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Diuretic activity of the flavonoid pinostrobin previously identified from the species *Renealmia alpinia*

Carolina Lopera-Londoño [a*], Cristhian Melan [a], Julieta Vásquez [a], Alejandro Serna [a], Arley C. Patiño [a,b], Dora M. Benjumea [a,b]

a. Research Group in Toxinology, Pharmaceutical, and Food Alternatives, Faculty of Pharmaceutical and Food Sciences, University of Antioquia, Medellín, Antioquia, Colombia.
b. Faculty of Pharmaceutical and Food Sciences, Pharmacy Department, University of Antioquia, Medellín, Antioquia, Colombia.

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* **Corresponding Author:**
Carolina Lopera-Londoño
Email: carolina.loperal@udea.edu.co

Abstract:

Renealmia alpinia is a plant traditionally employed as antiophidic, pain reliever, antipyretic, and antiemetic. Its main metabolites are flavonoids, with pinostrobin as the most abundant compound of this plant species. In this study, we determined the diuretic activity of pinostrobin, previously identified from the species *Renealmia alpinia* in normal mice.

The evaluation of the diuretic activity of the flavonoid pinostrobin was carried out at doses of 5, 10, and 20 mg/kg of body weight. The diuretic activity was evaluated using the Kau *et al*. (1984) method with modifications made by Benjumea *et al*. (2005), using mice instead of rats. Metabolic cages equipped with graduated cylinders were used to measure the volume of urine excretion at 2, 4, and 6 hours. At 6 hours, pH and conductivity were measured, and the concentration of excreted sodium, potassium, magnesium, and calcium ions was estimated by ion chromatography. Creatinine, albumin, blood urea nitrogen, and urea of mice blood were analyzed.

There was an increase of 32% and 38% at a dose of 10 and 20 mg/kg, respectively, in the urinary excretion of water by pinostrobin, an elimination of sodium concentration similar to furosemide at 5 and 10 mg/kg, An increase in potassium concentration of 52% at 10 mg/kg and a higher magnesium excretion of 85% at 5 mg/kg, were statistically significant compared to furosemide.

Pinostrobin showed a diuretic effect increasing the volume of urinary excretion in mice and excretion of sodium, potassium, magnesium, and calcium ions, with a possible mechanism in the loop of Henle.

Keywords: Diuretics; Flavonoid; Furosemide; Medicinal plant; Pinostrobin; Urinary excretion.

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1. Introduction

Renealmia alpinia (Rottb.) Maas (Zingiberaceae) has been traditionally used to treat the effects of snake bites in the Embera-Katíos indigenous tribes of Antioquia and Chocó in Colombia [1]. The plant has been found to have neutralizing, anti-deworming, anti-hemorrhagic, and analgesic effects. Among the main metabolites found are flavonoids, which have shown various biological activities [2]. On the other hand, an acute oral toxicity study carried out in mice for 14 days with a dose of 2,000 mg/kg of the ethanol extract showed neither signs of toxicity, nor alterations in the organs and no animal deaths occurred [3].

Diuretics such as furosemide are commonly used to treat hypertension, chronic kidney disease, edema, congestive heart failure, and stroke. However, these agents are associated with electrolyte disturbances, including hyponatremia, hypokalemia, and hypercalcemia. Flavonoids are polyphenolic compounds and secondary metabolites found in plants, which have also shown diuretic effects [4-6]. Among these, there is pinostrobin (5-hydroxy-7-methoxy flavanone) which was discovered

over six decades ago in the species *Pinus strobus* L. and has demonstrated anti-inflammatory, gastroprotective, antioxidant, antimicrobial, antiviral, anticarcinogenic, as well as antifungal activities [7] Additionally, a study showed that pinostrobin could be responsible for the analgesic activity attributed to *R. Alpinia* [8].

Other flavonoids have been studied due to their diuretic potential. The epicatechin flavonoid obtained from the *Garcinia achachairu* species was investigated for its diuretic and saluretic properties [9]. The results showed that the biological effects of this flavonoid might be due to endogenous vasodilators, thus highlighting the role of prostanoids. Moreover, the methoxy flavonoids obtained from the methanolic extract of *Orthosiphon stamineus* showed diuretic activity and proved to be promising antagonist ligands of the adenosine A1 receptor [10]. Other studies that have isolated flavonoids include the species of *Marchantia convoluta* [6], *Spergularia purpurea* Pers. [11], *Helichrysum* spp. [12], *Satureja montana*, *Lepidium sativum*, *Crataegus songarica* and *Verbascum nigrum* [14-17], as well as the sinensetin and 30-hydroxy-5,6,7,40-tetramethoxyflavone extracted from *Orthosiphon stamineus* [13].

Flavonoids could be used as new therapeutic alternatives to treat some cardiovascular and renal diseases. The kidney-protecting activity of *R. alpinia* extract has been evaluated in mice, demonstrating an increase in the volume of urine excretion, sodium and potassium ion concentrations.[18] Therefore, the present study was undertaken to evaluate the diuretic activity of the flavonoid pinostrobin previously isolated from *R. alpinia* as its main compound, comparing a commercial sample with furosemide in mice.

2. Materials & Methods

2.1. Reagents

The flavonoid pinostrobin (≥ 99.0%) was purchased from Sigma Aldrich Corporation (St. Louis, MO, USA). The furosemide used as the reference drug, was obtained from Genfar S.A. (Cali, Colombia).

2.2. Animals

Female and male Swiss mice (20-25 g) were obtained from the bioterium at Sede de Investigación Universitaria (SIU) of the University of Antioquia. Experiments were performed under the University of Antioquia Ethics Committee (Medellín, Colombia) approval number 113 (October 12th, 2017). Experiments were also performed following the Canadian Council Guidelines for the care of experimental animals (1998) and the United States National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (2011) [19,20].

2.3. Diuretic Activity

The method of Kau *et al*. (1984) with modifications made by Benjumea *et al*. (2005) was used [21,22]. A total of 36 mice were used and fed with a standard pellet diet (Labdiet®), water *ad libitium* and were maintained in a light-dark cycle of 12h/12h at 22 °C. The animals were distributed in groups as follows: a control group that received furosemide and three groups treated with pinostrobin (with three different doses). Each group had nine mice placed in three metabolism cages. In each metabolism cage, three mice were housed to obtain an adequate urinary excretion volume and the subsequent measurement of the parameters. The ethics committee did not consider necessary the use of a negative control group for this study.

Animals fasted with free access to water for 6 hours. Then, all animals received an overload of 0.9% NaCl orally, using an orogastric tube at a rate of 5 mL/100 g of weight. The control group received furosemide intraperitoneally (20 mg/kg) to measure the diuretic-electrolytic activity. The groups to be evaluated received doses of 5, 10, and 20 mg/kg of body weight orally of the flavonoid in 5% of Tween 80 [4] The animals were housed in metabolism cages equipped with graduated cylinders to measure the volume of excreted urine, recording it at 2-hours intervals for 6 hours. Then, urine pH and density were measured. Urine samples were later frozen for conductivity and electrolyte concentration evaluations. These parameters were measured once for the whole experiment.

Electrolyte concentrations (Na^+, K^+, Mg^{2+}, Ca^{2+}) and conductivity were estimated from the urine sample obtained at the end of the experiment and expressed as ppm/10 g of body weight. Accumulated urine excretion was calculated using body/weight relations and expressed as mL/10 g of body weight. Conductivity was expressed in mS/cm.

2.4. Biochemical Analysis

Creatinine, albumin, blood urea nitrogen (BUN), and urea were analyzed in the blood of Swiss Webster mice treated with pinostrobin at doses of 5, 10, 20 mg/kg and furosemide at doses of 20 mg/kg. The samples were obtained by cardiac puncture. The experimental animals were placed into an anesthetic induction chamber with isoflurane. Then, under anesthesia effects, blood samples were taken using 1mL syringes with 23G needles and transferred to BD® vacuum tubes for serum.

2.5 Analytical Procedures

The concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+} were measured with ion chromatography. The instrument was calibrated with a standard of 100mg / L NH_4, Br, Ca, K,

Li, Na, Mg, Mn, Sr in 0.001 mol HNO_3 / L IC multi-element standard VII Certipur, with batch number HC69087422 and reference 110322.0100. pH and conductivity were determined directly in fresh urine samples using a Metrohm 848 titrino plus pH meter and a HACH HQ 40D conductivity meter.
Density estimation was performed by weighing on a Mettler Toledo analytical balance and measuring the urinary volume with a Transferpette BRAND micropipette.

2.6 Statistical Analysis

Results are expressed as the mean values ± standard error of the mean (SEM). The statistical analysis was carried out using the software SPSS v.24 and a one-way ANOVA followed by the Tukey range test. Values of $p < 0.05$ in relation to the control group were considered statistically significant.

3. Results and Discussion

3.1. Effect on urine volume

The different diuretic parameters evaluated for pinostrobin, and furosemide are shown in Tables 1-3. Table 1 shows the urinary volume excretion (mL/10g/6h) for pinostrobin and furosemide. Pinostrobin at doses of 10 and 20 mg/kg increased the volume of urinary excretion by 32% and 38%, respectively, in relation to furosemide.

Table 1. Effects of oral administration of pinostrobin on urinary volume excretion.

Group	n	V (mL/10 g/6 h)	Diuretic Index
Furosemide (20 mg/kg)	9	0.70 ± 0.064	1.0
Pinostrobin (5 mg/kg)	9	0.68 ± 0.029	0.96
Pinostrobin (10 mg/kg)	9	0.92 ± 0.087	1.32
Pinostrobin (20 mg/kg)	9	0.97 ± 0.088	1.38

The results represent the mean ± SEM. Diuretic index = urinary volume treated group / urinary volume control group. n = 9, number of animals used in each group. * p <0.05 in relation to the control group (one-way ANOVA).

The results of the present study showed an important diuretic effect of furosemide by increasing the volume of urinary excretion and the sodium ion at 20 mg/kg. Furthermore, the mechanism involved in the diuretic action is mediated by the inhibition of the $Na^+/K^+/2Cl^-$ co-transporter in the thick ascending limb of the loop of Henle [23]. When observing the results obtained with pinostrobin at different concentrations, it exhibited a significant water excretory effect dose-dependently, with notable increases at 20 mg/kg. Pinostrobin thus had a clear and significant dose-dependent diuretic effect, with values very similar to those of furosemide.

Table 2. Effects of oral administration of pinostrobin on urinary electrolyte excretion

Group	n	Na (ppm/10g/6h)	K (ppm/10g/6h)	Mg (ppm/10g/6h)	Ca (ppm/10g/6h)	Saluretic index				Na/K
						Na	K	Mg	Ca	
Furosemide (20 mg/kg)	9	350.74 ± 6.73	103.19 ± 14.59	6.40 ± 0.37	4.17 ± 0.29	1.0	1.0	1.0	1.0	3.40
Pinostrobin (5 mg/kg)	9	294.63 ± 2.10	135.98 ± 4.63	11.86 ± 0.72*	2.89 ± 0.52	0.84	1.32	1.85	0.69	2.17
Pinostrobin (10 mg/kg)	9	287.72 ± 35.91	157.22 ± 11.66*	9.39 ± 2.23	3.97 ± 0.85	0.82	1.52	1.47	0.95	1.83
Pinostrobin (20 mg/kg)	9	265.43 ± 11.58*	154.25 ± 14.16	7.49 ± 0.69	3.54 ± 0.67	0.76	1.49	1.17	0.85	1.72

The results represent the mean ± SEM. Saluretic index (SI) = ppm treated group / ppm control group. n = 9, number of animals used in each group. * p <0.05 in relation to the control group (one-way ANOVA).

Table 3. Effects of oral administration of pinostrobin on conductivity, pH and density

Group	n	Conductivity (mS/cm)	pH	Density (g/mL)
Furosemide (20 mg/kg)	9	12.72 ± 0.06	6.52 ± 0.07	1.02 ± 0.007
Pinostrobin (5 mg/kg)	9	10.21 ± 0.64*	6.83 ± 0.10	1.01 ± 0.009
Pinostrobin (10 mg/kg)	9	10.44 ± 0.16*	7.33 ± 0.21*	0.99 ± 0.003*
Pinostrobin (20 mg/kg)	9	9.78 ± 0.23*	7.29 ± 0.07*	0.99 ± 0.002*

The results represent the mean ± SEM. n = 9, number of animals used in each group.
* p <0.05 in relation to the control group (one-way ANOVA).

3.2. Effect on urinary electrolyte excretion

Table 2 shows the content of Na^+, K^+, Mg^{2+}, Ca^{2+} in urine (ppm/10g/6 h) for pinostrobin and furosemide. None of the pinostrobin doses had a greater excretion of Na+ when compared to furosemide. However, doses of 5 (p=0.227) and 10 mg/kg (p=0.152) of pinostrobin did not show statistical differences with values of 84 and 82% of electrolytic excretion, respectively. While pinostrobin, at a dose of 20 mg/kg, showed a statistically significant decrease (p <0.05).

Regarding the electrolytic excretion of Na^+, at 5 and 10 mg/kg of pinostrobin, similar values to furosemide were observed. Therefore, implying the natriuretic effect, which is a characteristic trait of a diuretic substance. Its mechanism could involve the inhibition of the $Na^+/K^+/2Cl^-$ co-transporter present in the luminal membrane of the loop of Henle in the nephron, thus allowing sodium not to be reabsorbed and thereby eliminated to a great extent. Several studies have demonstrated the diuretic effect of other flavonoids, such as those of the plant species *Solidago virgaurea* L.S. Gigantea Ait., *S. canadensis* var. canadensis, and *S. canadensis* var. "Scabra" [5]. Flavonol isoquercitrin from *Tropaeolum majus* [4], as well as hesperidin, a flavanone present in *Citrus* sp. [24] and luteolin, were found in a wider variety of natural products [25].

Pinostrobin at doses of 5, 10, and 20 mg/kg produced an increase in the K^+ excretion of 32, 52, and 49%, respectively, in relation to furosemide, which is statistically significant at the dose of 10 mg/kg (p <0.05). For the Mg^{2+} ion, increases in the concentration of 85, 47, and 17% were found at the doses of 5, 10, and 20 mg/kg, respectively, which were statistically significant compared to furosemide at the lowest dose (p <0.05) (Table 2). As the dose of pinostrobin increased, the ion concentration decreased, thus demonstrating a dose dependence of this magnesium-sparing effect. While for the Ca^{2+} ion concentrations, the three doses of pinostrobin were slightly lower than furosemide, with values of 69, 95, and 85%.

Likewise, the Na/K ratio indicates that furosemide had a higher Na^+ excretion in relation to K^+. While pinostrobin at a dose of 5 mg/kg had the highest ratio when compared to its higher doses, pinostrobin ratios were lower in 10 and 20 mg/kg doses, assuming a higher excretion of K^+ over Na^+.

The increase in K^+ excretion of pinostrobin for the three doses, being significant at 10 mg/kg (p <0.05), could be explained by its reabsorption at the collecting tubule level, an effect that occurs through the Na^+/K^+ pump of the basolateral membrane of the distal convoluted tubule [23]. A clinical highlight of importance here is that hypokalemia may cause metabolic acidosis, rhabdomyolysis, leg cramps, weakness, paresis or ascending paralysis, constipation or intestinal paralysis, as well as respiratory failure, electrocardiographic changes (U waves, T wave flattening, and ST-segment changes), cardiac arrhythmias and heart failure [26].

A marked excretory effect of magnesium ion was observed in the animals treated with pinostrobin for the three doses administered, statistically significant at 5 mg/kg (p <0.05). It should be noted that a reduction in intracellular magnesium might produce a decrease in the internal rectification and therefore, an increase in potassium conductance out of the cell lead to its consequential loss, as evinced by the results [27]. Hypomagnesemia manifests in neuromuscular complications (paresis, tremors, seizures, and paresthesia), cardiovascular complications (non-specific changes in the T wave, U-waves, and QT wave prolongation) and arrhythmias (ventricular extrasystoles, ventricular tachycardia, and ventricular fibrillation) [28].

The calcium-sparing effect observed with pinostrobin for the three doses (similar with furosemide effect) may be associated with reabsorption of these ions in the loop of Henle. The high concentrations of K^+ generated by both the $Na^+/K^+/2Cl^-$ co-transporter and the Na^+/K^+ pump in the tubular lumen allows the formation of channels through which the Ca^{2+} ion enters the blood. This effect prevents the generation of positive electric potential, required for the reabsorption of Ca^{2+} and Mg^{2+} cations and promotes their elimination. When passing through the distal convoluted tubule, the high charge of Na^+, K^+, Mg^{2+}, Ca^{2+} ions from the loop of Henle enhances ion exchange because of the electrolyte imbalance that travels through the urinary fluid, thus causing the reabsorption of sodium due to potassium saturation in the distal convoluted tubule [23].

Furthermore, the parathyroid hormone (PTH) acting on its receptors, usually promotes the increase of calcium channels through which calcium passes from the tubular lumen to the tubule. The calcium accumulated in the tubule is entered into the blood using a Na^+/Ca^{2+} exchanger, causing calcium to decrease at the end of the urinary elimination, thereby affecting the results. The PTH and the two sodium exchangers in the distal convoluted tubule, are known to play an important role in this pair of ions (calcium and sodium) [23].

3.3 Effect on conductivity, pH, and density

Table 3 shows other urinary excretion parameters such as conductivity, pH and density of the urine sample at 6 hours. Concerning conductivity, an indirect measurement of the total ion content in the three doses was carried out. The values obtained at 5 (p <0.01), 10 (p <0.01) and 20 mg/kg (p <0.01) were statistically lower in relation to furosemide, thus accounting for a lower concentration of ions in the urine. Similarly, the density values were lower, and the doses of 10 (p <0.05) and 20 mg/kg (p <0.01) were statistically lower than

furosemide. On the contrary, there was an increase in the pH values at the three doses in relation to furosemide, with statistical differences at 10 (p <0.01) and 20 mg/kg (p <0.01).
Conductivity tests showed a consistent correlation of a greater number of excreted ions in the animals treated with furosemide than in those treated with pinostrobin at the three doses and were statistically significant (p <0.01). Pinostrobin exerted greater water excretion, butwhen measuring conductivity to a greater volume, its excretion value decreased. This means, the concentration of the different ions in the samples might have been affected by inverse proportionality (as the solvent increases, the ppm of ions decreases).
On the contrary, there was a pH increase for the three doses, with a significant difference at 10 and 20 mg/kg (p <0.01). This might be explained by the increase in urinary volume at 32 and 38%, the alkaline tendency of pinostrobin in the presence of chloride (not directly measured, but present), and the increase in sodium ions. This is given as a common ion effect, in which the displacement of "Le Châtelier" occurs. The acid-base dissociation of water is affected and shifted to the right of the reaction while reducing the pOH to around 6.70. These values are within normal ranges of urine pH in mice (5.5-7.5) [29]. However, this could have clinical consequences regarding the possible interactions with the concomitant use of substances, both acidic and basic, thus causing either reabsorption or a more rapid elimination with subsequent toxicity levels or losses of pharmacological effects.

3.4 Effect on serum parameters

The results obtained from the analysis of creatinine, albumin, blood urea nitrogen (BUN), and urea are summarized in Table 4. Reference ranges were given for each serum parameter, intended for the interpretation of the results [30]. Creatinine levels for pinostrobin and furosemide were within the reference values, while albumin values were below the reference. BUN and urea values were below the reference value at the doses of 5 and 10 mg/kg of pinostrobin, but at 20 mg/kg, the values were within the reference.

Table 4. Effects of oral administration of pinostrobin on the serum parameters.

	Creatinine (mg/dL)	Albumin (g/L)	BUN (mg/dL)	Urea (mg/dL)
Reference range	0.2-0.9	25-30	8-33	25-45
Furosemide 20 mg/Kg	0.33	18	10.78	23.06
Pinostrobin 5 mg/Kg	0.55	22	6.93	14.83
Pinostrobin 10 mg/Kg	0.35	16	7.68	16.43
Pinostrobin 20 mg/Kg	0.49	15	13.43	28.74

Blood creatinine values showed a protective effect of furosemide and pinostrobin at all doses, as the values were within the reference range for this parameter. Albumin and BUN values are interrelated. Low BUN values are usually associated with liver failure and a low-protein diet. Therefore, the values shown in our results may be due to metabolic factors that animals presented during tests, such as stress or metabolic diseases [31].
The results in the present study did not contemplate a negative control group due to the regulations on the number of animals approved by the ethics committee. Therefore, the results and conclusions are taken with precaution, as we cannot ensure that an unknown variable may have been adversely affecting the animals during the experiment. Additionally, higher doses of pinostrobin could not be studied because of the limited amount of compound available. Further studies are thus required using higher doses to demonstrate the diuretic dose-dependent effect of pinostrobin.

4. Conclusion

Pinostrobin demonstrated an important dose-dependent water excretion effect at 10 and 20 mg/kg, reaching a remarkable increase in urine volume at the highest concentration. Additionally, it showed a sodium excretory effect similar to furosemide, except at 20 mg/kg. The water and sodium excretion effects demonstrated the diuretic effect of pinostrobin, whose mechanism could be involved in the loop of Henle while inhibiting the reabsorption of sodium ions at this level. Therefore, pinostrobin could be considered for more exhaustive future studies as a candidate for new diuretic drug with the potential for treating renal or cardiovascular disorders. Nonetheless, there should be special considerations regarding hypomagnesemia and hypokalemia due to possible clinical manifestations, considering the advantage of non-observable increases in calcium excretion.

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References

1. Otero R, Fonnegra R, Jimenez SL, Nuñez V, Evans N, Alzate SP, Garcia ME, Saldarriaga M, Del Valle G, Osorio RG, Diaz A, Valderrama R, Duque A, Velez HN. Snakebites and ethnobotany in the northwest region of Colombia: Part I: traditional use of plants. *J Ethnopharmacol*. 2000;71 (3): 493-504.

2. Gómez-Betancur I, Benjumea D. Traditional use of the genus Renealmia and Renealmia alpinia (Rottb.) Maas (Zingiberaceae)-a review in the treatment of snakebites. Asian Pac J Trop Med. 2014;7S1: S574-82.
3. Patiño AC, López J, Aristizábal M, Quintana JC, Benjumea D. Evaluation of the inhibitory effect of extracts from leaves of Renealmia alpinia Rottb. Maas (Zingiberaceae) on the venom of Bothrops asper (mapaná). Biomédica. 2012;32 (3): 365-74. Spanish.
4. Gasparotto Junior A, Gasparotto FM, Boffo MA, Lourenço EL, Stefanello MÉ, Salvador MJ, da Silva-Santos JE, Marques MC, Kassuya CA. Diuretic and potassium-sparing effect of isoquercitrin—An active flavonoid of Tropaeolum majus L. J Ethnopharmacol. 2011;134 (2): 210–5.
5. Chodera A, Dabrowska K, Sloderbach A, Skrzypczak L, Budzianowski J. Effect of flavonoid fractions of Solidago virgaurea L on diuresis and levels of electrolytes. Acta Pol Pharm. 1991;48 (5–6): 35—37.
6. Xiao J, Jiang X, Chen X. Antibacterial, anti-inflammatory and diuretic effect of flavonoids from Marchantia convoluta. African J Tradit Complement Altern Med. 2005;2 (3): 244–52.
7. Patel NK, Jaiswal G, Bhutani KK. A review on biological sources, chemistry and pharmacological activities of pinostrobin. Nat Prod Res. 2016;30 (18): 2017–27.
8. Gómez-betancur I, Cortés N, Benjumea D, Osorio E, León F, Cutler SJ. Antinociceptive activity of extracts and secondary metabolites from wild growing and micropropagated plants of Renealmia alpinia. J Ethnopharmacol. 2015;165: 191–7.
9. Mariano LNB, Boeing T, da Silva RCMVAF, Cechinel-Filho V, Niero R, da Silva LM, de Souza P, Andrade SF. Preclinical evaluation of the diuretic and saluretic effects of (-)-epicatechin and the result of its combination with standard diuretics. Biomed Pharmacother. 2018;107: 520-525.
10. Yuliana ND, Khatib A, Link-Struensee AMR, Ijzerman AP, Rungkat-Zakaria F, Choi YH, Verpoorte R. Adenosine A1 receptor binding activity of methoxy flavonoids from Orthosiphon stamineus. Planta Med. 2009;75 (02): 132-136.
11. Jouad H, Lacaille-Dubois MA, Lyoussi B, Eddouks M. Effects of the flavonoids extracted from Spergularia purpurea Pers. on arterial blood pressure and renal function in normal and hypertensive rats. J Ethnopharmacol. 2001;76 (2): 159-163.
12. Çubukçu B. Helichrysum species as choleretic, chologogue crude drugs. Acta Pharmaceutica Turcica. 2002;44: 145–150.
13. Hossain MA, Rahman SM. Isolation and characterisation of flavonoids from the leaves of medicinal plant Orthosiphon stamineus. Arabian Journal of Chemistry. 2015;8 (2): 218-221.
14. Stanic G, Samaržija I. Diuretic Activity of Satureja montana subsp. montana extracts and oil in rats. Phytotherapy Research. 1993;7 (5): 363-366.
15. Patel U, Kulkarni M, Undale V, Bhosale A. Evaluation of diuretic activity of aqueous and methanol extracts of Lepidium sativum garden cress (Cruciferae) in rats. Trop J Pharm Res. 2009;8 (3): 215-219.
16. Younis W, Alamgeer, Schini-Kerth VB, da Silva DB, Gasparotto Junior A, Bukhari IA, Assiri AM. Role of the NO/cGMP pathway and renin-angiotensin system in the hypotensive and diuretic effects of aqueous soluble fraction from Crataegus songarica K. Koch. J Ethnopharmacol. 2020;249: 112400.
17. Kalinina SA, Elkina OV, Kalinin DV, Syropyatov BY, Dolzhenko AV. Diuretic activity and toxicity of some Verbascum nigrum extracts and fractions. Pharm Biol. 2014;52 (2): 191-198.
18. Patiño AC. Renealmia alpinia (Rottb.) Maas (Zingiberaceae) una especie vegetal inhibidora del envenenamiento ocasionado por algunas serpientes colombianas: de la medicina tradicional a la validación experimental [thesis]. [Medellin]: Universidad de Antioquia: 2013. 124 p. Spanish.
19. Canadian Council on Animal Care. Guidelines On-choosing an Appropriate Endpoint Experiments Using Animals for Research, Teaching and Testing. The Council [Internet]. 1998 [cited 2017 Oct 10]. Available from: https://www.ccac.ca/Documents/Standards/Guidelines/Appropriate_endpoint.pdf
20. National Institutes of Health. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington DC: National Academic Press [Internet]. 2011 [cited 2017 Oct 10]. Available from: https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf
21. Kau ST, Keddie JR, Andrews D. A method for screening diuretic agents in the rat. J Pharmacol Methods.1984;11 (1): 67-75.
22. Benjumea D, Abdala S, Hernandez-Luis F, Pérez-Paz P, Martin-Herrera D. Diuretic activity of Artemisia thuscula, an endemic canary species. J. Ethnopharmacol. 2005;100 (1-2): 205–209.
23. Katzung B, Masters S, Treveor A, Araiza Martínez M. Basic and clinical pharmacology. 12th ed. Mexico: MacGraw Hill; 2013.
24. Galati EM, Trovato A, Kirjavainen S, Forestieri AM, Rossitto A, Monforte MT. Biological effects of hesperidin, a Citrus flavonoid. (Note III): antihypertensive and diuretic activity in rat. Farmaco. 1996;51 (3): 219-221.
25. Boeing T, da Silva LM, Mariott M, Andrade SF, de Souza P. Diuretic and natriuretic effect of luteolin in normotensive and hypertensive rats: Role of muscarinic acetylcholine receptors. Pharmacol Rep. 2017;69 (6): 1121-1124.
26. Kardalas E, Paschou SA, Anagnostis P, Muscogiuri G, Siasos G, Vryonidou A. Hypokalemia: a clinical update. Endocr Connect. 2018;7 (4): 135–146.
27. Pérez González E, Santos Rodríguez F, Coto García E. Homeostasis of the magnesium. Physiology, etiopatogenia, clinic and treatment of the hypomagnesemia. Nefrología (Madr.). 2009;29 (6): 518-524.
28. Rondón-Berríos H. Hypomagnesemia. An Fac Med. 2006;67 (1): 38-48.
29. Cohen SM. Role of urinary physiology and chemistry in bladder carcinogenesis. Food Chem Toxicol. 1995;33 (9): 715–30.
30. University of Arizona. Clinical Pathology University Animal Care [Internet]. [cited 2019 Nov 12]. Available from: https://uac.arizona.edu/services/veterinary-services/pathology/clinical-pathology-0
31. Quimby FW, Luong RH. Clinical Chemistry of the Laboratory Mouse. *The Mouse in Biomedical Research*. 2007; 171–216.