3.30	71.25	6.55	76.00	6.98	81.75	4.57
BUN (mg/dL)	20.00	1.41	16.50	1.29	21.00	2.94	19.50	1.29	20.00	4.69
CREAT (mg/dL)	0.68	0.06	0.80	0.07	0.80	0.14	0.93	0.13	0.78	0.10
TB (mg/dL)	0.25	0.10	0.34	0.01	0.33	0.07	0.23	0.05	0.34	0.10
TP (mg/dL)	7.15	0.13	7.08	0.23	7.35	0.26	7.33	0.26	7.38	0.37
ALB (mg/dL)	4.43	0.10	4.19	0.34	4.38	0.28	3.99	0.39	3.65	0.52
GLO (mg/dL)	2.65	0.13	2.90	0.15	2.95	0.26	3.14	0.27	2.98	0.53
ALT (U/L)	39.00	2.16	35.50	3.70	38.50	1.91	40.00	5.29	37.00	2.94
AST (U/L)*	47.75	0.96	46.00	1.41	40.75	5.12	37.50	3.87	42.00	6.98
LDH (U/L)	439.75	20.25	414.25	91.34	447.75	70.32	440.25	73.79	462.75	56.36
ALKP (U/L)	380.50	56.24	278.50	64.07	295.75	46.08	286.25	23.26	298.00	58.76
GGT (U/L)	72.50	4.43	76.00	2.45	81.00	2.58	78.25	14.08	75.00	12.19
LAC	1.83	0.17	1.63	0.43	1.60	0.27	1.50	0.36	1.80	0.22
CK (U/L)	354.75	93.40	334.75	71.80	308.00	86.61	228.50	81.38	189.75	36.15
Na (mEq/L)	146.75	1.50	146.00	0.82	148.00	1.41	147.50	4.20	148.75	2.22
K (mEq/L)	4.08	0.44	4.28	0.73	4.13	0.30	4.25	0.13	4.05	0.31
CL (mEq/L)	109.00	4.24	106.50	1.29	112.00	2.16	108.75	2.36	106.50	4.20
Ca (mEq/L)*	10.48	0.13	10.04	0.03	10.05	0.29	10.18	0.45	10.28	0.74
P (mEq/L)*	5.58	0.57	5.50	0.43	5.45	0.58	4.48	0.67	3.88	0.59

Data are presented as means ± 1 standard deviation (SD).
Statistical analysis revealed significant differences (*$P < 0.05$) between the different age groups in AST ($F_{4,6.87} = 6.880$, $P = 0.015$), Ca ($F_{4,6.22} = 8.613$, $P = 0.011$) and P ($F_{4,7.45} = 5.649$, $P = 0.021$).

of senile animals.[6,32-39] In the other cases, the results obtained from the evaluation of the effects of the different drugs on the monkeys' physiological constants showed that for Groups 1, 2 and 3 there were statistically significant differences in HR and RR between KA and KX, and the control group (Figures 1 and 2). HR and RR decreased significantly when the combinations KA and KX were administered, regardless of age, while for SP and DP we observed that all age groups assessed showed significant differences upon comparing the anesthetic mixtures (Figures 3 and 4), including decreases in SP and DP under the anesthetic mixtures KA and KX, though this was less evident in TZ compared to the values obtained with K.

These findings are consistent with data reported in the literature, which affirm that acepromazine—a derivative of the phenothiazines—achieves its effect by bonding to dopamine receptors in the brain, thus depressing the CNS by affecting the basal ganglia, hypothalamus, limbic system, brainstem, and reticular activation system. This, in turn, produces sedation and muscular relaxation while also reducing motor activity. However, the secondary effects described include hypotension, bradycardia, and a slight depression of the respiratory system.[3,6,9,40-42] These changes were observed in experimental Group A in our study. There are also descriptions that xylazine, an α2 adrenoceptor agonist, reduces HR, causes hypotension, and decreases cerebral venous blood and intracranial pressure, thus depressing the CNS by reducing cerebral blood flow. In addition, this has been shown to decrease the number of respirations per minute by depressing the respiratory centers of the CNS, findings that are consistent with the reduction in SpO_2 observed in Group 5.[43,44] According to Settle et al,[45] depression of the HR due to administration of xylazine can be attributed to the combined action of the baroreceptor reflex and vasoconstriction mediated by the α2 receptor. The vasopressor effect of the α2 adrenoceptors produces greater vagal activity while inhibiting central sympathetic activity. With regard to ketamine, this drug has been associated with a tendency to stimulate the cardiovascular system by presenting sympathomimetic properties that cause an increase in HR, cardiac output, and arterial and central venous pressure,[26,46] which is related to the increases in HR, SP and DP recorded in the experimental group that received ketamine alone. However, these changes fall within the normal physiological parameters of this NHP species.[25,26] With respect to blood pressure, acepromazine has been seen to decrease arterial pressure in animals because it inhibits nervous control of blood vessels by blocking adrenergic α receptors.[17,47] Similarly, reports show that ketamine causes arterial hypertension, which is also consistent with the findings of this study.

In addition, there are reports that one of the main changes and/or effects generated by administering anesthetic drugs to NHPs is

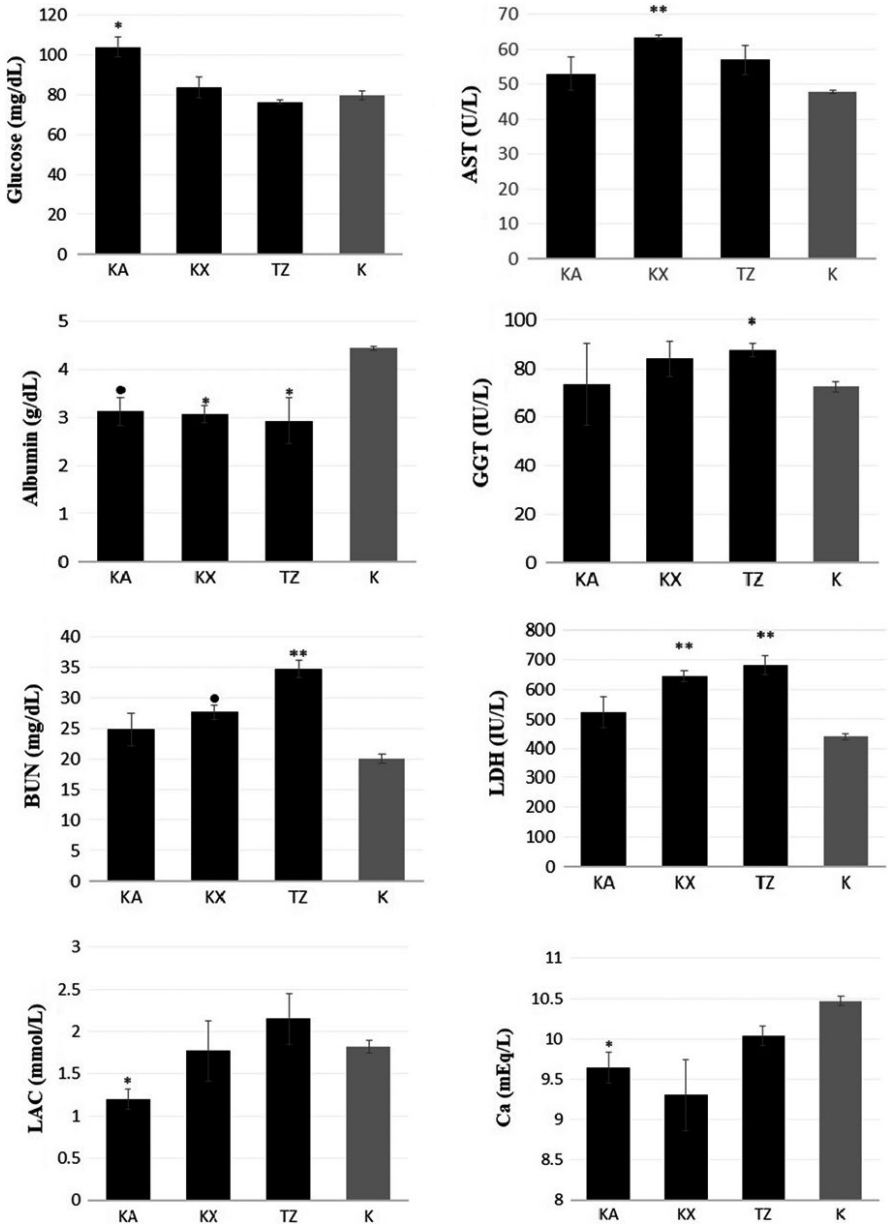

FIGURE 5 Effect of anesthetic mixture on blood biochemistry in male rhesus monkeys (0-1 y). Statistical analysis of ANOVA shows the significant differences between KA, KX and TZ against the K control group, using Bonferroni and Games-Howell post hoc tests, distinguished through the Levene analysis. •$P = 0.05$, *$P = 0.001$, **$P = 0.0001$

hypothermia, often associated with hypocalcemia, metabolic acidosis, and hyperglycemia. Drug-induced hypothermia reduces the metabolic rate and slows the biotransformation of drugs and anesthesia, thus prolonging the effects of these substances.[8] Our results show that statistically significant differences in this regard occurred only in Group 3 when KX was compared to the control group. It is clear that while no significant differences appeared among the other groups for KA and KX, temperature always remained below the values obtained for the control group, though without exceeding the normal range (36-40°C).[48,49]

Differences among age groups were evident for several of the biochemical measures recorded; however, results show that the same statistical differences did not appear in all treatments administered among the same age groups. This finding emphasizes the importance of considering the interaction of each anesthetic mixture with each age group.

Our results show statistical differences in experimental Group A (KA) for GLU (Table 2), a finding consistent with reports in the literature which affirm that the phenothiazines exert a hyperglycemic effect by blocking insulin's action.[50] We observed that in all age groups treated with KA, GLU increased compared to the other anesthetic mixtures administered. Only Group 1 showed a statistical difference between KA and the control group (K) (Figure 5).

In the case of BUN, no age-dependent statistical differences occurred, though the statistical analyses dependent on anesthetic mixture showed that this factor was higher in the groups treated with the anesthetic mixtures than in the control group for all age groups (Figures 5-9). This increase is attributed to the fact that most anesthetic agents, such as benzodiazepines or neuromuscular blockers, reduce the urinary rate and glomerular filtrate to various degrees, though this effect tends to be transitory, not clinically relevant, and preventable with good hydration.[51]

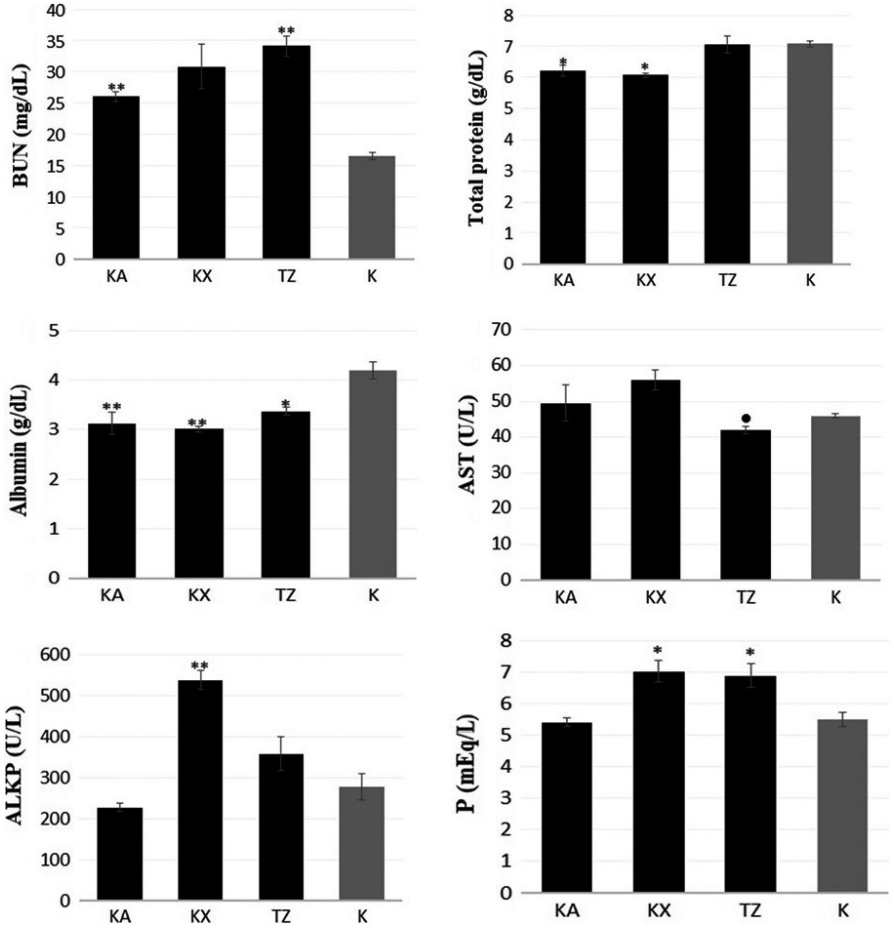

FIGURE 6 Effect of anesthetic mixture on blood biochemistry in male rhesus monkeys (1-3 y). Statistical analysis of ANOVA shows the significant differences between KA, KX and TZ against the K control group, using Bonferroni and Games-Howell post hoc tests, distinguished through the Levene analysis. •$P = 0.05$, *$P = 0.001$, **$P = 0.0001$

The statistical analyses that compared the age groups found no significant differences for TP, ALB and GLOB, but for ALB they showed a significant increase in the control group (K) versus the anesthetic mixtures, with significant differences in Groups 1, 2, 3 and 4 among all the mixtures compared to the control group (Figures 5-8). Observations of Groups 2 and 3 also found significant differences in TP (Figures 6 and 7). As reported previously, studies show that ketamine can have various effects on blood biochemistry, which can include hypoproteinemia.[6,9,52] Our results obtained with ketamine are similar to those reported in the literature,[1,2,30,33] although we also observed a decrease in TP and ALB values for the anesthetic mixtures, which suggests an influx of liquid into the vascular space.[52]

Turning to the hepatic enzymes ALT and AST, which we used as general indicators of liver function, ALT especially showed statistical differences related to age in experimental Groups B (KX) and D (K) (Tables 3 and 5). According to the literature, rhesus and vervet monkeys do not present age-related changes in ALT and AST.[13,53] However, other studies have found that ALT levels decrease with age, as seen in this study in experimental Group A (KA) and because ALT levels in Group 5 were lower than those of all other age groups assessed. These changes can be attributed to a reduction in liver mass or liver function in senile monkeys.[19,54] However, in experimental Group B (KX), ALT levels increased in Group 5, though the difference was not statistically significant (Table 3). Regarding AST, this enzyme presented statistical differences in experimental Groups B (KX) and D (K), mainly between the lower age groups and the older animals (Tables 3 and 4). Earlier studies showed that AST tends to increase due to the muscular irritation caused by intramuscular administration of drugs. There are also reports that many medications tend to alter AST values, but the AST levels obtained in our different age groups were similar to those reported by Buchl and Howard,[55] whose study utilized ketamine only as the chemical restraint for the animals and reported statistical changes attributable to age. The statistical analyses that contrasted the anesthetic mixtures revealed that these –mainly KX and TZ– increased AST, LDH, and GGT activity in Group 1 (Figure 5), while observations of Group 2 showed the same finding for AST and ALKP (Figure 6). In Group 3, we observed the same phenomenon for AST, but GGT decreased in TZ and increased in KA (Figure 7). In Group 4, AST increased in KA and TZ, while LDH was reduced in all anesthetic mixtures compared to the control group, K, and GGT showed the same behavior as in Group 3 (Figure 8). Finally, Group 5 showed a significant increase of LDH in KX, but a decrease of GGT in TZ; the same behavior seen in Groups 3 and 4 (Figure 9).

There are reports that muscular lesions increase the activity of muscular enzymes. Our results therefore suggest that this increase may be due to local myotoxicity caused by intramuscular injection.[3,9] Other work shows that ketamine affects hepatic metabolism through the metabolization process that, when combined with proteases, can contribute to a drug-induced hepatitis that

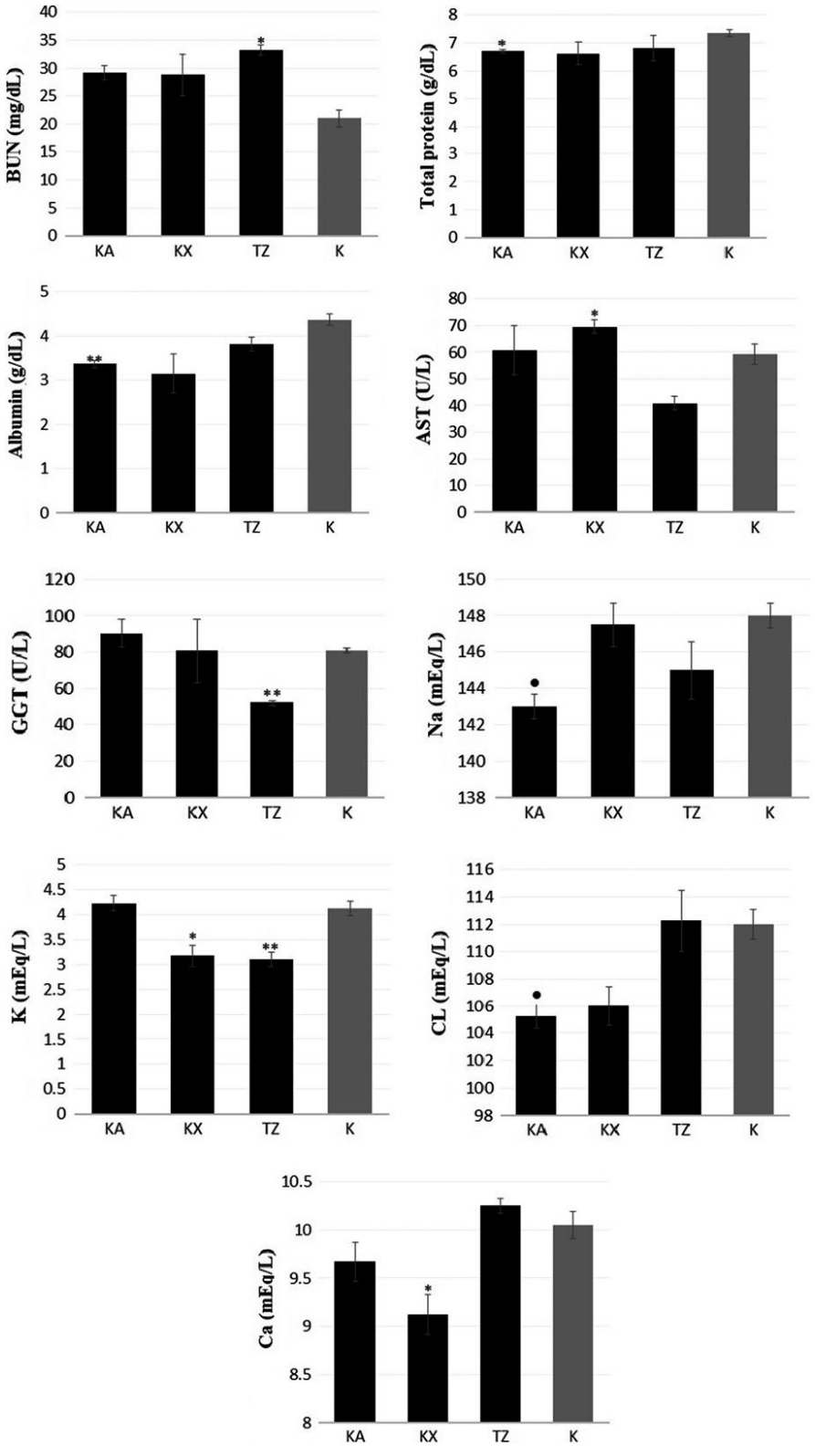

FIGURE 7 Effect of anesthetic mixture on blood biochemistry in male rhesus monkeys (3-5 y). Statistical analysis of ANOVA shows the significant differences between KA, KX and TZ against the K control group, using Bonferroni and Games-Howell post hoc tests, distinguished through the Levene analysis. •P = 0.05, *P = 0.001, **P = 0.0001

raises levels of hepatic enzymes. Ruíz et al,[56] meanwhile, state that pre-anesthetics like acepromazine or xylazine present phenomena of hypotension due to interaction with receptors in the autonomic nervous system (ANS) that can reduce blood perfusion in different organs, including the liver and kidneys, thus causing alterations in their functioning. This may explain the variations that the present study observed in the hepatic enzymes. There are reports that AST—an enzyme with great metabolic activity present in tissues—is released into circulation when cell lesions or cell death occur.[51] Numerous medications can increase AST levels. In this regard, our observations show that all the anesthetic mixtures presented increases compared to Group D (K), such that

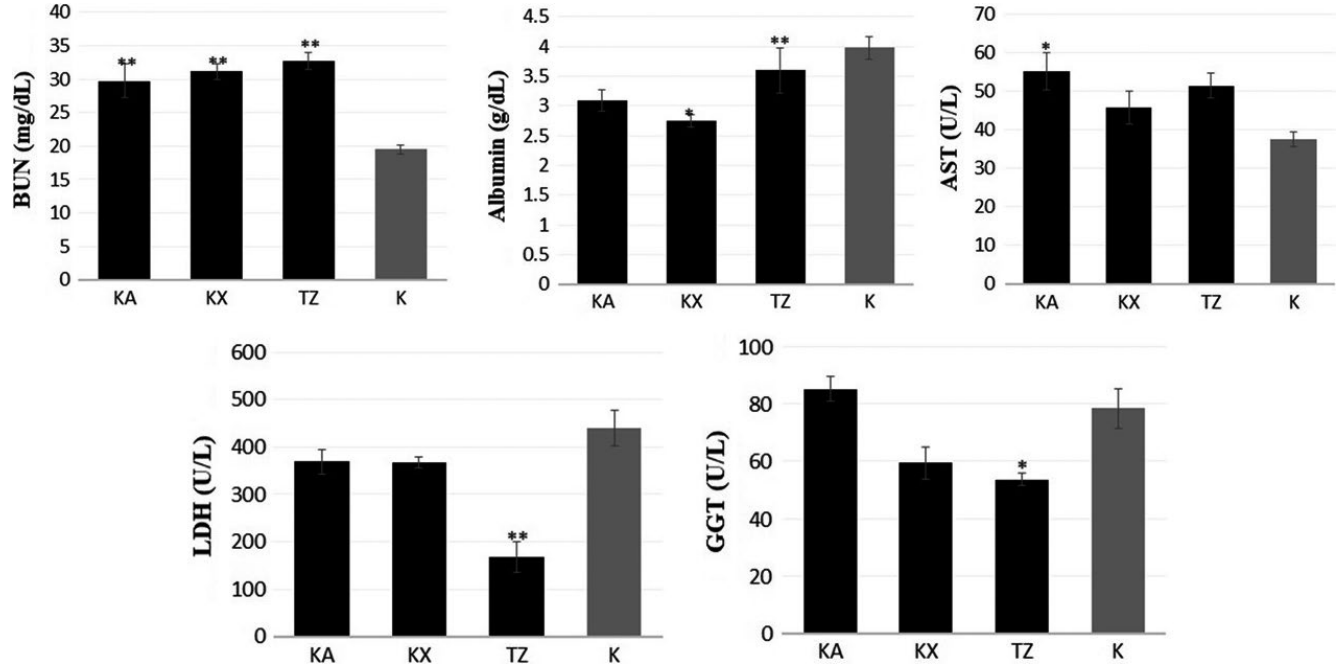

FIGURE 8 Effect of anesthetic mixture on blood biochemistry in male rhesus monkeys (5-15 y). Statistical analysis of ANOVA shows the significant differences between KA, KX and TZ against the K control group, using Bonferroni and Games-Howell post hoc tests, distinguished through the Levene analysis. *P = 0.001, **P = 0.0001

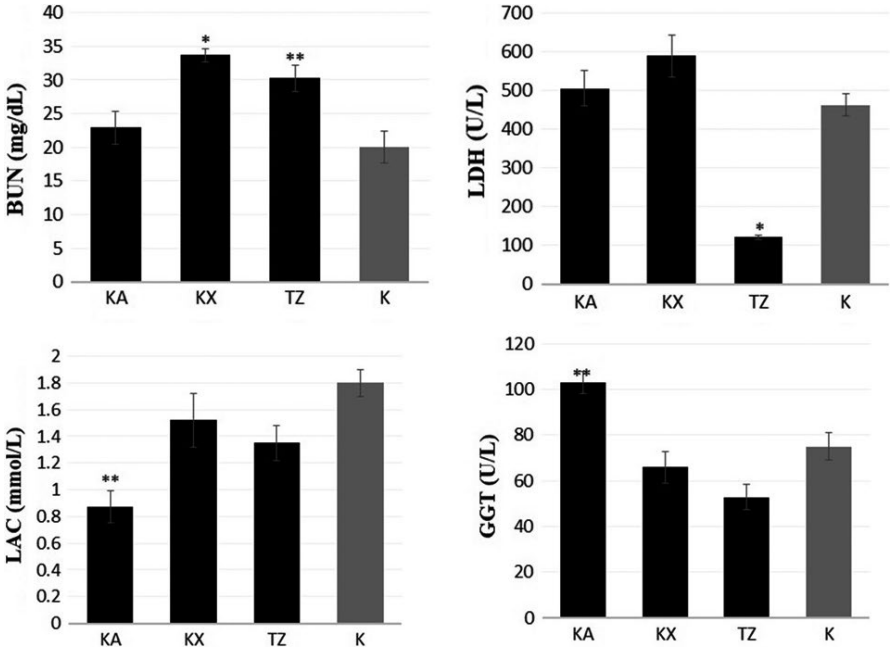

FIGURE 9 Effect of anesthetic mixture on blood biochemistry in male rhesus monkeys (+15 y). Statistical analysis of ANOVA shows the significant differences between KA, KX and TZ against the K control group, using Bonferroni and Games-Howell post hoc tests, distinguished through the Levene analysis. *P = 0.001, **P = 0.0001

interaction with muscle relaxants could tend to influence the release of this enzyme and have an effect on the myotoxicity that occurs with intramuscular administration of these compounds.

In the case of the analyses that compared the age groups, the enzymes LDH, GGT, and CK, which exist in greater concentrations in skeletal muscle tissue, showed statistical differences in Groups B (KX) and C (TZ), principally between Groups 1 and 2, which manifested higher concentrations of these enzymes compared to the older age groups (Tables 3-5). There are reports that both enzymes tend to increase due to muscular lesions caused by intramuscular administration of drugs and the toxicity of these substances for muscles.[3,9,57,58] Increases in CK have also been associated with a decrease in body temperature.[46] This relates to descriptions in various articles which mention that the anesthetic combinations of ketamine-xylazine and tiletamine-zolazepam lower body temperature,[8,48,49] and while the values we obtained are similar to those reported for CK and LDH, these statistical differences could be related to the anesthetic mixtures utilized.[9,13,16,17,19,26,28,30,40,48,55] Finally, our study

observed changes in electrolytes, including a decrease in Ca in the cases of KA and KX. This finding has been reported previously in other NHP species when KX is applied.[59] With respect to P, this increased in the groups treated with KX and TZ compared to controls. CL, meanwhile, increased in the group treated with TZ. Only a few studies have reported these variations in work with NHPs. In relation to the analyses that compared the age groups, our study found that P decreased in the older animals compared to the infant and juvenile groups in KX, TZ and K. This is also consistent with reports in the literature.[11] Descriptions of other animal species confirm that the benzodiazepines reduce renal blood flow, which could cause the increase seen in the group treated with TZ, given that the kidney is the main excretion site of this electrolyte. With regard to the increase in K with administration of KA, this can be attributed to the fact that increases in K in plasma are mediated by alpha adrenoceptors and, since acepromazine is an α2 adrenoceptor agonist, it could generate these variations through competition in the same receptors.[60] There are reports in the literature of an increase in P concentrations after administration of K in baboons, which has been attributed to the decomposition of phosphocreatine after a muscular lesion, stress or the decomposition of the excretion of inorganic phosphorus.[59,61] Other authors have suggested that the benzodiazepines reduce rates of glomerular filtration and urine production in humans,[62] so that these changes in electrolytes could be related to the use of the tranquilizers and sedatives applied in this study. It is important to emphasize that while our results reveal significant differences among the different anesthetic mixtures compared to controls, they remained within the ranges of normality published previously.[2,9,10,13,16,17,19,25,28,30,45,48]

The statistical differences observed among the different age groups evaluated due to the utilization of different anesthetic mixtures were consistent with many reports in the scientific literature, where it is well-documented that neonate and juvenile mammals are more susceptible than adults to the toxic effects of various substances. This is attributed to the fact that young animals have not yet fully developed their biotransformation and excretion mechanisms, while those in advanced age suffer a reduced efficiency of those same mechanisms. The distribution pattern of the drugs can be altered in neonates and geriatric animals due to modifications of the volume of fluids in their organs and changes related to adipose and non-adipose tissues.[56] Understanding the effects of age and different drugs on the biochemical parameters of serum will make it possible to determine the limits between normal changes and alterations related to disease, and to identify other factors or conditions that could affect these parameters.

The results obtained in this study demonstrate the need to evaluate the changes generated by different anesthetics and anesthetic mixtures in rhesus monkeys, especially when these analytes are the central objects of interest. The results of the present study may also make it possible to select the anesthetic that will produce the fewest changes in the variables of interest. Moreover, refining techniques of physical and chemical restraint will allow greater certainty and wider safety margins when drawing and analyzing samples for blood chemistry and physiological constants, which are basic instruments commonly used in processes of clinical diagnostics, inclusion criteria in diverse experiments, and zootechnical handling and animal welfare.

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None.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

All authors listed on title page have participated in the formulation of the investigation and also have read the manuscript, attest to the validity and legitimacy of data and its interpretation. Conceptualization and study design: BHG and AIC; methodology: BHG, AMH, ACU, and AIC; investigation: BHG and AIC; analysis and interpretation of the data: BHG, HBJ, and AIC, formal analysis: BHG, AP, HBJ, and MAS; writing (original draft): BHG, AP, HBJ, MAS, and AIC; writing (review and editing): HBJ and AIC; visualization: HBJ, MAS, and AP; statistical analysis: BHG, AMH, ACU; and AIC; critical revision of manuscript: HBJ, AP, MAS, and AIC; study supervision: AIC.

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