v. 2, n. 19, 2022

International Journal of Health Science

PHARMACOLOGICAL PROFILE OF LOOP DIURETIC IN EXPERIMENTAL MODEL OF HEART FAILURE

Jhônata Costa Moura
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA, São Luís, MA, Brazil

Vinícius Santos Mendes
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA, São Luís, MA, Brazil

Gabriel Gomes Oliveira
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA, São Luís, MA, Brazil

Gabriel Antônio Bezerra Costa e Souza
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA, São Luís, MA, Brazil

Beatriz da Silva Ferreira de Lima
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA, São Luís, MA, Brazil

Nicolas Melo de Cerqueira Salgado
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA, São Luís, MA, Brazil

Lara Possapp Andrade
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA, São Luís, MA, Brazil

Ludmila Tavares dos Santos Silva
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA, São Luís, MA, Brazil

Isabella Teixeira Lopes
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA, São Luís, MA, Brazil

Elder Teles Teixeira
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA, São Luís, MA, Brazil

Marilene O. R. Borges

Antonio Carlos R. Borges

Rachel Melo Ribeiro
Laboratory of Pharmacology, Department of Physiological Science, Federal University of Maranhão-UFMA
Graduate Program in Health Sciences, Federal University of Maranhão-UFMA
São Luís, MA, Brazil

Abstract: Loop diuretics such as furosemide are first-line drugs for patients with decompensated heart failure (HF). The study aims to describe the implementation and standardization of an experimental model in diuresis at the Laboratory of Research and Graduate Studies in Pharmacology at the Federal University of Maranhão. Rats were randomly divided: furosemide (furosemide) group received 10 mg/kg furosemide, without CI; The Heart Failure Group (Vehicle-HF) received 10ml/kg of water and the Heart Failure-Furosemide Group (Furosemide-HF) received 10mg/kg of furosemide. IC was induced by isoproterenol for seven days. Oral treatment took place for 7 days to assess food and water intake, weight gain, urinary volume, excretion and diuretic activity. The rodents with HF showed cardiac hypertrophy and body weight loss in the induction process. Regarding the impacts of HF on water consumption, the animals with heart disease showed intense water intake compared to the Furosemide Group. During treatment, there was a significant drop in water consumption in these groups, a change that is not evident on the last day of treatment among the groups evaluated. There was no change in feed consumption. The HF-vehicle and HF-furosemide groups showed a reduction in 24-hour urinary volume, urinary excretion and diuresis, on the 7th day of treatment, when compared to the healthy group, treated with the diuretic. The results allow to identify the deleterious impacts of the induction of CI on the renal capacity of the animals, reducing the urinary volume and urinary excretion, where the diuretic activity of furosemide was compromised without it being able to induce with the expected effectiveness, its activity on the animal renal system. The data studied identified patterns in the diuresis protocol in animals with heart failure, with possibilities for future pharmacological investigations.

Keywords: Loop diuretics, Heart failure, Health.

INTRODUCTION

Heart failure (HF) is a syndrome that can manifest itself in different ways and be the result of several other cardiovascular or systemic diseases that cause cardiovascular impairment. HF can present itself in a chronic or acute form, the latter having decompensation and congestion as its most common and prevalent manifestations. Thus, diuretics are some of the first-line drugs in the treatment of decompensated patients, especially loop drugs and thiazides (1-3).

However, these drugs are related to several adverse effects, such as hypovolemia, which can cause low renal perfusion and hypotension, electrolyte disturbances, and even diabetes (2, 4). Thus, continuous research and experimentation with new drugs and substances of synthetic or plant origin, which can perform similar diuretic effects and with fewer side effects, is essential.

In this context, phytotherapics represent an important class of drugs with great potential for the discovery of new molecules, since Brazil has enormous botanical diversity, which can be used in the treatment of diseases in a safe, more natural and accessible way, once their therapeutic activity is proven (5).

To understand the pathophysiology and effectiveness of available pharmacotherapy, experimental animal models are created, tested and evaluated to mimic the clinical conditions of various diseases, including HF (6, 7). Regarding diuresis protocols, specifically, methodologies need to be compared in order to allow a higher degree of reliability (7), since many variables (e.g. urinary excretion, weight gain, etc.) and collection methodologies require standardization (8, 9).

Such models allow not only, together with clinical research, the discovery of new drugs (10), but also contribute to a better understanding of a gap regarding this topic: the effect that HF has on pharmacodynamic and kinetic characteristics (11). In the literature, several advances have been made in this aspect within population studies (12, 13), but within experimental research, studies and models that can significantly detect such changes are still scarce.

Thus, the application and development of experimental models that support the discovery of new drugs and increase the existing pathophysiological and pharmacokinetic understanding is essential. As a result, this study aims to describe the findings, the implementation and standardization of an experimental model in diuresis in the Laboratory of Research and Post-graduation in Pharmacology of Federal University of Maranhão (LPPF-UFMA).

METHODOLOGY

ANIMALS

Male rats (Rattus norvegicus) of the Wistar strain were used. The animals were obtained from the colonies of the Central Animal Facility of the Federal University of Maranhão, where the experiments were conducted. The animals were housed in polypropylene cages, provided with beds of shavings and housed in climate-controlled racks. The animals had free access to food and water ad libitum, and the room temperature was maintained around 22 ºC, with a 12 h light-dark cycle. In the metabolic cages, the animals had free access to water and feed.

The procedures are in accordance with the federal law 11.794 of October 08, 2008, with the regulations of the National Council for Control and Animal Experimentation, and were approved by the Ethics Committee on Animal Experimentation of the Federal University of Maranhão, according to protocol nº 23115.010680/2020-78.

EXPERIMENTAL DESIGN

The treatment of the animals occurred by gavage, for seven consecutive days of furosemide in animals induced or not to heart failure. Rats (n=12) were randomly divided into three groups: healthy furosemide group (Furosemide) received furosemide 10mg/kg/day; heart failure group (HF-Vehicle) received water 10ml/kg/day and heart failure furosemide group (HF-Furosemide) received furosemide 10mg/kg/day.

INDUCTION OF HEART FAILURE AND ESTIMATION OF CARDIAC HYPERTROPHY

Experimental induction of congestive heart failure in HF-vehicle and HF-Furosemide animals was performed by administering low doses (5mg/Kg for 7 consecutive days) of isoproterenol, diluted in 2mL of 0.9% NaCl isotonic solution subcutaneously daily (WANG et al., 2019). At the end of the experiment, the hearts were removed, washed with phosphate buffered saline (PBS) solution (in mM): 137 NaCl; 2.7 KCl; 4.3 Na2HPO4; 1.47 KH2PO4, pH 7.4 and dried with filter paper. Subsequently, they were weighed on precision analytical balance to obtain the relative weight (Organ Weight) / (Body Weight), to estimate the presence of cardiac hypertrophy.

FEED AND WATER CONSUMPTION AND PONDERAL VARIATION

During treatment, the daily feed intake and water volume ingested by the animals were checked. Also, twice a week, the weight of the animals over seven days was checked. In parallel, the ponderal variation was calculated with the difference between the initial weight (referring to the weight of the animals before the beginning of the treatment) and the final weight (weight of the animals at the end of the 7-day treatment), to corroborate the induction of heart failure with the estimate of hypertrophy.

STANDARDIZATION OF EXPERIMENTAL DIURETIC ANALYSIS

Diuretic activity was determined according to the method published by Liu et al., 2019. Thus, after undergoing a 15-day adaptation period, all animals underwent a 6-h urine collection in metabolic cages after being loaded with distilled water, in which those that showed a urinary excretion greater than 40% of the ingested content were selected for the study.

After selection, each animal was housed individually in a metabolic cage for a period of 7 days with water and feed ad libitum. For each animal the 24 h urine was collected daily for determination of the daily volume, The urine of the last 24h of the experiment were used to determine the Urinary Volume (measured urine volume), the urinary excretion (Urine Volume 24h / 24h*60min*100) and the diuretic action of each group (urinary excretion of the group / urinary excretion of the negative control group). Urine physicochemical analysis was performed using the 24 h urine samples from all animals. Blood samples were collected from the abdominal aorta and later submitted to centrifugation (3500 rpm for 10 min) Sera were stored at -20 ºC for subsequent analysis of electrolyte concentration (Na+, K+ and Cl-).

URINE PHYSICOCHEMICAL ANALYSIS

For urine analysis, reagent strips were used to evaluate pH and specific density (1000-1030).

STATISTICAL ANALYSIS

Data were analyzed in GraphPad Prism® 6.0 software and presented as mean ± standard error of means and analyzed by analysis of

variance (ANOVA) for multiple comparisons, followed by Tukey's test. Differences with $P < 0.05$ indicated statistical significance.

RESULTS

HYPERTROPHY AND WEIGHT VARIATION IN INDUCTION OF HEART FAILURE

The present study found that there was an increase in the heart weight/body weight ratio (hypertrophy estimate) of animals that underwent heart failure induction by the use of isoproterenol (vehicle HF and furosemide HF, Figure 1.A). Also, the results show no effect of furosemide on the myocardial hypertrophy of the animals. After HF induction and treatment with loop diuretic, there is a reduction in final body weight (Figure 1.B), as expected for the experimental model and the use of furosemide. The furosemide group without HF showed less body weight reduction between the first and last days of treatment.

IMPACTS OF THE HEART FAILURE INDUCTION PROTOCOL ON FEED AND WATER CONSUMPTION AND WEIGHT GAIN

Over the 7 days of treatment, it was observed that the groups did not present statistically different absolute weights ($p>0.05$), as shown in Figure 2.

As shown in Figure 3, during the time spent in metabolic cages, there were no significant differences in feed intake. There was no increase or decrease for any of the groups during the 07 days, not even differences between the groups. It was concluded that consumption was equivalent between the groups throughout all the days of treatment.

Regarding the impacts of heart failure induction on water consumption (Figure 4), it can be observed that at the beginning of treatment, animals with cardiac disorder presented intense water consumption compared to the Furosemide (healthy) group. No difference was observed between the HF-vehicle and HF-furosemide groups. However, during the treatment, it is possible to notice a significant drop in the water consumption of these groups, so that on the last day of treatment there is no longer a statistically significant difference between the consumption of the HF-vehicle, HF-furosemide and Furosemide groups.

PHYSICAL-CHEMICAL ANALYSIS OF URINE

The physical-chemical analysis of the urine showed that the pH values were within the expected values for the respective Furosemide groups (7 ± 0.5); IC-Vehicle (7) and HF-Furosemide (7 ± 0.5). The same was observed for the specific gravity of urine, Furosemide (1.008 ± 0.002); IC-Vehicle (1.005) and HF-Furosemide (1.005).

ASSESSMENT OF URINARY PARAMETERS

Regarding urinary volumes, it was observed that the groups submitted to the heart failure protocol, that is, HF-vehicle and HF-furosemide, showed statistically significant reductions in 24-hour urinary volume. Urinary excretion is also a measure of 24-hour diuresis, whose unit relates volume to time of excretion. The result of urinary excretion is shown in Figure 6, which also showed a negative impact resulting from the induction of HF.

Diuretic activity relates the urinary excretion of the evaluated group with the negative control group. It is then possible to observe the damage to renal function associated with the development of heart failure, as in Figure 7, all failure groups (HF-vehicle and HF-Furosemide) had a diuretic action of less than 1, thus indicating that

renal excretion was reduced. to levels below the negative control.

DISCUSSION

This study aimed to implement the diuresis model in animals with heart failure in the period of seven days of treatment, evaluating the diuretic profile of furosemide in healthy animals and with HF. Our results show that there is an alteration in the diuretic capacity of this drug in animals submitted to this cardiovascular disorder, with a reduction in the urinary volume excreted and less diuretic activity.

Regarding the weight evolution of the animals, there was no significant difference in the weights between the groups. However, it was observed that the animals showed a weight gain during the 7 days of treatment. Highlighting a greater weight gain for the Furosemide group (Figure 1). These findings are corroborated by DE ALMEIDA et al., 2018(14), where they observed that the use of hydrochlorothiazide in normotensive and SHR Wistar rats showed no significant difference between the groups over the 7 days of treatment, thus showing that the treatments with hydrochlorothiazide or notophagine did not alter the weight gain of these animals, thus reducing the probability of causing excessive dehydration and consequently an indirect weight loss.

Continuing, on the consumption of water and feed, DE ALMEIDA et al., 2018(14), in their study showed that the use of diuretics did not change the consumption of water and feed among the groups treated with diuretics when compared to the control group. Thus, these findings are in line with our results, in which no significant difference was observed between the groups in relation to feed consumption (Figure 3). Thus, it is assumed that the induction of heart failure did not significantly affect the feed intake of the animals. Regarding water consumption, significant differences were observed between the healthy group and the IC groups at the beginning of treatment (Figure 4). Thus, it is believed that the increase in water consumption by animals with induced heart failure occurred due to the cardiorenal repercussions of the pathology, with increased water retention and consequent increase in water consumption by animals.

Regarding the results of diuretic activity, the healthy furosemide group stood out, presenting higher values in the three analyzed parameters: urinary volume, urinary excretion in percentage and diuretic action. Corroborating these findings, Liu et al., 2019(15), in their study on the diuretic effect of the ethanolic extract of Logopsis supina in normotensive rats, observed that the volume of diuresis in the furosemide group was significantly increased. This property can be explained by the mechanism of action of loop diuretics, based on the reduction of solute concentration in the medullary interstitium and, as a consequence, there is a reduction in water reabsorption in the collecting ducts, promoting diuresis (16).

In the present study, we used an isoproterenol-induced HF animal model. Isoproterenol is a non-selective β-agonist synthetic substance used for the treatment of bradyarrhythmias (17). The use of this substance causes an increase in free radicals in myocytes, oxidative stress, apoptosis and consequent necrosis of cardiac tissue, which justifies its ability to induce HF (18).

One of the expected physiological consequences of HF is the reduction of urinary volume, a significant marker of renal dysfunction, a pathophysiological context involved in the cardiorenal syndrome (19, 20). Therefore, our results show that the groups induced to HF showed a marked reduction in the urinary parameters analyzed (Figures 5,

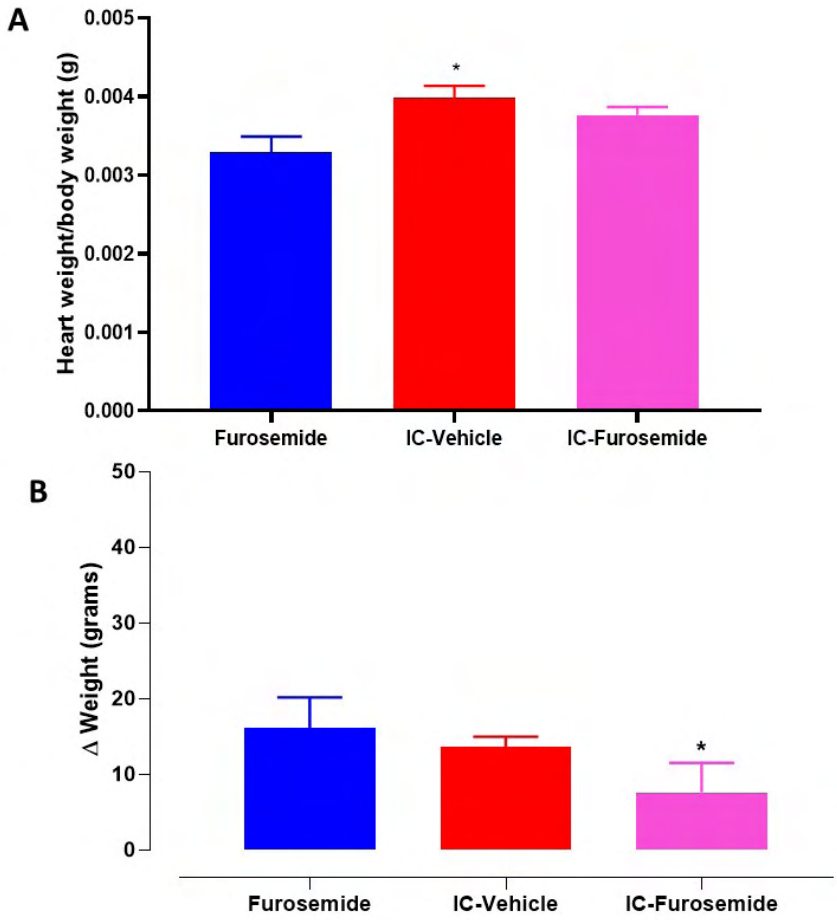

Figure 1: (A) Heart weight to body weight ratio (B)Variation of initial and final body weight (g) of animals. ANOVA, Tukey. *p<0.05 (n= 3-4).

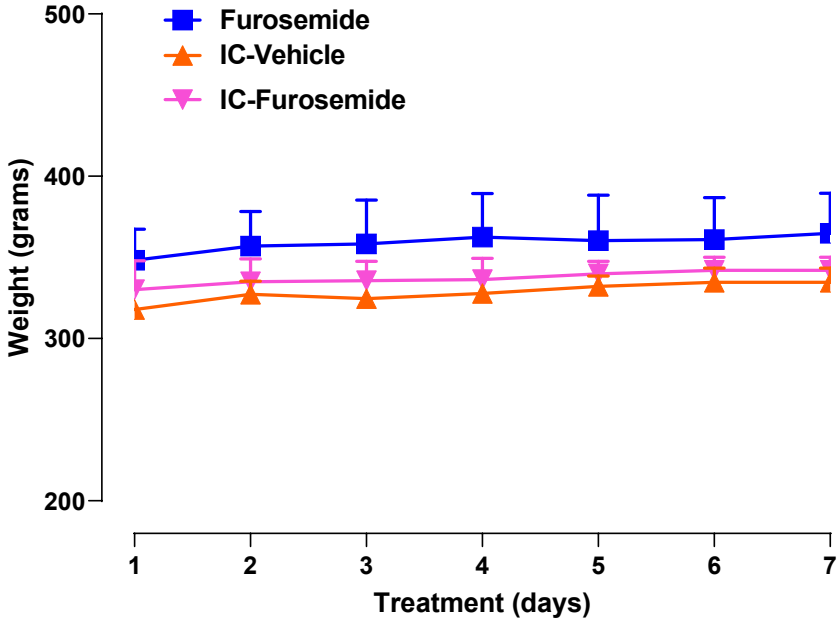

Figure 2: Weight evolution over 07 days. ANOVA, Tukey. *p<0.05 (n=3-4).

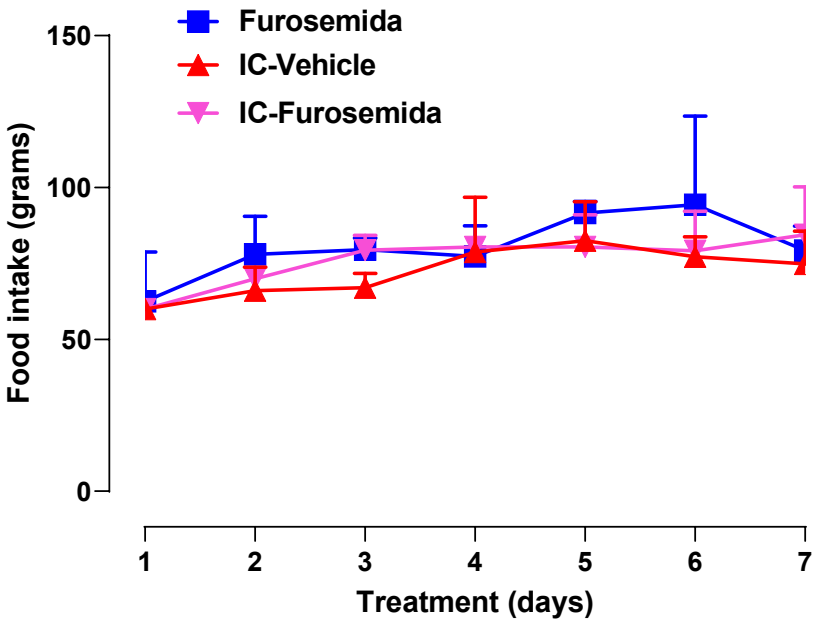

Figure 3 – Assessment of feed water consumption of animals submitted to heart failure treated with furosemide. ANOVA, Tukey. *p < 0.05 (n = 3-4).

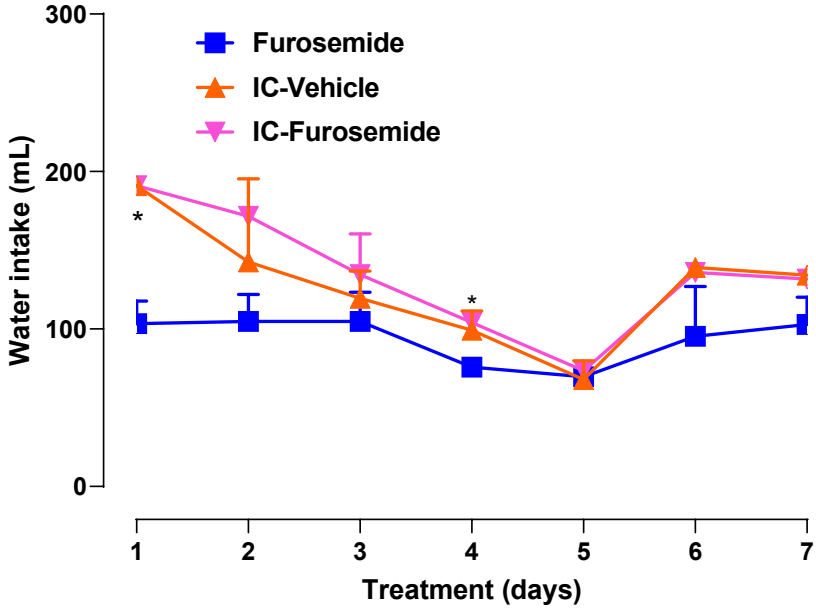

Figure 4 – Assessment of water consumption in animals submitted to heart failure treated with furosemide. ANOVA, Tukey. *p < 0.05 (n = 3-4).

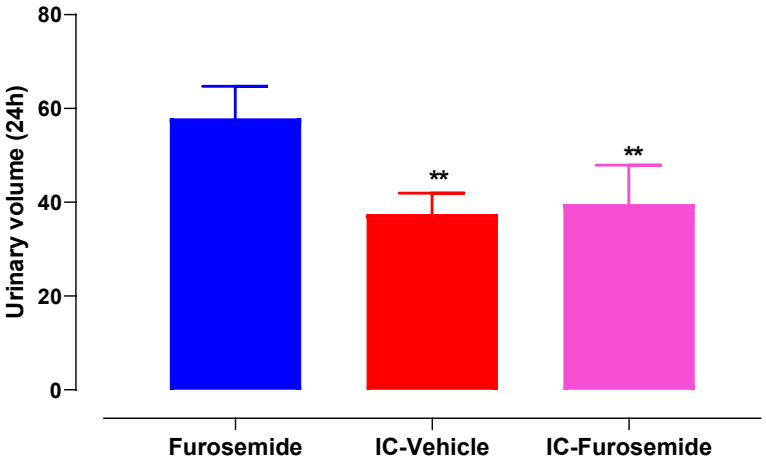

Figure 5 - 24-hour urinary volume of water in animals submitted to heart failure treated with furosemide. ANOVA, Tukey. *p<0.05; **p<0.01 (n=3-4).

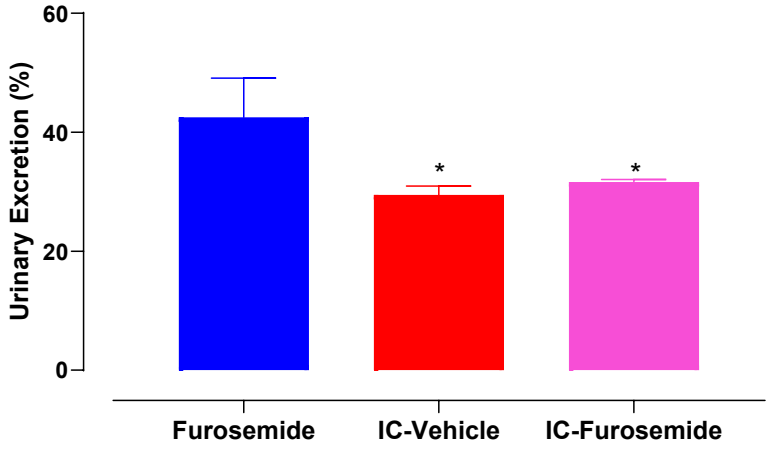

Figure 6: Urinary excretion of animals submitted to heart failure treated with furosemide. ANOVA, Tukey. *p<0.05; **p<0.01 (n=3-4).

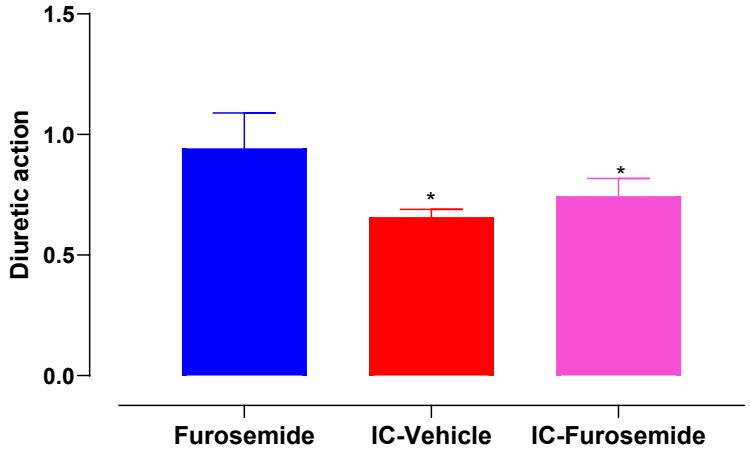

Figure 7 - Diuretic activity of animals submitted to heart failure treated with furosemide. ANOVA, Tukey. *p<0.05 (n=3-4).

6 and 7), and these results have already been exposed and discussed in previous studies (21, 22). Thus, the observed results are in agreement with the literature.

In continuity, the results obtained for the urinalysis in relation to specific density and urinary pH, showed that the treatment with diuretic was not able to change these parameters. Similar results were observed by YANG et al., 2021(23), where they observed that treatment with aqueous soluble fraction of Lagopsis supina (40-320 mg/kg) or furosemide (10 mg/kg) was not able to change the pH of the animal urine. Similar to these results, DE ALMEIDA et al., 2018(14) point out in their study that they did not find changes in pH values and urinary density.

CONCLUSION

Thus, the present study identified patterns involved in the diuresis protocol in animals with isoproterenol-induced heart failure. It is a new line of research in our laboratory and opens up possibilities for new pharmacological investigations at the University. It was possible to identify the deleterious impacts of HF induction on the renal function of the animals, reducing urinary volume and excretion. There was also intense water intake by the sick animals, especially in the first days of treatment, right after the HF induction protocol. More studies are needed to better understand the pharmacological changes that occurred for furosemide in this experimental model.

THANKS

We thank the Foundation for Research Support and Scientific and Technological Development of Maranhão (FAPEMA), Professor Antonio Carlos Romão Borges and Marilene Oliveira da Rocha Borges.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Barroso WKS, Rodrigues CIS, Bortolotto LA, Mota-Gomes MA, Brandão AA, Feitosa ADdM, et al. Diretrizes Brasileiras de Hipertensão Arterial–2020. 2021;116:516-658.

2. Calazans RM, Sepulvida MBdC, Quadrado EB, Miranda RDJABdC. Use of Diuretic Therapy in Patients with Decompensated Heart Failure and Acute Kidney Injury. What to do in this Dilemma? : SciELO Brasil; 2021. p. 725-6.

3. Piardi DS, Butzke M, Beck-da-Silva LJAHFC. Diuretics and Clinical Management of Congestion in Heart Failure: A Review. 2021;1(1):55-62.

4. de Andrade AEA, Binda NS, da Silva JFJBJoHR. Análise da associação entre diuréticos tiazídicos e o desenvolvimento do diabetes mellitus do tipo 2 Analysis of the association between thiazide diuretics and the development of type 2 diabetes mellitus. 2021;4(6):24170-87.

5. Farias PAM, da Silva IA, de Queiroz ALFG, Silva JG, Melo ERD, de Melo Barros JMJBJoD. Propriedades terapêuticas de plantas do gênero Syagrus: uma revisão integrativa Therapeutic properties of plants of the genus Syagrus: an integrative. 2021;7(8):76999-7010.

6. Cops J, Haesen S, De Moor B, Mullens W, Hansen DJHFR. Current animal models for the study of congestion in heart failure: an overview. 2019;24(3):387-97.

7. Bacmeister L, Schwarzl M, Warnke S, Stoffers B, Blankenberg S, Westermann D, et al. Inflammation and fibrosis in murine models of heart failure. 2019;114(3):1-35.

8. Onishi A, Fu Y, Patel R, Darshi M, Crespo-Masip M, Huang W, et al. A role for tubular Na+/H+ exchanger NHE3 in the natriuretic effect of the SGLT2 inhibitor empagliflozin. 2020;319(4):F712-F28.

9. Rao VS, Ivey-Miranda JB, Cox ZL, Riello R, Griffin M, Fleming J, et al. Natriuretic equation to predict loop diuretic response in patients with heart failure. 2021;77(6):695-708.

10. Teerlink JR, Diaz R, Felker GM, McMurray JJ, Metra M, Solomon SD, et al. Cardiac myosin activation with omecamtiv mecarbil in systolic heart failure. 2021;384(2):105-16.

11. Felker GM, Ellison DH, Mullens W, Cox ZL, Testani JMJJotACoC. Diuretic therapy for patients with heart failure: JACC state-of-the-art review. 2020;75(10):1178-95.

12. Abdel Jalil MH, Abdullah N, Alsous MM, Saleh M, Abu-Hammour KJBJoCP. A systematic review of population pharmacokinetic analyses of digoxin in the paediatric population. 2020;86(7):1267-80.

13. Kassem I, Sanche S, Li J, Bonnefois G, Dubé MP, Rouleau JL, et al. Population Pharmacokinetics of Candesartan in Patients with Chronic Heart Failure. 2021;14(1):194-203.

14. de Almeida CLB, Cechinel-Filho V, Boeing T, Mariano LNB, da Silva LM, de Andrade SF, et al. Prolonged diuretic and saluretic effect of nothofagin isolated from Leandra dasytricha (A. Gray) Cogn. leaves in normotensive and hypertensive rats: role of antioxidant system and renal protection. 2018;279:227-33.

15. Liu Z, Yang L, Li L, Wei R, Luo X, Xu T, et al. Diuretic and antidiuretic activities of ethanol extract and fractions of Lagopsis supina in normal rats. 2019;2019.

16. Fiorini EA, de Souza Siragusi RH, Junior EAL, Franco RPJRdECeMVeZdC-S. Utilização da furosemida em bolus e em infusão contínua em cães e gatos: revisão de literatura. 2017;15(1):54-60.

17. Szymanski MW, Singh DP. Isoproterenol. 2018.

18. Allawadhi P, Khurana A, Sayed N, Kumari P, Godugu CJPR. Isoproterenol-induced cardiac ischemia and fibrosis: Plant-based approaches for intervention. 2018;32(10):1908-32.

19. Ronco C, Bellasi A, Di Lullo LJAickd. Cardiorenal syndrome: an overview. 2018;25(5):382-90.

20. Costanzo MRJHFC. The cardiorenal syndrome in heart failure. 2020;16(1):81-97.

21. Peng D-F, Tang S-Y, Hu Y-J, Chen J, Yang LJE, Medicine T. Pathophysiological model of chronic heart failure complicated with renal failure caused by three-quarter nephrectomy and subcutaneous injection of isoprenaline. 2013;5(3):835-9.

22. Zheng G, Cai J, Chen X, Chen L, Ge W, Zhou X, et al. Relaxin ameliorates renal fibrosis and expression of endothelial cell transition markers in rats of isoproterenol-induced heart failure. 2017;40(7):960-6.

23. Yang L, He Z-W, He J-WJJoE. The chemical profiling of aqueous soluble fraction from Lagopsis supina and its diuretic effects via suppression of AQP and RAAS pathways in saline-loaded rats. 2021;272:113951.