

Effects of double intravenous furosemide administration at different doses on clinical variables, electrocardiographic indices and serum electrolytes in miniature donkeys

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Article Info	Abstract
<p>Article history:</p> <p>Received: 15 June 2020 Accepted: 06 October 2020 Available online: 15 January 2023</p> <p>Keywords:</p> <p>Electrocardiographic indices Furosemide Intravenous administration Macro-minerals Miniature donkey</p>	<p>The intravenous (IV) usage of diuretics such as furosemide may cause changes in clinical variables, electrocardiographic (ECG) indices and some serum electrolytes concentrations in miniature donkeys. The purpose of this study was to evaluate the effects of double intravenous (IV) administration of furosemide at different doses on the clinical variables, ECG indices and serum electrolytes levels in miniature donkeys. A total of 21 clinically and para-clinically healthy miniature donkeys with a mean age of 6.00 ± 1.00 years and weight of 95.00 ± 5.00 kg were used. The animals were randomly divided into three groups of control (normal saline) and low- (1.00 mg kg^{-1}) and high-dose (2.00 mg kg^{-1}) of furosemide. Two IV injections with 12-hr interval were administered in each animal. The clinical and ECG parameters were measured at 0 (baseline), 2 (T2), 24 (T24) and 48 (T48) hr after drug administration. Serum concentrations of electrolytes were measured at T0, T24 and T48. The results of this study showed no changes in clinical variables (heart rate, rectal temperature and respiratory rate) and ECG indices (amplitude and duration of P, QRS and T waves and duration of PR, QT and RR intervals) as well as no significant changes in serum electrolytes (sodium, potassium, chloride, phosphorus, calcium and magnesium) levels. Although no significant effect on clinical variables, electrolytes levels and ECG indices after double IV administration of furosemide (1.00 and 2.00 mg kg^{-1}) in miniature donkeys was observed, absence of these effects does not mean that furosemide had no pharmacological effect.</p>

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Introduction

Miniature donkeys have been kept for transportation as well as milk production. This species differs from other equids because of small body size. Today, they are very wanted as companion animals.^{1,2}

Diuretics are prescribed to excrete additional extra-cellular fluids and decrease hypertension by increasing urine flow and sodium excretion. They are used in many veterinary treatments, such as congestive heart failure, udder and pulmonary edemas, ascites and acidosis or alkalosis as well as dilution and faster excretion of toxins. Also, diuretics are effective on renal glomerular filtration rate.^{3,4} These drugs are functionally categorized into different groups including loop, osmotic, potassium retainers and carbonic anhydrate inhibitors. The loop

diuretics are very powerful drugs that increase tubular excretion of sodium, chloride and other electrolytes from kidney and enhance urine flow. Furosemide is considered to be one of the widely used diuretics having some side effects, particularly decreases in serum potassium and sodium levels.^{5,6}

There is limited information regarding the effect of double intra-venous (IV) furosemide administration on clinical variables, electrocardiographic (ECG) indices and serum electrolytes levels in equidae, especially in miniature donkeys.^{7,8}

The purpose of the present study was to investigate the effects of double intravenous (IV) administration of furosemide at different doses on clinical variables, ECG indices and some serum electrolytes concentrations in miniature donkeys.

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Materials and Methods

Animals. This study was carried out in the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran. All ethical considerations including utilizing animals were considered cautiously. Also, the trial convention was affirmed by the Animal Welfare Committee (which was covered Institutional Animal Care and Use Committee approval) of the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman (institutional approval number: IR.UK.VETMED.REC.1399.007). Miniature donkeys were selected from the Large Animal Research Center of the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman. The donkeys had been domesticated and information including date of birth and history of vaccination and health status had been recorded precisely.

These animals were kept under the same nutritional and management conditions under the same husbandry. They were fed a mixed-grain ration (Khorakedam Co., Kerman, Iran) at a rate of 500 g per 50.00 kg daily with alfalfa hay *ad libitum*. During the study, the feed was distributed *ad libitum* in two meals at 9:00 AM and 6:00 PM. Fresh water was also provided *ad libitum*. Four weeks before the start of the experiment, all animals were orally treated with broad-spectrum anti-parasitic agents including 0.20 mg kg⁻¹ ivermectin and 10.00 mg kg⁻¹ praziquantel (Wormnil; Cipla Ltd. Co, Mumbai, India) to control possible internal and external parasites. Ten days before commencing the study, the animals were monitored for behavior. Also, three days before the start of the experiment, animals were evaluated for normal health condition using clinical and para-clinical (hemato-biochemistry and fecal parasitology) examinations. The experiment was carried out in the morning. The ambient temperature and relative humidity during the experiments were 19.00-21.00 °C and 12.00 - 14.00%, respectively. The study was performed in an outdoor covered 6.00 × 7.00 m² area. The animals were weighed before each treatment for calculation of drug dose. The donkeys were moved to the experiment area and restrained in a quiet standing position on a soft and comfortable mattress. Skin over the left jugular vein was clipped and scrubbed with povidone-iodine for IV injection and blood sampling.

Experimental procedures. The current study was conducted on 21 clinically and para-clinically healthy miniature donkeys with a mean age of 6.00 ± 1.00 years and weight of 95.00 ± 5.00 kg. The animals were randomly divided into three groups (seven animals in each group) including control (normal saline) and low- (1.00 mg kg⁻¹) and high-dose (2.00 mg kg⁻¹) of furosemide (Aburaihan Pharma Co., Tehran, Iran). Two IV injections with 12-hr interval were administered in each animal. The clinical variables including heart rate (HR) respiratory rate (RR) and rectal temperature (RT) and ECG indices (amplitude

and duration of P, QRS and T waves and duration of PR, QT and RR intervals) were measured at 0 (baseline), 2 (T2), 24 (T24) and 48 (T48) hr after drug administration. Serum concentrations of different electrolytes (sodium, potassium, chloride, phosphorus, calcium and magnesium) were measured at T0, T24 and T48.

Clinical findings and ECG indices. The HR was evaluated using stethoscope (Classic II SE, Littmann Co, USA) on the left side of chest wall (5th inter-costal space, behind the olecranon) for 1 min. The RR was assessed using direct observation of the thoraco-abdominal movement for 1 min. The RT was measured using a digital thermometer inserted into the rectum. Two large animal internists who were unaware of the drug doses evaluated the clinical variables. The ECG was obtained from all animals by bipolar base-apex lead system similar to Gunther-Harrington *et al.*, method.⁷

Biochemical parameters. Blood samples were taken from each animal via jugular vein and kept in test-tube with no anti-coagulant. For blood biochemistry, samples were kept at 24.00 °C for one hr and then, centrifuged (10 min, 3,000 g). Sera were separated and stored at - 20.00 °C for one day (before variables measurement). Sera were analyzed for sodium and potassium values by flame photometry (FLM, Ontario, Canada). Serum concentrations of chloride and phosphorus were measured using ultraviolet spectrophotometer. Atomic absorption spectrophotometry (AA-670; Shimadzu, Tokyo, Japan) was used to determine calcium and magnesium levels.

Statistical analysis. Data (clinical variables, ECG indices and electrolytes levels) were expressed as mean ± standard deviation. Data normality was analyzed by Kolmogorov-Smirnov test before statistical analysis. Data obtained from the same time between different groups were analyzed using one-way analysis of variance (ANOVA). Also, repeated measures of ANOVA were applied to compare the quantitative data at different time points in each group. The SPSS Software (version 23.0; IBM Corp., Armonk, USA) was used for all statistical analyses. Statistically significance level was considered to be $p < 0.05$.

Results

Changes in clinical variables and ECG indices are shown in Tables 1 and 2, respectively. There was no statistically significant difference in clinical variables (HR, RR and RT) and ECG indices (amplitude and duration of P, QRS and T waves and duration of PR, QT and RR intervals) among different groups at all time points. Changes in electrolytes concentrations in different groups are shown in Table 3. There were no significant differences in serum electrolytes (sodium, potassium, chloride, phosphorus, calcium and magnesium) concentrations among different groups at any time point. All parameters didn't change significantly from baseline in all groups after drug administration.

Table 1. Clinical variables immediately before (baseline) and up to 48 hr following double intravenous administration of low- (1.00 mg kg⁻¹) and high-dose (2.00 mg kg⁻¹) of furosemide as well as normal saline (control) in miniature donkeys.

Variables	Groups	Baseline	2 hr	24 hr	48 hr
Heart rate (beats per min)	Control	73.00 ± 10.00	73.00 ± 10.00	74.00 ± 9.00	75.00 ± 9.00
	Low-dose	75.00 ± 9.00	75.00 ± 8.00	74.00 ± 5.00	75.00 ± 8.00
	High-dose	74.00 ± 10.00	72.00 ± 6.00	72.00 ± 5.00	73.00 ± 8.00
Respiration rate (breaths per min)	Control	22.00 ± 4.00	22.00 ± 4.00	21.00 ± 3.00	23.00 ± 4.00
	Low-dose	23.00 ± 2.00	23.00 ± 1.00	22.00 ± 1.00	23.00 ± 2.00
	High-dose	22.00 ± 4.00	23.00 ± 4.00	23.00 ± 2.00	22.00 ± 4.00
Rectal temperature (°C)	Control	37.80 ± 0.50	37.80 ± 0.40	37.70 ± 0.50	38.00 ± 0.40
	Low-dose	37.90 ± 0.20	37.80 ± 0.20	37.70 ± 0.20	37.80 ± 0.20
	High-dose	37.90 ± 0.40	37.90 ± 0.20	37.90 ± 0.40	37.90 ± 0.40

Table 2. Electrocardiographic (ECG) indices immediately before (baseline) and up to 48 hr following double intravenous administration of low- (1.00 mg kg⁻¹) and high-dose (2.00 mg kg⁻¹) of furosemide as well as normal saline (control) in miniature donkeys.

ECG indices	Groups	Baseline	2 hr	24 hr	48 hr	
Amplitude (mV)	P	Control	0.23 ± 0.06	0.24 ± 0.06	0.24 ± 0.06	0.23 ± 0.06
		Low-dose	0.29 ± 0.06	0.24 ± 0.05	0.27 ± 0.08	0.28 ± 0.07
		High-dose	0.21 ± 0.08	0.21 ± 0.05	0.21 ± 0.06	0.22 ± 0.06
	QRS	Control	2.03 ± 0.40	2.06 ± 0.43	2.06 ± 0.30	2.06 ± 0.30
		Low-dose	1.89 ± 0.10	1.88 ± 0.18	1.92 ± 0.10	1.88 ± 0.10
		High-dose	2.12 ± 0.20	2.05 ± 0.13	2.10 ± 0.20	2.10 ± 0.20
	T	Control	0.63 ± 0.13	0.62 ± 0.12	0.64 ± 0.09	0.62 ± 0.13
		Low-dose	0.64 ± 0.08	0.66 ± 0.07	0.65 ± 0.05	0.64 ± 0.08
		High-dose	0.72 ± 0.07	0.72 ± 0.06	0.70 ± 0.05	0.69 ± 0.10
Duration (sec)	P	Control	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01
		Low-dose	0.11 ± 0.02	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.02
		High-dose	0.11 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
	QRS	Control	0.10 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.10 ± 0.01
		Low-dose	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01
		High-dose	0.11 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
	T	Control	0.12 ± 0.02	0.12 ± 0.01	0.12 ± 0.02	0.12 ± 0.01
		Low-dose	0.13 ± 0.02	0.12 ± 0.02	0.13 ± 0.01	0.13 ± 0.01
		High-dose	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.02	0.13 ± 0.01
Interval (sec)	PR	Control	0.18 ± 0.02	0.17 ± 0.02	0.17 ± 0.01	0.18 ± 0.02
		Low-dose	0.18 ± 0.02	0.18 ± 0.01	0.20 ± 0.01	0.19 ± 0.01
		High-dose	0.16 ± 0.02	0.20 ± 0.01	0.19 ± 0.02	0.17 ± 0.02
	QT	Control	0.46 ± 0.04	0.46 ± 0.04	0.47 ± 0.02	0.47 ± 0.04
		Low-dose	0.47 ± 0.04	0.48 ± 0.04	0.49 ± 0.04	0.45 ± 0.04
		High-dose	0.46 ± 0.02	0.49 ± 0.05	0.50 ± 0.05	0.48 ± 0.02
	RR	Control	0.92 ± 0.16	0.97 ± 0.08	0.96 ± 0.08	0.90 ± 0.20
		Low-dose	0.90 ± 0.07	0.93 ± 0.14	0.96 ± 0.10	0.80 ± 0.30
		High-dose	0.84 ± 0.13	0.93 ± 0.05	0.88 ± 0.06	0.82 ± 0.10

Table 3. Serum electrolytes concentrations immediately before (baseline) and up to 48 hr following double intravenous administration of low- (1.00 mg kg⁻¹) and high-dose (2.00 mg kg⁻¹) of furosemide as well as normal saline (control) in miniature donkeys.

Variables	Groups	Baseline	24 hr	48 hr
Calcium (mg dL ⁻¹)	Control	12.20 ± 1.90	12.60 ± 1.40	12.10 ± 1.70
	Low-dose	11.10 ± 1.70	11.70 ± 1.30	11.60 ± 1.50
	High-dose	11.40 ± 2.00	11.40 ± 1.80	12.10 ± 1.80
Phosphorus (mg dL ⁻¹)	Control	4.00 ± 0.40	4.20 ± 0.40	4.00 ± 0.40
	Low-dose	3.80 ± 0.20	4.20 ± 0.10	3.90 ± 0.20
	High-dose	3.90 ± 0.40	3.90 ± 0.50	4.20 ± 0.20
Sodium (mEq L ⁻¹)	Control	133.00 ± 4.00	135.00 ± 3.00	132.00 ± 4.00
	Low-dose	134.00 ± 10.00	136.00 ± 7.00	128.00 ± 9.00
	High-dose	135.00 ± 7.00	141.00 ± 4.00	137.00 ± 7.00
Potassium (mEq L ⁻¹)	Control	3.70 ± 0.60	4.10 ± 0.50	4.10 ± 0.70
	Low-dose	3.60 ± 0.30	4.50 ± 0.50	3.70 ± 0.40
	High-dose	4.30 ± 0.50	4.40 ± 0.30	4.20 ± 0.60
Chloride (mEq L ⁻¹)	Control	100.00 ± 11.00	99.00 ± 5.00	101.00 ± 8.00
	Low-dose	104.00 ± 6.00	104.00 ± 4.00	100.00 ± 5.00
	High-dose	103.00 ± 9.00	99.00 ± 7.00	105.00 ± 8.00
Magnesium (mg dL ⁻¹)	Control	2.05 ± 0.50	2.10 ± 0.60	1.80 ± 0.40
	Low-dose	2.10 ± 0.60	2.30 ± 0.30	2.00 ± 0.50
	High-dose	1.70 ± 0.50	2.30 ± 0.50	1.80 ± 0.30

Discussion

Electrolyte disturbances due to extensive administration of potent diuretics may lead to cardiac dysrhythmia.^{7,9} Hypokalemia is one of the common electrolyte disturbances as a side effect of diuretics.^{6,7} Changes in ECG after furosemide administration are largely unknown and there have been limited studies in equidae.⁷ The dose of furosemide used in the current study was based on the studies comparing it in other equidae.^{8,10,11} The 48-hr time course chosen for the present study was based on literature about the pharmacokinetic characteristic of furosemide in equidae.^{8,11,12} Changes of hemato-biochemical parameters following furosemide have been reported in humans,¹³ goats,¹⁴ cattle,¹⁵ ponies¹⁶ and horses.^{4,17,18}

Electrolytes values were within normal physiological range (for this species at this age) at baseline in all groups. This is in accordance with the findings of Samimi and Samimi and Tajik.^{19,20} In our study, no changes in serum electrolytes concentrations were recorded up to 48 hr. In a study by Gunther-Harrington *et al.*, IV administration of furosemide at 250 mg kg⁻¹ reduced potassium levels in racing horses.⁷ In another study in mature horses, hypocalcemia was observed after single IV furosemide (1.00 mg kg⁻¹) administration.¹² A decrease in serum levels of calcium, phosphorous, chloride and sodium was observed 24 hr after IV administration of furosemide in exercised Thoroughbreds horses.⁶ Also, a decrease in serum levels of sodium, chloride and potassium was observed in goats 5 hr after IV furosemide (10.00 mg kg⁻¹) administration.¹⁴ In addition, serum sodium and potassium levels were decreased after IV administration of furosemide (5.00 and 10.00 mg kg⁻¹) in high-yielding Holstein cows.¹⁵ The reasons for controversial findings between these studies and the current study may be due to differences in age, sex, species, physiological and management conditions, and dosage of furosemide.³ Hinchcliff and Muir have discussed the importance of the effect of different physiological condition on furosemide pharmacokinetic parameters.⁵

In this study, all clinical variables and ECG indices at baseline were within the physiological range of miniature donkeys reported by Samimi and Tajik.²⁰ No significant changes in clinical variables and ECG indices were observed in any studied groups. In contrast to the current study, Gunther-Harrington *et al.*, have observed significant changes in PR and RR intervals after single IV administration of furosemide (250 mg kg⁻¹) in racing horses.⁷ Gunter-Harrington *et al.*, have shown changes in HR and ECG indices without affecting cardiac biomarkers following furosemide administration in racing horses.⁷ Pourjafar *et al.*, have observed significant changes in PR, RR and ST intervals 5 hr after single dose of IV administration of furosemide (10.00 mg kg⁻¹) in goats.¹⁴

Chalmeh and Mazrouei Sebdani have reported that PR, RR, QT and ST intervals and also P and R amplitudes after single dose of IV administration of furosemide (5.00 and 10.00 mg kg⁻¹) change in high-yielding dairy cows.¹⁵ In addition, prolonged durations of P wave and QRS complex were observed during hypokalemia in rat.⁹ It can be stated that changes in ECG indices following furosemide administration were due to alterations in electrical conduction and/or action potential of myocardium following electrolyte imbalances.^{7,16} Akita *et al.*, have confirmed the ECG changes during furosemide-induced hypokalemia.⁹ These results suggest that myocardial excitabilities in the atria and ventricles may be affected by extra-cellular potassium level in rat.^{7,9}

It should be noted that absence of comparisons of furosemide with other diuretics as well as non-use of furosemide at higher and consecutive doses can be considered as limitations of the present study. A 24-hr interval between first and second sampling time does not provide a complete view of the effects of furosemide (at these doses) on electrolytes values. It would be better if more frequent measurements of electrolytes were considered especially during first 24 hr. In addition, more investigations about pharmacological effects of furosemide in miniature donkeys are required.

In conclusion, although no significant effect on clinical variables, electrolytes levels and ECG indices after double IV administration of furosemide (1.00 and 2.00 mg kg⁻¹) in miniature donkeys was observed, absence of these effects doesn't mean that furosemide had no pharmacological effect.

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Conflict of interest

The authors declare that there were no conflict of interest and financial disclosure associated with this research or its contents.

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