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Original Research

Diuretic Effect of the Aqueous Extract of *Cymbopogon nardus* (L.) Rendle Compared with Furosemide in Wistar Rats

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Abstract

Cymbopogon nardus (L.) Rendle is one of the medicinal plants widely used in folk medicine to cure certain diseases. The current work aims to assess the diuretic potential of *C. nardus* using animal models. Urine electrolytes (sodium and potassium), serum electrolytes (sodium, potassium, and chloride), blood urea, creatinine, creatinine clearance, osmolar clearance, urinary osmolarity, and urine flow were determined. To assess the diuretic effect of the studied plant, four groups of rats were used (n = 6). The control group received 10 mL of water, the second and third groups received two doses of the plant (100 and 150 mg/kg bw), and the last group received furosemide (10 mg/kg bw), the experiment sustained for seven days. Urine flow and electrolytes levels were studied. The single dose of *C. nardus* extract significantly increased urine flow after oral administration. In addition, daily administration of both doses of *C. nardus* significantly elevated urine excretion, opposite to the first group. *C. nardus* enhanced the urine elimination of sodium and potassium. Importantly, both doses have no effect on serum potassium level. Creatinine clearance was significantly elevated in a dose-dependent manner. This information will be considered as a keystone for further studies forward in applying new process to isolate active compounds of *C. nardus* responsible for its biological properties.

Keywords: *Cymbopogon nardus*; Diuretic effect; Aqueous extract

Introduction

Natural herbal products have a long history in health maintenance and disease treatment. *Cymbopogon* species occupy an important role in traditional medicine as natural remedies for many human ailments [1]. There are 144 species of *Cymbopogon*, characterized by their high content of essential oil, and grow in different tropical and subtropical areas such as Africa, Australia and others [2]. *Cymbopogon nardus* (L.) Rendle is native to Ceylon as one of the *Cymbopogon* species, which is commonly used as an insect repellent [3,4].

C. nardus is widely used as a food ingredient [5]. It is also used in Chinese medicine for the treatment of fever rheumatism, digestive problems, and in aromatherapy to treat colds and headaches [6].

C. nardus has various biological properties such an antifungal and antibacterial effect [7-10]. In the literature, citronella has been also used as an antipyretic, antidiabetic, antioxidant and anticholinesterase [1,11]. Mounting evidence showed that *C. nardus* essential oil exhibited an excellent cytotoxic effect on human epidermal cell line HaCaT [12]. Additionally, the GC-MS analysis showed that the citronellal is the major com-

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pound of Cn essential oil and exhibits significant antifungal effect against *Candida albicans* by inhibiting biofilm formation and disrupting the preformed biofilms [13]. Owing to its phytochemical compounds, *C. nardus* counteracts the deleterious effects of lead acetate on mice testis [14]. The medicinal benefit of plants is related to their content of bioactive component. On account of this, more attention has been paid to determine the phytochemical profile of *C. nardus*, including phenolic compounds, flavonoids, tannins, terpenoids, phenylpropanoids, and alkaloids [15]. Citronellal, citronellol, and geraniol are abundant components detected in *C. nardus* [16]. The same findings are evoked by Saptura et al, who found 29 active compounds using GC-MS, including carbamate, carbinol, neophytadiene, trans-geraniol, phenol-methoxy [17]. The phytochemical complexity of *C. nardus* makes it a good source of active components with remarkable health benefits.

C. nardus is widely used in folk medicine to treat and prevent several human diseases. Several scientific reports demonstrated the *C. nardus* controls glycaemia by inhibiting carbohydrate hydrolyzing enzymes [18]. Additionally, *C. nardus* has been shown to be effective against obesity by controlling the gene expression of C/EBPα, FAS, SERBP-1, HSL, ATGL, and PPARγ [19].

This plant is used for its diuretic effect in Moroccan traditional medicine. Since no experimental research has been conducted to examine the diuretic effect of *C. nardus*. In fact, diuretic drugs are clinically important in case of heart failure, renal failure, hypertension, edema, and electrolyte homeostasis maintenance [14,15]. Within this frame, the current research was designed to evaluate the impact of the aqueous extract of *C. nardus* on diuresis and electrolyte homeostasis in normal Albino rats and compared it with furosemide as standard drug.

Materials and Methods

Plant sampling

C. nardus leaves were gathered in Rabat (33°58’09”N 6°51’26”W), Morocco, in April 2018. Plant was identified by Professor Amina Bari as a botanist (FSDM, USMB, Fez, Morocco). Sample of plant deposited at the herbarium in the Department of Biology under number 2298/416-3/Rb.

Preparation of aqueous extracts

Leaves of *C. nardus* were air-dried and then ground to get a powder. The extraction process was carried out by mixing 10 g of the leaves powder with 100 mL of water. Then, the obtained combination was heated at 100 °C and agitated for 30 min. The filtrate was recovered and kept in the refrigerator.

Study design

Ethical approval

In vivo experiment was carried out using 24 Wistar rats weighing between 150 and 180 g. The animals are housed under standard conditions (25 ± 1 °C, 55 ± 5% of humidity, and 12 h/12 h cycle light /dark) at Faculty of Science, Dhar El Mahraz, Fez. The study respects the guidelines of animal use and care under Ethical Approval Committee, Faculty of Sciences Dhar El Mahraz, Fez, Morocco (USMBA-SNAMOPEQ 2017-03).

Diuretic effect

Rats weighed 190±12 g were allocated into four groups of six rats each. Fasted during 12 h, in individual metabolic cage is used for 24 h to adapt each rat to the new conditions before experimentation. The experiment design adopted as follows [22]:

- Group 1: control, received distilled water (10 mL/kg bw).
- Group 2: orally received 100 mg/kg bw of leaves aqueous extract of *C. nardus* (D1)
- Group 3: orally received 150 mg/kg bw of leaves aqueous extract of *C. nardus* (D1)
- Group 4: orally received 10 mg/kg bw of furosemide.

The measurement of urine volume was performed at 1, 2, 4, 6, and 24 h after each intervention.

Diuretic activity of daily doses

Aqueous extract of *C. nardus* leaves, furosemide, and distilled water were administered to four groups of rats during seven days. For each rat, the urine volume excreted daily by each rat was measured. Blood sampling was carried out by retro-orbital puncture under anesthesia on day 7.

Renal indices

After seven days of treatment, urine electrolytes (Sodium and potassium), serum electrolytes (Sodium, potassium, and chloride) using ion-selective potentiometry method (Architect c8000i biochemistry analyzer) (kit refs 1E49-01, LN9D28-02 and 1E48-20, respectively), blood urea (using kit number 7D75-30, urease/NADH method and creatinine (using kit number 7D64-20, picric acid/NaOH method, Architect c8000i biochemistry analyzer) were determined. Creatinine clearance, osmolar clearance, urinary osmolarity, and urine flow were determined. Free water reabsorption (TCH_2O) was determined following this formula [23]:

$TCH_2O = - (CH_2O)$

Statistical analysis

The findings are presented as mean ± SEM. Graph Pad Prism software (version 5.0; GraphPad software, Inc., San Diego, USA) was used to determine the statistical

difference using ANOVA test and student's t-test.

Results

Outcomes of single dose of Cymbopogon nardus or furosemide on urine volume in 24 h

Comparing with the control group, the administration of *C. nardus* extract with both doses D1 and D2 increased significantly ($p<0.05$) the urine volume throughout the study period in a dose-dependent manner. The administration of single dose increased significantly urine flow as the opposite to the control group. However, there is no significant difference in the urine volume measured at 24 h between the group treated with furosemide and the group treated with the aqueous extract at the dose of 100 mg/kg (Table 1).

Table 1. Urine volume during 24 h after a single oral dose of distilled water, aqueous extracts of Cymbopogon nardus with D1 (100 mg/ kg bw) and D2 (150 mg/ kg bw) and furosemide

Groups	1 h	2 h	4 h	6 h	24 h
Control	0.64	1.29^	1.73^	2.17^	6.23^
Extract D1	1.12*	4.09*^	7.07*^	8.1*^	17.14*^
Extract D2	3.14*+	6.12*+^	8.3*^	11.12*+^	23.24*+^
Furosemide	6.1*+#	10.07*+#	12.1*+#	15.14*+#	17.14*+#

*p<0.05 vs. control group. +p<0.05 vs. D1group. #p<0.05 vs. D2 group. ˆp<0.05 vs.1 h

Effect of sub-chronic treatment on urine volume

The results of sub-chronic treatment of rats with *C. nardus* extract on the urine volume are shown in Table 2. Both doses of *C. nardus* extract significantly affected the urine excretion opposite to the control group ($p<0.05$). This increase was starting on the first day of the treatment. It is clearly seen the urine volume is elevated by both of the studied doses than in the control group during seven days of experiment (Table 2). However, a remarkable diuretic effect was observed for seven days in the group treated daily with D2 ($p<0.05$).

Results of urinary electrolytes and creatinine excretion

As shown in table 3, daily treatment of the aqueous extract of *C. nardus* significantly increased the urine elimination of creatinine and electrolytes (Na+ and K+), opposite to the control ($p<0.05$). *C. nardus* extract enhanced the urine elimination sodium, potassium and creatinine than furosemide used as a standard drug ($p<0.05$).

Table 2. Diuretic activity of repeated administrations of plant extracts and furosemide

Groups	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Control	5.15 ± 0.11	5.36 ± 0.36	5.82 ± 0.25	6.14 ± 0.52	6.52 ± 0.41	6.73 ± 0.27^	6.92 ± 0.33^	7.08 ± 0.18^
Extract D1	5.08± 0.21	11.86± 0.24*^	12.52 ± 0.34*^	13.87 ± 0.27*^	14.20 ± 0.37*^	16.65 ± 0.18*^	16.94 ± 0.22*^	17.42 ± 0.31*^
Extract D2	5.11 ± 0.10	15.69 ± 0.25*+^	19.72 ± 0.13*+^	21.85 ± 0.33*+^	23.57 ± 0.19*+^	24.60 ± 0.25*+^	26.70 ± 0.51*+^	27.38 ± 0.29*+^
furosemide	5.17 ± 0.36	14.96 ± 0.19*+^	15.33± 0.40*+#^	19.87 ± 0.39+#^	20.72 ± 0.26+#^	21.68 ± 0.14+#^	21.98 ± 0.22+#^	22.46 ± 0.17+#^

*p<0.05 vs. control group. +p<0.05 vs. D1group. #p<0.05 vs. D2 group. ˆp<0.05 vs. Day1

Table 3. Effect of daily administration of aqueous extracts of Cymbopogon nardus and furosemide on urinary excretion of creatinine, sodium and potassium measured on day 7

Groups	Urine creatinine (mg/dL)	Concentration of ions (mEq/L)		Saluretic index	
		Sodium	Potassium	Sodium	Potassium
Control	44.3 ± 0.4	87.7 ± 0.7	64.2 ± 1.1	1.00	1.00
Extract D1	50.0 ± 0.1 *	138.2 ± 1.1 *	85.5 ± 0.9 *	1.57	1.33
Extract D2	54.0 ± 0.2 *	147.3 ± 1.3*+	91.5 ± 1.3*+	1.67	1.42
Furosemide	49.1 ± 0.3*#	124.8 ± 0.9*+#	72.1 ± 0.8*+#	142.1	1.12

*p<0.05 vs. control group. +p<0.05 vs. D1group. #p<0.05 vs. D2 group

Effect on plasma electrolytes level

Table 4 shows the results of serum electrolytes levels. It is clearly seen that there are no significant changes between different groups in the plasma electrolytes levels, creatinine, and urea, as compared to the control group (Table 4).

Results of creatinine clearance

The results of creatinine clearance are summarized in table 5. The treatment of the obtained results did not show any significant modification in creatinine clearance between day 1 and day 7 in the control group. However, the administration of aqueous extract of *Cymbopogon nardus* caused an increase in creatinine clearance that was significant as the opposite to the first and fourth groups ($p<0.05$) (Table 5). The D2 was more effective to increase significantly creatinine clearance than the D1 of the same studied extract ($p<0.05$).

Table 4. Effect of aqueous extracts of Cymbopogon nardus and furosemide on plasma level of electrolytes (sodium and potassium), urea and creatinine on day seven

Groups	Plasma electrolytes level (mEq/dL)		Blood urea (mg/dL)	Creatinine (mg/dL)
	Sodium	Potassium		
Control	138.4 ± 1.1	6.1 ± 0.3	32.9 ± 1.3	0.89 ± 0.02
Extract D1	136.9 ± 0.9	6.4 ± 0.1	32.7 ± 0.8	0.92 ± 0.05
Extract D2	140.0 ± 1.7	6.2 ± 0.5	33.0 ± 1.5	0.97 ± 0.01
Furosemide	139.5 ± 1.3	6.1 ± 0.2	32.7 ± 1.4	1.79 ± 0.03

Table 5. effect of aqueous extract of Cymbopogon nardus and furosemide on creatinine clearance (mL/min) measured on the first day and the day 7

Groups	Day 1	Day 7
Control	0.23 ± 0.02	0.24 ± 0.02
Extract D1	0.28 ± 0.01*	0.65 ± 0.01 *^
Extract D2	0.54 ± 0.04*+	1.05 ± 0.07*+^
Furosemide	0.51 ± 0.02*+#	0.96± 0.02*+#^

*p<0.05 vs. control group. +p<0.05 vs. D1group. #p<0.05 vs. D2 group. ˆp<0.05 vs. Day1

Results of plasma and urine osmolarity and clearance of free water

The aqueous extract of *C. nardus* administrated for seven days did not induce significant modifications in the urine osmolarity, plasma osmolarity, and increase significantly the osmolar clearance and decreased water clearance as the opposite to the first group ($p<0.05$). It is worthy to be noted that the both doses were most effective than the standard drug used (furosemide).

Table 6. effect of aqueous extract of Cymbopogon nardus and furosemide on plasma osmolarity, urine osmolarity, osmolar clearance and clearance of free water on day seven

Groups	Posm (mOsm/L)	Uosm (mOsm/L)	Cosmolar (µL/min)	CH_2O (µL/min)	TCH_2O (µL/min)
Control	289.64 ± 5.5	300.24 ± 5.2	5.08	-0.28	0.28
Extract D1	287.44 ± 3.8	298.24 ± 4.3	12.58*	-0.49*	0.49*
Extract D2	285.87 ±4.1	296.24 ± 1.7	19.89*+	-0.88*+	0.88*+
Furosemide	287.24 ± 3.8	293.58 ± 5.3	15.93*+#	-0.34*+#	0.34*+#

*p<0.05 vs. control group. +p<0.05 vs. D1group. #p<0.05 vs. D2 group.

Discussion

The diuretic effect of aqueous extract of *C. nardus* leaves was tested in Wistar rats using single and daily administration of two studied doses sustained seven days. The diuresis effect of both doses was compared with standard medication (furosemide) and distilled water. The treatment of rats showed a significant diuretic effect of both doses administered on the day 1 or daily for seven days. However, the D2 was more potent as a diuretic than D1 and furosemide, which confirms the claimed diuretic property of the plant tested. Additionally, the daily oral administration of the aqueous extract elevated the urine elimination of Na^+ and K^+. D2 showed a higher activity than produced the standard medication. Furthermore, all interventions, including furosemide, decreased urine osmolarity and had no significant effect on plasma osmolarity. The mechanism of action responsible for the diuretic effects of the plant extract is not known until now, and further investigations are required. However, the diuretic effect of the studied extract could be explained by the same mechanism of action of furosemide on

sodium and potassium. It has been proved that furosemide affects normal diuresis by controlling the $Na^+/K^+/2Cl^-$ implicated in the co-transporter system of the loop of Henle [16].

C. nardus, also known as lemongrass is a popular plant cultivated in different areas in the world. Several populations use leaves to treat different disease such as nervous disturbances with tea prepared with fresh or dry leaves [17]. It is a potential source of bioactive substances with high antioxidant effect [18]. Previous studies have shown that some bioactive compounds of C. citratus such as saponins, tannins, and flavonoids, can induce diuresis and natriuresis by inhibiting co-transporter $Na^+/K^+/2Cl^-$ in the ascending loop of Henle [19]. Thus, the diuretic effect might be attributed to the presence of some bioactive compounds in the aqueous extract of *C. nardus*. Based on the numerous scientific published reports reviewed that citronellal, citronellol and geraniol are the major active ingredients of *C. nardus* providing different health benefits, including antimicrobial, anti-inflammatory, diuretic, and stimulant activities [28-30]. The administration of geraniol exhibited considerable protective effect against renal ischemia/reperfusion injury in rats by boosting their antioxidant defense system [31]. In the same context, geraniol has proved its ability to protect the kidney against nephrotoxicity induced by cyclosporine A and showed to be a good agent to improve the kidney function [32]. Additionally, citronellol is an active ingredient of *C. nardus* essential oil with a wide range of pharmacological activities, including antimicrobial, anti-lice, anti-inflammatory, antispasmodic, antidiabetic, nephropretective, and antinociceptive effects [33]. The synergistic effect between the active components of *C. nardus* could be the main reason for its remarkable beneficial properties accounting diuretic effect.

The administration of aqueous extract stimulates the sodium elimination without affecting the normal range of potassium, this effect is considered the main key factor to select the most effective diuretic agent [15]. Additionally, the plant extract significantly increased the osmolar clearance and decreased water clearance compared to the control group. This might be due to a decreased synthesis of antidiuretic hormone (ADH) [20]. However, it is also possible that the water extract of citronella exerts its diuretic activities via the excessive production of prostaglandins type E. This type of prostaglandins can inhibit the action of ADH at the collector channels which consequently increasing the urinary flow and the excretion of electrolytes [21].

In spite of the widespread use of *C. nardus*, there are no pharmacological investigations on the diuretic effect has been carried out. Due to the similarity of the urinary system between human and rats, we chose to explore the *C. nardus*'s diuretic effect on the rat model. Results showed that the aqueous extracts of *C. nardus* have a dose-dependent diuretic effect and increased urinary sodium and potassium as compared with control group.

Conclusion

The present experimental investigation demonstrated that oral administration of aqueous extract of *C. nardus* exhibited strong ability to increase diuresis without inducing hypokalemia after single and daily administration of two doses in normal rats. Investigation justifies for the first time the use of *C. nardus* in traditional medicine as a diuretic. Future studies are needed to identify the active compounds responsible for this effect.

Conflict of Interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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