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Potential effect of *Silybum marianum* L. and *Cistus ladaniferus* L. extracts on urine volume, creatinine clearance and renal function

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ABSTRACT

Objective: To investigate the diuretic and renal effects of *Silybum marianum* L. and *Cistus ladaniferus* L. in normal rats. **Methods:** Four groups of rats were used in each experiment. The first group received water, the second group received *Cistus ladaniferus* L. extract (100 mg/kg b.wt), the third group received *Silybum marianum* L. extract (100 mg/kg b.wt), and the fourth group received furosemide (10 mg/kg b.wt). Variables including urine volume, plasma and urine sodium, potassium and creatinine, and creatinine clearance were measured. Two experiments were conducted. A single dose of each intervention was used and the variables were measured during 24 h, and the interventions were given daily for a total of 8 d and the variables were measured during various intervals. **Results:** The single dose of each plant extract increased urine volume at all-time intervals and increased urine sodium and potassium excretion without affecting plasma sodium and potassium ($P<0.05$). On the day 8 after daily administration, the plant extracts induced a significant diuresis and natriuresis without affecting serum electrolytes ($P<0.05$), while furosemide caused hypokalemia. Both plant extracts significantly increased creatinine clearance ($P<0.05$). **Conclusions:** *Silybum marianum* L. and *Cistus ladaniferus* L. increase creatinine clearance and have a significant diuretic effect without affecting serum electrolytes. *Silybum marianum* L. is more potent than furosemide or *Cistus ladaniferus* L.

1. Introduction

Milk thistle [*Silybum marianum* L. (*S. marianum*)] is a member of the Asteraceae/Compositae family. It is an annual or biennial native shrub. The plant mainly grows around the Mediterranean regions. In Morocco, the Milk thistle is known locally as “chouk J’mal” or “Guandoule”, and it is distributed in all over Moroccan regions[1]. Many studies have demonstrated that *S. marianum* has antimicrobial activity and a favorable effect on inflammation, oxidative stress, and elevated blood sugar[2-7]. *S. marianum* extract [100mg/(kg•d)] was found effective in the prevention of CCL₄-induced nephrotoxicity in rats[8]. Furthermore, it was found that the extract improves kidney

functions and reduces proteinuria[9,10]. These effects were attributed to anti-inflammatory and antioxidant properties of silymarin[11-14]. In the clinical setting, it improves antioxidants in diabetic patients[15].

Milk thistle is rich in silymarin, which is a bioflavonoid complex extract from the seeds. The medicinal benefit of silymarin has been tested in burn, cancer, dyslipidemia, and neurological and bone diseases[16]. Furthermore, silymarin has been used for a long time in the treatment of liver and kidney diseases[17,18]. It has been used

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for prevention of kidney injury by mushroom poisoning, ischemia/reperfusion and adriamycin and cisplatin toxicity[19-23]. Silymarin could prevent renal cell injury resulted from incubation with high glucose concentration[23]. In diabetic rats, it decreases podocyte superoxide formation and increases the activity of antioxidant enzymes in renal tissue[24]. In acute kidney injury, silymarin prevents kidney damage induced by oxidative stress, glomerular and tubular cell injury and apoptosis[25,26]. Another study found that silymarin decreases prostaglandin and leukotriene biosynthesis, and it also inhibits cyclooxygenase III[27].

Cistus ladaniferus L. (*C. ladaniferus*) is a medicinal plant that grows in the Mediterranean region. It belongs to the Cistaceae family, commonly known as rock-rose. The plant is a medium sized tree, and in north-east of Morocco, it is known as Touzal, and in the other regions of Morocco as Targala and Bu-zgzaw. Several studies showed that *C. ladaniferus* has a favorable effect in oxidative stress, cancer, elevated blood pressure, colic, and elevated blood pressure, and was used as an antimicrobial agent[28-31]. In Morocco, it is used in diarrheal diseases and in acid reflux, as well as an antispasmodic agent[32].

S. marianum and *C. ladaniferus* are well known in Moroccan traditional medicine as having a diuretic effect; however, no scientific data have been published to confirm their effect. The use of diuretics in the clinical setting is important and common, especially, in cases of hypertension, fluid overload due to renal, hepatic, or cardiac reasons, and in cases of electrolyte disturbance.

Therefore, the objective of this study was to evaluate the effect of a single dose or repeated doses of oral administration of aqueous extracts of *S. marianum* flowers and *C. ladaniferus* leaves on plasma electrolytes, urine volume, creatinine clearance and urinary excretion of electrolytes in normal rats.

2. Materials and methods

2.1. Rats

Adult male Wistar rats (150–220 g) were used. The animals were provided by the Animal House Breeding Center, Department of Biology, Faculty of Sciences, Fes, Morocco. The animals were housed under standard environmental conditions [(25±1) °C, (55±5)% humidity and 12 h/12 h light/dark cycle] and were maintained with free access to water and laboratory rat food. All the experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care, and approval from the Ethical Committee, Faculty of Sciences, Fes, Morocco was obtained.

2.2. Plant collection

The plants used in this study were collected from their natural habitat, i.e., from the Sefrou region, named Taounate. The plants were identified and authenticated as *C. ladaniferus* and *S. marianum* by Professor Amina Bari, Faculty of Science, Fes, Morocco.

2.3. Extract preparation

After identification of the plants, flowers of *S. marianum* and leaves of *C. ladaniferus* were dried at room temperature in the shade and then ground to get a coarse powder. Ten grams of the dried powder of *S. marianum* flowers were mixed with 100 mL of water, and the mixture was boiled at 100 °C under reflux for 30 min. The decoction obtained was centrifuged, filtered and frozen at 20 °C. The same procedure was applied to get the extract of *C. ladaniferus* leaves (10 g dry powder extracted with 100 mL of water).

2.4. Reference drug

Furosemide (Lasilix, Pharma 5, Morocco), a loop diuretic, was used as the reference drug.

2.5. Diuretic activity of a single dose of interventions

Each animal was placed in an individual metabolic cage for 24 h prior to the commencement of the experiment for adaptation. After overnight fasting with free water, the rats were divided into four groups, six rats in each group. The first group received orally distilled water (10 mL/kg b.wt), and served as the control group. The second and the third groups were treated with oral administration of 100 mg/kg b.wt of *S. marianum* flowers extract and *C. ladaniferus* leaves extract, respectively. The fourth group was treated with an oral dose of 10 mg/kg b.wt of furosemide. Urine was collected in a graduated cylinder and measured at 1, 2, 4, 6, and 24 h after the administration of interventions. The extracts or furosemide were delivered orally by gavages.

2.6. Diuretic activity of daily doses of interventions

The animals were placed individually in metabolic cages. Four groups, six rats each, were used. Each rat in the first group received every morning *S. marianum* flowers extract (100 mg/kg b.wt) and in the second group received *C. ladaniferus* leaves extract (100 mg/kg b.wt) by oral gavages. The third group was treated with furosemide (10 mg/kg b.wt), and the fourth group received water (10 mL/kg b.wt). The interventions were given daily for a maximum of 8 d by gavages. For each rat, 24-hour urine output was collected daily in a graduated cylinder and its volume was measured. The urine samples were subjected to further testing, including measurement of urine sodium and potassium concentration with the use of a flame photometer. Blood samples were collected in capillary tubes containing EDTA by retro-orbital puncture under light diethyl ether anesthesia on day 8. Plasma was separated by centrifugation at 10 000 × g for 10 min and was analyzed for sodium, potassium, creatinine and urea with the use of an autoanalyzer. Creatinine clearance was calculated from the plasma and urine creatinine level on day 8. Serum and urine osmolality were measured by freezing point depression method using osmometer. Osmolar clearance was

determined with the use of the following equation:

$$\text{Osmolar clearance} = \text{Urinary osmolarity} \times \text{Urine flow} / \text{Plasma osmolarity} \quad (1)$$

Free water clearance was determined by the following formula:

$$\text{Free water clearance} = \text{Urine volume} \times (1 - \text{urine osmolarity/plasma osmolarity}) \quad (2)$$

T_{CH₂O} (free water reabsorption) was assessed by C_{H₂O} according to the following formula:

$$T_{\text{CH}_2\text{O}} = - (C_{\text{H}_2\text{O}}) \quad (3)$$

2.7. Statistical analysis

The data are expressed as mean ± SEM. Statistical comparisons between the multiple groups were performed with one-way analysis of variance (ANOVA) (Graph Pad Prism 5 software followed by post hoc “Tukey’s Multiple Comparison Test”. Student *t*-test was used to compare between two groups. *P*<0.05 was considered statistically significant.

3. Results

3.1. Diuretic activity of a single dose of plant extracts and furosemide

The urine volume was significantly higher in the groups treated with either *C. ladaniferus* or *S. marianum* extract than that collected in the control group during all time intervals (*P* all<0.05, Table 1). Steady increases in the urine volume were obtained throughout the study period, and the maximum increment was noticed after 24 h post the administration of the interventions.

The urine volume measured after a single dose of furosemide was higher than that obtained in the control group at all time intervals and higher than the urine volume measurements in the *C. ladaniferus* or *S. marianum* group during the period from 1–6 h after the treatment (*P*<0.05, Table 1). However, the urine volume measured at 24 h was not different significantly between the *C. ladaniferus* and furosemide

groups. Moreover, the volume was significantly higher in the *S. marianum* group as compared to the furosemide or *C. ladaniferus* groups (*P*<0.05). Therefore, *S. marianum* had the highest diuretic activity after 24 h.

3.2. Diuretic activity of repeated administrations of plant extracts and furosemide

The effect of repeated doses of aqueous extracts of *C. ladaniferus* and *S. marianum* on the urine volume is shown in the Table 2. The results showed that both *C. ladaniferus*, and *S. marianum* caused significant increase in the urine volume that was obvious on the first day of treatment compared to the control (*P*<0.05) and profound effects were obtained on day 8. The diuretic effect of daily dose of furosemide was higher than the effect of *C. ladaniferus* (*P*<0.05). However, the urine volume of *S. marianum* group was higher than that of *C. ladaniferus* extract or furosemide from day 1 to day 8 (*P*<0.05).

3.3. Effects of interventions on urinary electrolytes and creatinine excretion

The effect of the interventions on the urinary excretion of creatinine, sodium and potassium are summarized in the Table 3. The daily treatment of the aqueous extract of *C. ladaniferus*, or *S. marianum* caused a significant increase in the excretion of creatinine, sodium and potassium compared to the control group (*P*<0.05). In a similar manner, furosemide produced a significant increase in urine creatinine, sodium and potassium excretion when compared to the control group (*P*<0.05). Interestingly, the effect of *S. marianum* extract was higher than that of *C. ladaniferus* extract or furosemide.

3.4. Effects of the interventions on blood electrolytes, urea and creatinine

No significant changes were noticed on the blood levels of sodium, potassium, creatinine and urea after 8 d of administration of the

Table 1

Urine volume during 24 h after a single oral dose of aqueous extracts of *C. ladaniferus* leaves, *S. marianum* flowers, or furosemide (mL).

Groups	1 h	2 h	4 h	6 h	24 h
Control	0.51±0.07	1.16±0.18 [^]	1.60±0.18 [^]	2.04±0.11 [^]	6.10±0.26 [^]
<i>C. ladaniferus</i> group	4.10±0.33 ⁺	7.08±0.24 ^{##}	9.06±0.47 ^{##}	12.08±0.32 ^{##}	18.10±0.48 ^{##}
<i>S. marianum</i> group	2.08±0.13 ⁺⁺	5.05±0.26 ^{##+}	8.03±0.17 ^{##}	9.06±0.25 ^{##+}	24.20±0.42 ^{##+}
Furosemide group	7.06±0.48 ^{##}	11.03±0.31 ^{##}	13.06±0.40 ^{##}	16.10±0.39 ^{##}	18.10±0.29 ^{##}

⁺*P*<0.05 vs. control group. ⁺*P*<0.05 vs. *C. ladaniferus* group. ^{##}*P*<0.05 vs. *S. marianum* group. [^]*P*<0.05 vs. 1 h urine volume.

Table 2

Daily urine volume after oral dose of the extracts of *C. ladaniferus* leaves, *S. marianum* flowers, or furosemide (mL/24 h).

Groups	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Control	5.50±0.20	5.80±0.23	6.18±0.38	7.10±0.39	7.20±0.48	6.90±0.18	6.91±0.11	7.23±0.13 [^]	7.11±0.21 [^]
<i>C. ladaniferus</i> group	5.00±0.29	12.20±0.60 ^{##}	12.83±0.16 ^{##}	14.03±0.48 ^{##}	14.60±0.39 ^{##}	17.08±0.63 ^{##}	17.18±0.23 ^{##}	18.03±0.82 ^{##}	18.00±0.64 ^{##}
<i>S. marianum</i> group	5.01±0.39	16.05±0.92 ^{##+}	20.08±0.71 ^{##+}	22.06±0.49 ^{##+}	24.03±0.57 ^{##+}	25.06±0.31 ^{##+}	27.01±0.27 ^{##+}	28.05±0.58 ^{##+}	28.01±0.37 ^{##+}
Furosemide group	5.45±0.25	15.21±0.42 ^{##+}	16.35±0.27 ^{##+}	20.19±0.40 ^{##+}	21.25±0.56 ^{##+}	22.21±0.31 ^{##+}	22.55±0.39 ^{##+}	23.05±0.26 ^{##+}	23.06±0.33 ^{##+}

⁺*P*<0.05 vs. control group ⁺*P*<0.05 vs. *C. ladaniferus* group ^{##}*P*<0.05 vs. *S. marianum* group. [^]*P*<0.05 vs. day 0.

Table 3

Effect of daily administration of aqueous extracts of *C. ladaniferus* leaves, *S. marianum* flowers, and furosemide on urinary excretion of creatinine, sodium and potassium measured on day 8.

Groups	Urine creatinine (mg/dL)	Urine concentration of ions (mEq/L)		Saluretic index	
		Sodium	Potassium	Sodium	Potassium
Control	46.5±0.9	90.7±1.8	62.9±1.2	1.00	1.00
<i>C. ladaniferus</i> group	52.3±0.1*	141.2±0.9*	84.2±1.4*	1.55	1.33
<i>S. marianum</i> group	56.2±0.2*	150.3±1.2* ⁺	90.2±1.2* ⁺	1.65	1.43
Furosemide group	51.3±0.1 [#]	127.8±1.2* [#]	70.8±0.2* [#]	1.40	0.80

Saluretic index = test mEq/L/control mEq/L. **P*<0.05 vs. control group. ⁺*P*<0.05 vs. *C. ladaniferus* group. [#]*P*<0.05 vs. *S. marianum* group.

Table 4

Effect of daily administration of the aqueous extracts of *C. ladaniferus* leaves, *S. marianum* flowers, or furosemide on plasma levels of sodium, potassium, urea and creatinine in normal rats on day 8.

Groups	Plasma electrolyte level (mEq/dL)		Blood urea (mg/dL)	Creatinine (mg/dL)
	Sodium	Potassium		
Control	145.3±1.2	5.3±0.4	35.1±0.6	0.92±0.02
<i>C. ladaniferus</i> group	144.2±1.3	5.4±0.1	35.0±1.3	0.95±0.10
<i>S. marianum</i> group	143.5±0.8	5.1±0.7	35.1±0.5	1.01±0.10
Furosemide group	141.1±1.5	4.3±0.4	34.9±0.6	0.82±0.10

aqueous extracts of *C. ladaniferus* leaves or *S. marianum* flowers (Table 4). However, furosemide lowered serum potassium.

3.5. Effect of interventions on creatinine clearance

Creatinine clearance was measured on the first day and the last day of the treatment. On day 8, a significant increase in the creatinine clearance was observed in the groups treated with *C. ladaniferus*, *S. marianum* extracts or furosemide as compared to day 1 in the same group and to the control group (Table 5). The increase in creatinine clearance was significantly higher in the group treated by *S. marianum* extract or furosemide as compared to the group treated with *C. ladaniferus* on the day 8 (*P*<0.05). No change in creatinine clearance between day 1 and day 8 was observed in the control group.

3.6. Effect on plasma and urine osmolality and clearance of free water

After 8 days of the treatment, there was no significant effect of the plant extracts or furosemide on the plasma osmolality (Table 6). Both plant extracts caused a mild reduction in the urine osmolality, while furosemide caused more and significant reduction in the urine osmolality (*P*<0.05). Free water clearance was significantly decreased by both plant extracts (*P*<0.05), but it was significantly

elevated by furosemide. Therefore, furosemide increased significantly free water clearance and decreased significantly urine osmolality (*P*<0.05). In addition, the extracts of *C. ladaniferus*, *S. marianum* and furosemide produced a significant increase in the osmolar clearance when compared to the control group (*P*<0.05); the effect of *S. marianum* was higher than that of furosemide or *C. ladaniferus*.

Table 5

Effect of oral administration of aqueous extracts of *C. ladaniferus* leaves, *S. marianum* flowers, and furosemide on creatinine clearance measured on day 1 and on day 8 after the treatment (mL/min).

Groups	Day 1	Day 8
Control	0.19±0.04	0.20±0.02
<i>C. ladaniferus</i> group	0.41±0.02*	0.66±0.01* [^]
<i>S. marianum</i> group	0.40±0.01*	1.05±0.01* [^]
Furosemide group	0.58±0.04* [#]	0.93±0.08* [#]

**P*<0.05 vs. control group. ⁺*P*<0.05 vs. *C. ladaniferus* group. [#]*P*<0.05 vs. *S. marianum* group. [^]*P*<0.05 vs. day 1.

4. Discussion

The present study showed that both plants have a significant diuretic effect when used in a single dose or during daily doses over a period of 8 d. The diuretic activity of *S. marianum* was

Table 6

Effect of daily administration of aqueous extracts of *C. ladaniferus* leaves, *S. marianum* flowers, or furosemide on plasma osmolality, urine osmolality, osmolar clearance and clearance of free water on day 8 of treatment.

Groups	Plasma osmolality (mOsm/L)	Urine osmolality (mOsm/L)	Cosmolar (μL/min)	CH ₂ O (μL/min)	TCH ₂ O (μL/min)
Control	290.6±1.1	301.2±0.2	5.10±0.13	-0.14±0.90	0.14±0.90
<i>C. ladaniferus</i> group	288.4±0.9	299.2±0.2*	12.90±0.20*	-0.40±1.20*	0.40±1.20*
<i>S. marianum</i> group	287.0±1.0	297.2±0.1*	20.10±1.14* ⁺	-0.60±0.18*	0.60±0.18*
Furosemide group	292.2±0.2	290.8±0.1*	14.90±0.80* [#]	0.08±0.13*	-0.08±0.13* [#]

**P*<0.05 vs. control group. ⁺*P*<0.05 vs. *C. ladaniferus* group. [#]*P*<0.05 vs. *S. marianum* group.

higher than that of furosemide or *C. landaniferus*. In addition, both plants increased urinary excretion of sodium, potassium and creatinine and the effect of *S. marianum* was more potent than the effects of furosemide or *C. landaniferus*. Interestingly, both plants did not cause changes in the plasma sodium or potassium while furosemide caused hypokalemia. These findings are important since both plants might have a potential to be used as a future diuretic without hypokalemia, which is a common finding with the use of loop diuretics or hydrochlorothiazide. Because there was no effect of the plant extract on plasma electrolytes and plasma osmolarity, it might be suggested that the active principle(s) in both plants may have a potassium-sparing effect. Furthermore, both plants increased creatinine clearance and studying their effect in kidney failure might explore their potential to increase creatinine clearance or ameliorate acute/chronic kidney failure.

Basically, diuresis is an increase in urine volume and a loss of electrolyte in urine. These might be a result of suppression of renal tubular reabsorption of water and electrolyte. The mechanism of action of the plant extracts is not known and requires further investigations. However, both plants might have a similar action to loop diuretics because they increased urinary potassium and sodium excretion that was similar to furosemide. It is well known that furosemide increases urine volume and urinary excretion of sodium and potassium by inhibiting $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symporter in the thick ascending loop of Henle.

Furthermore, both plants and furosemide increased osmolar clearance and decreased urine osmolarity. This means that these interventions increased urine osmoles such as sodium, potassium, urea or glycoses and/or decreased water excretion. However, the main difference was that the plant extracts decreased free water clearance while furosemide increased free water clearance, which was accompanied by high osmolar excretion as compared to the control. Furosemide decreased urine osmolarity and caused diluted urine as compared to the plant extracts. Interestingly, the plant extracts increased osmolar clearance and decreased free water excretion; therefore, hypernatremia and dehydration are less likely a consequence of diuresis caused by both plant extracts, especially *S. marianum*. This might be due to high antidiuretic hormone associated with the use of the plant extracts. If this is true, *S. marianum* will be useful in case of hypernatremia and dehydration such as diabetes insipidus. This opinion needs further investigation. However, hyponatremia and hypokalemia are a common finding in diuresis with the use of loop or thiazide diuretics. Both plant extracts did not cause hypokalemia or hyponatremia.

The plant extracts caused a significant increase in the urine volume beginning from the second hour while the single dose of furosemide induced a significant diuresis within the first hour of administration. The difference in the onset of diuretic action might be related to the gastrointestinal absorption characteristics of the plant active ingredient(s). With the use of daily administration of the interventions, furosemide and plant extracts induced a significant diuresis from day 1 ($P < 0.05$). The urine output continued to increase throughout the 8-day period, and the effect of *S. marianum* extract

was more potent than furosemide or *C. landaniferus*.

The chemical components responsible for the diuretic, natriuretic and kaliuretic activities of the *C. landaniferus* or *S. marianum* extracts have not been studied. However, a preliminary phytochemical analysis of both plants revealed that the plants contain flavonoids^[33,34]. Data showed that flavonoids have a diuretic activity^[35]. For example, isoflavonoids, including genistein and daidzein, cause inhibition of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransporter and increase natriuresis and kaluresis^[36]. Furthermore, another study showed that flavonoids induce diuresis and sodium excretion^[37]. Therefore, the diuretic activity of the *C. landaniferus* and *S. marianum* extracts might be attributed to the presence of flavonoids. Further studies are needed to explore the mechanism of action and the active ingredient.

The result of the study provides the first scientific evidence of diuretic potential of aqueous extracts of *C. landaniferus* leaves and *S. marianum* flowers without hypokalemia or electrolyte disturbance. *S. marianum* caused significantly higher increase in the urine creatinine, urine volume, creatinine clearance, and urine sodium and potassium excretion as compared to furosemide or *C. landaniferus* ($P < 0.05$). It is important to study further these plant extracts, especially *S. marianum*, to identify their active components responsible for the diuresis.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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