

Diuretic effect of alkaloids fraction extracted from *Selaginella lepidophylla* (Hook. et Grev.) Spring

[Efecto diurético de la fracción con contenido de alcaloides extraída de *Selaginella lepidophylla* (Hook. et Grev.) Spring]

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Abstract: The aerial parts of *Selaginella lepidophylla* (Hook. et Grev.) Spring, are used in Mexican folk medicine to treat renal diseases. The aim of this study was to measure the diuretic response of an aqueous extract (200 mg/kg) and alkaloids fraction at different doses (10, 40 y 100 mg/kg) of this plant and compare it with that induced by furosemide (4 mg/kg). Extract, alkaloids fraction, furosemide and vehicle were administered orally to adult rats and the effects in sodium, potassium and water balance were measured. The extract, the alkaloids fraction and the furosemide produced important and significant increments in urinary excretion of sodium, potassium and water with respect to control group. This increment was dose dependent of the alkaloids fraction, the highest dose produced a major effect. Potassium excretion increased but it was less than the one induced by furosemide. These results suggest that the aqueous extract and rich fraction in alkaloids from *S. lepidophylla* induce diuretic response.

Keywords: diuretic, water excretion, *S. lepidophylla*, alkaloids, furosemide.

Resumen: Las partes aéreas de *Selaginella lepidophylla* (Hook. et Grev.) Spring, son usadas en la medicina tradicional mexicana para tratar enfermedades renales. El objetivo del estudio fue medir la respuesta diurética de un extracto acuoso (200 mg/kg) y de diferentes dosis de la fracción de alcaloides (10, 40 y 100 mg/kg) de esta planta y compararla con la inducida por furosemida (4 mg/kg). El extracto, la fracción de alcaloides, la furosemida y el vehículo fueron administrados por vía oral a ratas adultas y se midieron los efectos en el balance de sodio, potasio e hídrico. El extracto, la fracción de alcaloides y la furosemida produjeron importantes y significativos incrementos en la excreción urinaria de sodio, potasio y agua con respecto al grupo testigo. Este incremento fue dependiente de la dosis de la fracción de alcaloides, la dosis más alta produjo el mayor efecto. El incremento en la excreción de potasio fue menor al de furosemida. Los resultados sugieren que el extracto acuoso y la fracción rica en alcaloides de *S. lepidophylla* inducen una respuesta diurética.

Palabras Clave: diurético, excreción de agua, alcaloides, *S. lepidophylla*, furosemida.

Recibido | Received: November 6, 2012

Aceptado en versión corregida | Accepted in revised form: May 23, 2013

Publicado en línea | Published online: January 30, 2014.

Declaración de intereses | Declaration of interests: This work was partially supported by the Secretaría de Investigación y Posgrado, IPN. I. Contreras-León had scholarship from CONACYT (Mexico).

Este artículo puede ser citado como / This article must be cited as: ME Melendez-Camargo, I Contreras-León, R Silva-Torres. 2014. Diuretic effect of alkaloids fraction extracted from *Selaginella lepidophylla* (Hook. et Grev.) Spring. *Bol Latinoam Caribe Plant Med Aromat* 11(3): 92 – 99.

INTRODUCTION

The use of plants to heal is a common practice among the Mexican population and other countries. In Mexico, specially because of the geographic situation which provides a varied diversity of them and the traditional culture inherited from the Mayas and other ethnic groups (Martínez, 1996; Mickel and Smith, 2004). There are a great number of species with potent possibility to produce some therapeutic effects among them is *Selaginella lepidophylla* (Hook. et Grev.) Spring (Selaginellaceae-Pteridophyta), a desert and semidesert plant, native of the regions that goes from Texas to South America (Mickel and Smith, 2004; Vazquez et al., 2005), it grows in rocky soil and it needs little water. *Selaginella lepidophylla* has several common names such as: false rose of Jerico, resurrection plant, dinosaur plant, siempre viva (evergreen), stone flower and doradilla (goldenish), (Van Dijck et al., 2002). In its native habitat is noted for its ability to survive almost complete desiccation in the dry season. During which its stems curl into tight ball, it uncurled when exposed to moisture. It is an exotic house plant that is found in homes and can be dormant for years. Its beneficial effects have been known since the beginning of XX century. The plant was used to treat liver and kidney irritation (Martínez, 1996). Valenzuela-Avendaño et al., (2005) isolated intact RNA from *S. lepidophylla* partially hydrated. This plant has also been studied due to the fact that during extreme drought conditions, levels of trehalose up to 20% have been recorded in the leaves (Godwin and Smeekens, 1998; Van Dijck et al., 2002).

We carried out the ethnobotanical study of *Selaginella lepidophylla* (Hook. et Grev.) Spring in San Jose Xicohtencatl, municipality of Huamantla, Tlaxcala, Mexico and it was found that this plant is used like infusion in several urinary diseases. Also the specie was a new record from Tlaxcala state (Vazquez et al., 2005). Due to its popular use, in this study, the diuretic activity on aqueous extract and alkaloids fraction of *S. lepidophylla* was evaluated, by means of their aqueous and electrolyte balance in rats. In addition to creatinine clearance and acute toxicity was determined. The oral route was used because this is the way the people use it. In this study the diuretic effects of aqueous and alkaloid fractions are reported.

MATERIAL AND METHODS

Plant material

The plants were collected in May (2008) in San Jose Xicohtencatl, municipality of Huamantla, Tlaxcala, Mexico. *Selaginella lepidophylla* (Hook. et Grev.) Spring, (Selaginellaceae - Pteridophyta), was taxonomically authenticated by specialist at Escuela Nacional de Ciencias Biológicas (ENCB), Instituto Politécnico Nacional. A voucher specimen was deposited in the herbarium of ENCB (47098). The dried plant material was powdered.

Preparation of the aqueous extract

The dry powdered aerial part of *Selaginella lepidophylla* (500 g) was extracted with 1000 mL of distilled water for 5 minutes. Following filtration and concentration under vacuum, a brown sticky residue was obtained. The aqueous extract was used for the experiments. The secondary metabolites of aqueous extract were identified by phytochemical test with reagents for alkaloids such as: Dragendorff, Mayer, silicotungstic acid, Sonnenschein and Wagner, for the flavonoids by Shinoda reagent, the reductor sugars (Fehling and Benedict reagents), the tannins by Jelly reagent, the sesquiterpenolactones by ferric hydroximate reagent, the coumarins by Erlich reagent and quinones by ammonium hydroxide reagent (Domínguez, 1978).

Alkaloids and flavonoids were identified by ¹H nuclear magnetic resonance (NMR) spectra were used to confirmation of chemical structures of both metabolites in the crude and chloroform extracts. ¹H nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury-300 spectrometer (300 MHz and 75.4 MHz) (Varian Palo Alto, CA, USA), using CD₃OD and CDCl₃ as a solvent and tetramethylsilane (TMS) (Aldrich Chemical Co., Milwaukee WI, USA) as internal standard.

Alkaloid extract

The alkaloid extract was obtained from 40 g of aqueous extract in hot water and was successively extracted with hexane, ethyl acetate and butanol. From butanol extract was obtained a dark-brown mass. Butanol extract (20 g) was dissolved in mixture ethanol-water 1:1 w/w (100 mL) then was diluted with aqueous HCl (0.1 N) to give acidic solution and an insoluble part. The acid aqueous layer was then basified with aqueous 2N NaOH (pH 9) to produce free bases and extracted with CHCl₃ (three times, 100 mL) resulting two organic and aqueous

phases. The CHCl_3 fraction was evaporated under reduced pressure. This operation was repeated two times until was obtained 5 g of CHCl_3 extract (Ferrigni *et al.*, 1982).

Diuretic activity

Animals

Adult female Wistar rats were used. They were maintained in the animal house at room temperature (22 to 24 °C) and 50 to 55% relative humidity, with day/night cycles of 12 x 12 hours. They were fed with standard rodent diet and water *ad libitum*. Care and handling of the animals were in accordance with the Mexican Official Norm for Animal Care Handling (NOM-062-ZOO-1999). The Bioethical Committee for Research in experimental animals of the ENCB approved this protocol.

Effects of *Selaginella lepidophylla* on water balance in the rat

The experiments were carried out in unanesthetized adult female Wistar rats. These were distributed by means of a table of random numbers into six groups: control group received vehicle (distilled water, 1 ml/kg body weight (b wt)), furosemide-treated group (positive-control group, 4 mg/kg b wt), one group treated with the aqueous extract from *S. lepidophylla* at the dose of 200 mg/kg b wt and three groups treated with alkaloid fraction at the different doses (10, 40 and 100 mg/kg b wt). Furosemide and extracts were administered in aqueous solution (1ml/kg b wt). In all groups, the administration was oral and there were at least six animals per group.

At the beginning of the experiment, the urinary bladder was emptied by gentle compression of the abdomen ($t = 0$), as previously described (Melendez-Camargo *et al.*, 2004). In this paper adequacy of this method to obtain complete emptiness of urinary bladder was verified by opening the abdomen and direct inspection of the bladder.

Once the bladder was emptied, either the vehicle, furosemide or extracts were administered. The animals were kept during 6 h in metabolic cages in a quiet and warm environment (21 - 23 °C) under fluid and food deprivation. At the end of this period the sample of urine was obtained ($t = 6$ h) and collected into calibrated pipettes (Dade, Miami, Fla.), also by abdomen compression to ensure complete emptiness of the bladder. The sample was added to the spontaneously voided urine that was collected under mineral oil and ice-cooled to prevent evaporation. The

blood samples were obtained and plasma was separated from the cells by centrifugation for a period of 5 min at 4000 rpm (IEC MB centrifuge, Damon/IEC Division, Needham Heights, MA). Osmolalities of plasma and urine were measured in a vapor pressure osmometer (Wescor, Logan, Utah) by triplicate. Urine volume was also measured and then osmolal and free water clearances were calculated.

Effect of extracts on electrolyte balance in the adult rat

Sodium and potassium concentrations were measured in the urine and plasma samples (Flamephotometer Corning 400, Corning Medical and Scientific, England) from rats treated with aqueous extract and alkaloid fraction of *S. lepidophylla*. Urinary excretions and clearances of sodium and potassium were calculated (Melendez-Camargo *et al.*, 2004).

Effect of aqueous and alkaloid extract on glomerular filtration rate

Glomerular filtration rate (GFR) was estimated by the clearance of endogenous creatinine. To avoid the error due to tubular secretion of creatinine, only female animals (in which no secretion occurs) were used (Reyes *et al.*, 1998). Plasma and urinary creatinine were determined by the method of Jaffe alkaline picrate modified by Melendez-Camargo *et al.*, 2004. Creatinine clearance and fractional excretion of sodium (FE_{Na}) and potassium (FE_{K}) were calculated using the conventional equations (1 and 2):

$$\text{FE}_x = (\text{C}_x / \text{C}_{\text{creat}}) \times 100$$

Equation 1

$$\text{C}_x = (\text{U}_x \cdot \text{V} / \text{P}_x)$$

Equation 2

where FE_x is the fractional excretion (%) of substance x, C_x and C_{creat} are the clearance (ml/min) of substance x and creatinine. U_x , P_x , are the concentrations ($\mu\text{Eq/l}$) of substance x in urine and plasma, and V is the urinary flow (ml/min).

The amount of sodium and potassium in plant extracts

The content of Na^+ and K^+ was determined in aqueous and alkaloid extracts of the plant, as described previously by Melendez-Camargo *et al.*, 2004 (Flamephotometer Corning 400, Corning Medical and

Scientific, England) in the same concentration as administered to animals.

Acute Toxicity

Six groups of female mice from NIH strain of 25 ± 5 g of body weight were formed, a control group (vehicle) and five groups treated with different doses of aqueous extract (from 350 to 5000 mg/kg of body weight). The animals had access to food and water *ad libitum* and were observed for clinical signs during 24 hours following *per os* administration. Lethality was assessed using death within 7 days as an index of toxicity. Due the low yield of the extract of alkaloids only one group was treated at dose of 5000 mg/kg (b wt).

Statistical Analysis

The data are presented as mean \pm standard error (SEM) and the statistical significance between groups was analyzed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. The differences between groups were regarded as significant at $P < 0.05$.

RESULTS

The phytochemical analysis showed the presence of alkaloids, flavonoids, reductor sugars, tanines, sesquiterpenlactones, coumarins, and quinones in aqueous extract and the flavonoids were the major constituents. A brown sticky residue was obtained

from aqueous extract (yield: 17.92%). From buthanol extract was obtained a dark-brown mass in 18.6% of yield (7.46 g). The CHCl_3 fraction has a yield of 15.15% (3.03 g) and the main constituents were alkaloids.

The presence of alkaloids in the chloroform extract was evidenced by the Nuclear Magnetic Resonance (NMR). The protons of alkaloids showed two double and one multiple signals at 2, 2.1 and 2.2 ppm assigned probably protons of pyrrole alkaloids (Wang *et al.*, 2009). The presence of flavonoids were evidenced by the NMR spectrum showed one double signals at 6.3 ppm assigned to hydrogens of the ring A and one double signal at 7.0 ppm assigned to hydrogens of the ring B of the flavonoids (Kielhmann and Edmond, 1995).

Effect of aqueous and alkaloid extracts of *S. lepidophylla* on the water balance in the rat

An increase in the urinary volume was observed in rat groups treated with extracts and furosemide (Figure 1, $P < 0.05$). Osmolal clearance increased in groups treated with furosemide or aqueous and alkaloid extracts (Table 1, $P < 0.05$). Free water clearance decreased in both rat groups treated with extracts and furosemide (Table 1), the response in this last group was higher than in the groups treated with aqueous extract.

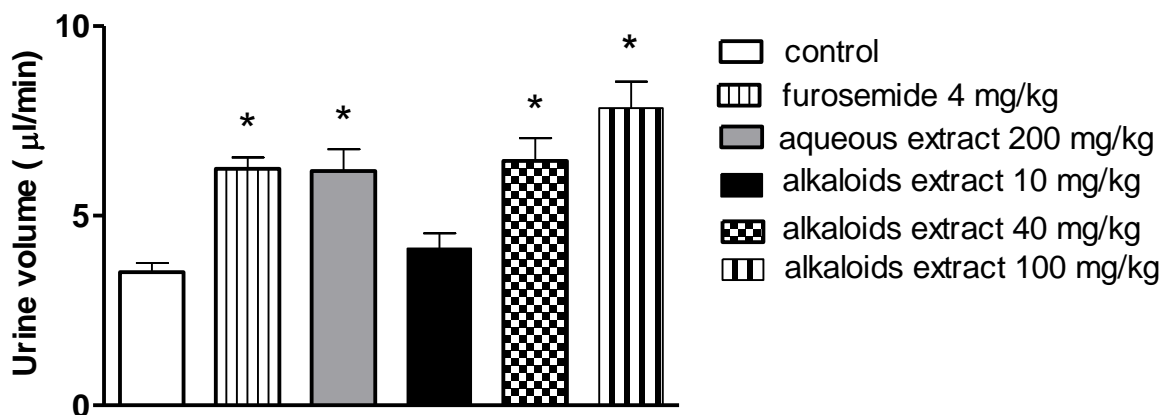


Figure 1

Urine volume in rats treated with aqueous and alkaloid extracts of *Selaginella lepidophylla*. Furosemide (4 mg/kg), aqueous (200 mg/kg) and alkaloid extracts (10, 40 and 100 mg/kg) of *S. lepidophylla*. Mean \pm standard error (SEM) are depicted by the heights of the bars and brackets. * $p < 0.05$.

Table 1
Effect of aqueous extract and rich fraction of alkaloids from *S. lepidophylla* on osmolal and free water clearances, FE_{Na} and FE_K

	Osmolal clearance ($\mu\text{l}/\text{min}$)	Free water clearance ($\mu\text{l}/\text{min}$)	$FE_{Na} \times 10^{-3}$ (%)	$FE_K \times 10^{-3}$ (%)
Control (vehicle) (14)	20.7 ± 2.7	-17.5 ± 2.9	101.4 ± 17.4	2.8 ± 0.4
Furosemide (4 mg/kg b wt), (15)	$32.2 \pm 2.6^*$	$-28.1 \pm 2.3^*$	$222.1 \pm 30.9^*$	$8.3 \pm 1^*$
Aqueous extract (200 mg/kg b wt), (15)	$31.7 \pm 2.4^*$	-21.3 ± 3.2	$240.5 \pm 24.6^*$	5.7 ± 0.6
Alkaloid extract (10 mg/kg b wt), (6)	21.7 ± 6.9	-16.9 ± 7.2	125.3 ± 31.3	$7.1 \pm 1.8^*$
Alkaloid extract (40 mg/kg b wt), (6)	25.2 ± 4.2	-23.1 ± 3.1	133.5 ± 30.4	$8.6 \pm 2.6^*$
Alkaloid extract (100 mg/kg b wt), (6)	$39.3 \pm 5.9^*$	$-31.74 \pm 5.0^*$	$257.5 \pm 52.5^*$	$8.9 \pm 3.0^*$

Mean \pm standard error (SEM) are shown. Figures in parenthesis indicate number of animals. $*P < 0.05$.

Effects of aqueous and alkaloid extracts of *Selaginella lepidophylla* on the electrolyte balance in the rat

Sodium clearance increased in the groups treated with extracts or furosemide. The increment was major on the extract rich in alkaloid at the highest dose and it was significantly different to control values (Figure 2A). The aqueous and alkaloid extracts increases the potassium clearance in all treated groups, (Figure 2B), all extracts induce a similar effect than furosemide.

Sodium and potassium fractional excretions (FE_{Na} and FE_K)

Significant increments in FE_{Na} and FE_K were seen in four experimental groups, the increments observed in FE_{Na} for the treated-group with the aqueous extract of

Selaginella lepidophylla at the doses of 200 mg/kg and alkaloid extract (100 mg/kg) were compared to effect of furosemide, this was not observed for FE_K where the increment was less than furosemide (Table 1).

The amount of sodium and potassium in extracts of *Selaginella lepidophylla*

In the aqueous extracts of *Selaginella lepidophylla* aerial part at the dose of 200 mg/kg, the potassium content was 4.5 mmol/l, the alkaloid extract (100 mg/kg) was 19 mmol/l. The content of sodium was 7.5 mmol/l and 28.7 mmol/l for the aqueous and alkaloid extract, respectively. The concentrations obtained did not affect the renal function (electrolyte and water balance).

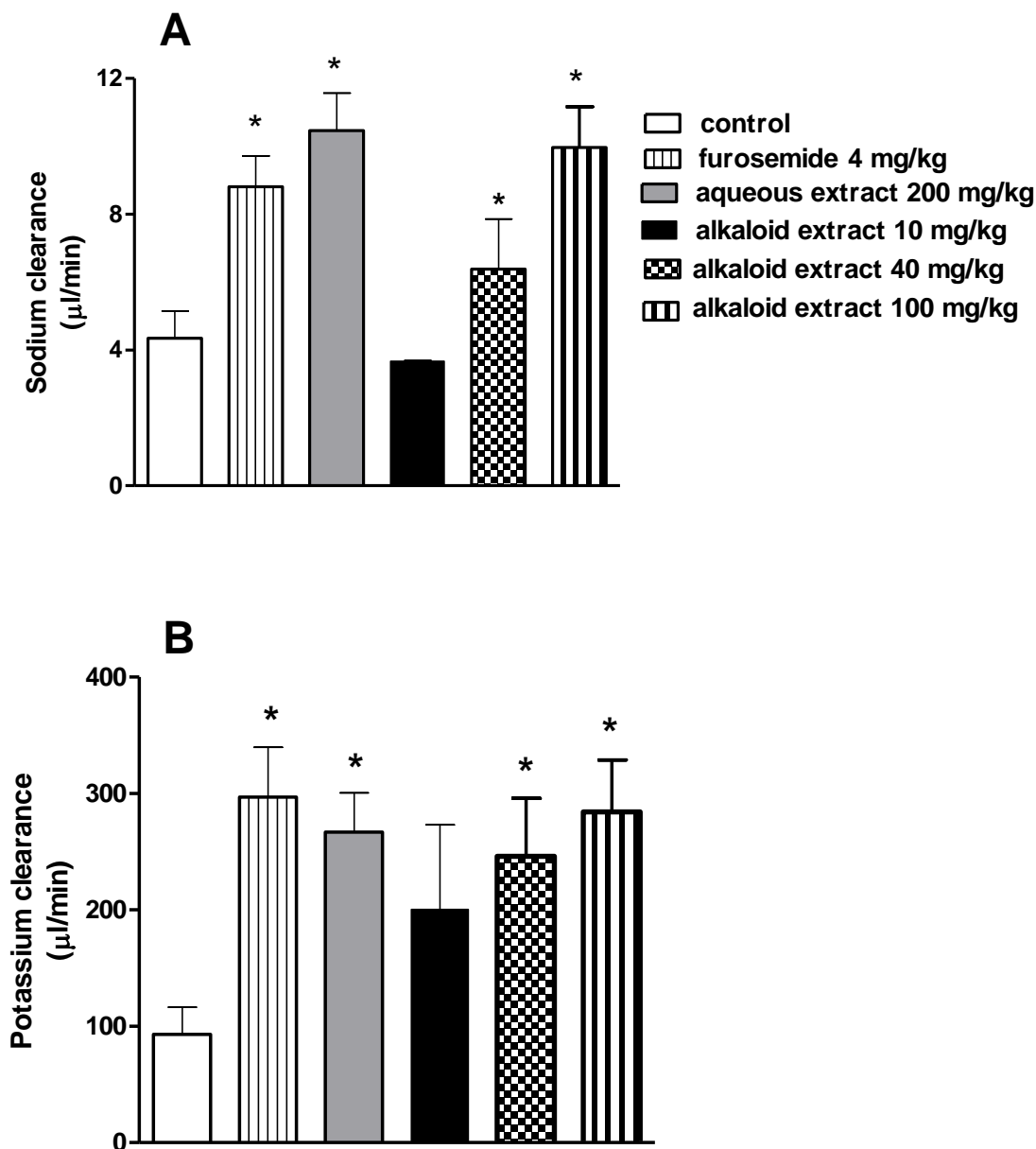


Figure 2

Sodium and potassium clearances in rats treated with aqueous and alkaloid extracts of *Selaginella lepidophylla*. A. Sodium clearances and B. Potassium clearances. Data are mean \pm standard error (SEM) are depicted by the heights of the bars and brackets, * $P < 0.05$.

DISCUSSION

In the present study the phytochemical analysis showed that the main constituents of this plant are alkaloids and flavonoids which are accordance to the reported composition of this genus (Chao *et al.*, 1987; Lin *et al.*, 2000; Ma *et al.*, 2001; Iturriaga *et al.*, 2006; Wang *et al.*, 2009). The pyrrole alkaloids are widely distributed in this *Selaginella* genus (Wang *et al.*,

2009). The signals obtained in 2, 2.1 and 2.2 ppm from alkaloid extract are characteristics the pyrrole alkaloids as reported by Grube and Köck (2006). The signals obtained in 6.3 and 7.0 ppm from aqueous extract are characteristics the flavonoids as reported by Kiehlmann and Edmond (1995).

Diuretics are drugs that increase the rate of urine flow and sodium excretion and are used to adjust the

volume and /or composition of body fluids in a variety of clinical situations, including hypertension, heart failure, nephritic syndrome and cirrhosis. Diuretics such as furosemide can increase the urinary flow rate, also they are strongly saluretic inasmuch as they increase sodium and chloride urinary excretion (Jackson, 2001), because of this furosemide was used as positive control in this study. On the other side, the herbal diuretics produce very little acute toxicity and in general they can be considered as mild and good drugs, in comparison to other diuretics used nowadays in the therapeutic (Melendez-Camargo *et al.*, 2004; Ranju *et al.*, 2011).

The most important thing is that a large number of these plants are rich in potassium, which would not lead to potassium depletion, thus giving the benefits of potassium sparing diuretic such as spironolactone, triamterene, etc., (Jackson, 2001; Melendez-Camargo *et al.*, 2004).

Through this work, the diuretic activity of the aqueous extract of the *S. lepidophylla* as described in Traditional Medicine for the treatment of renal and urinary tract diseases was demonstrated (Argueta *et al.*, 1994; Martínez, 1996; Vazquez *et al.*, 2005). *S. lepidophylla* induced an increment in the urine output and in the electrolyte excretion at highest doses of the alkaloid and aqueous extracts. These results reveal that the aqueous and alkaloid extracts (100 mg/kg) of *S. lepidophylla* produced an important diuretic action, although is not comparable with furosemide dose administered. The water excretion is higher in the presence of alkaloid extracts at doses of 100 mg/kg, and the response is dose-dependent. Both extracts induce an increment in sodium clearance. In contrast to furosemide, extracts do not affect in the same degree the potassium clearance, this response is lower in FE_K that in FE_{Na} , this potassium-sparing effect should be viewed as a favorable feature of the extract in regards to electrolyte excretion (Melendez-Camargo *et al.*, 2004).

In this study the GFR remained unchanged in all treated-groups, therefore this results suggest that the aqueous and alkaloids extracts produced diuretic action by direct renal tubular effect (Melendez-Camargo *et al.*, 2004; Jackson, 2001; Ranju *et al.*, 2011).

It is possible that flavonoids and alkaloids presents in the extracts of *S. lepidophylla* exerted diuretic effect by inhibiting tubular reabsorption of water and electrolytes as such action has been suggested for some other plants (Ranju *et al.*, 2011;

Hullatti *et al.*, 2011). Some of the plants with potent diuretic activity were found contain benzyl isoquinoline type of the alkaloids (Erdemgil *et al.*, 2001). Certain flavonoids were found to exert their diuretic activity by binding with adenosine A1 receptor associated with the diuretic action (Yuliana *et al.*, 2009). The diuretic activity of studied plant may be through any of these mechanisms since it is rich in alkaloids and flavonoids. Molecular and cellular mechanisms of the extract will be investigated.

The possibility of direct action of potassium content of *S. lepidophylla* extracts on diuretic effect is not considered since the K^+ content in the extracts was very low in comparison with the salt concentration obtained from other plants (Sripanidkulchai *et al.*, 2001).

In the toxicological evaluation this plant did not present toxic effects at the doses evaluated in this study, due to the results obtained is possible to consider that the aqueous and alkaloid extracts of *S. lepidophylla* are not toxic (Ecobichon, 1992).

These experiments justify the use of *Selaginella lepidophylla* as a diuretic in Traditional Medicine (Martínez, 1996; Argueta *et al.*, 1994).

CONCLUSIONS

Our results demonstrated that aqueous and alkaloid extracts from *S. lepidophylla* induce significant effects on urinary excretion of water and electrolytes and support the claims of diuretic efficacy extract in Mexican Traditional Medicine and probably the alkaloids and flavonoids are implicated in this activity. None of the doses of the extracts administered produced mortality or any behavioral disorders.

ACKNOWLEDGEMENTS

This work was partially supported by the Secretaría de Investigación y Posgrado, IPN. I. Contreras-Leon had scholarship from CONACYT (Mexico).

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