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Research Article

DIURETIC POTENTIAL OF AQUEOUS EXTRACT OF FRUITS OF *WITHANIA COAGULANS* DUNAL IN EXPERIMENTAL RATS

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ABSTRACT

Withania coagulans has been widely used in folklore medicines. The diuretic activity of the aqueous extract of fruits of *Withania coagulans* was studied by *in-vivo* Lipschitz test model with slight modifications using furosemide as a standard. The volumes of urine, urinary concentration of sodium, potassium and chloride ions were the parameters of the study. The results indicated significant ($P < 0.001$) increase in the urine volume by 79.12 % and 71.02 % in 500 mg/kg and 750 mg/kg doses respectively, when compared to control group. Urinary electrolyte excretions were increased with both the doses when compared to control. Both the doses showed significant excretion of electrolytes but on contrary; 500 mg/kg dose was found to be more significant as compared to 750 mg/kg dose. From the present study, the diuretic activity of the aqueous extract of *Withania coagulans* has been justified and further confirms its use as a diuretic agent.

Keywords: Diuretic activity, Furosemide, *Withania coagulans*

INTRODUCTION

Plants are important source of medicinal entities with potential therapeutic effects and many of them used have shown prominent diuretic activity[1]. *Withania coagulans* Dunal belonging to the family *Solanaceae* is the small shrub widely distributed in south Asia and India[2]. *Withania coagulans* commonly known as "Indian cheese maker" is a rigid, gray shrub 60-120 cm high. It is well known for its ethnopharmacological activities[3]. The fruits have been employed in diverse situations in men and animals and are widely used for milk coagulation which is attributed to the enzymatic charisma of the plant[4]. The volatile oil obtained from fruits of *W. coagulans* had antibacterial activity against *S. aureus* and *Vibrio cholera* and also found to have antihelminthic activity [5,6]. Withanolides isolated from ethanolic extract of the whole plant showed antifungal properties[7]. The aqueous extract of the plant have shown an anti-inflammatory activity in various rodent models[8]. Oral administration of aqueous extract of fruits of the plant significantly lowered blood sugar, serum cholesterol and hepatic LPO in diabetic rats as well as db/db mice [9, 10]. The aqueous extract and 3-β-hydroxy-2,3-dihydrowithanolide F of fruits of this plant has been shown to exert hepatoprotective activity against CCl_4 induced hepatotoxicity in adult albino rats[11]. The aqueous extract exhibited free radical scavenging activity in an in vitro system using DPPH[12]. It also showed hypolipidemic activity in triton induced hypercholesterolemic rats[13]. Phytochemically, *Withania coagulans* contains amino acids, essential oils, withanolides and coagulin[14]. The previous phytochemical investigation has led to isolation and identification of withanolides as coagulants P, Q and R[15,16]. The present study was undertaken to verify the efficacy of aqueous extract of *W. coagulans* as diuretic drug in experimental rats.

MATERIALS AND METHODS

Dried fruits of *Withania coagulans* were purchased from local market of Bangalore and authenticated by Prof. Balkrishna Gowda, Dept. of Forestry, University of Agriculture Science, Gandhi Krishi Vigyan Kendra, Bangalore, India. The voucher specimen has been kept in our department (SJPC/WC/01). After due authentification, the dried fruits (with persistent calyx and pedicle) were coarsely powdered using mechanical grinder.

Preparation of extract

The dried material (500 g) was extracted with distilled water at room temperature for 48 hr. Following filtration and concentration under vacuum, a brown sticky residue with coca like smell was obtained (yield: 13.3%). The residue was further dissolved in normal saline and used for experimental work.

Animals

Fresh Albino Wistar rats (150–200 g) of either sex used for the study were procured from Institute's animal house. Animals were housed in polypropylene cages and maintained under standard conditions (12 h light/dark cycle, 22 ± 2^0 C and 55 ± 5% relative humidity). They were fed with standard rat pellet diet and water *ad libitum*. The animals were maintained in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals) guidelines for the care and use of laboratory animals. The study was approved from the IAEC with proposal no. IJAHSM/IAEC/2009/02.

Diuretic activity

The methods of Lipschitz et al[17], Mukherjee et al[18] and Murugesan et al[19] were followed for the evaluation of diuretic activity. The animals were starved and also deprived of food for 18 hr prior to the experiment and were divided into four groups of animals containing six each. Out of the four groups, the first group served as control and was fed normal saline orally at 25 ml/kg body wt. The second group received the same amount of normal saline in which furosemide (10 mg/kg body wt.) was dissolved. The third and fourth groups received normal saline orally (25 mg/kg body wt.) in which *W. coagulans* at doses of 500 and 750 mg/kg body wt., respectively, was dissolved.

Immediately after administration of the drug, the rats were placed in metabolic cages (1 in each cage), specially designed to separate urine and fecal matter, and observed at room temperature of 25° ± 0.5 °C. During this period of the experiment, no food or water was made available to the animals. The total volume of urine excreted by the animals was collected and measured up to 8 hr after the administration. Urine samples were analyzed thereafter for Na^+, K^+ (cations) and Cl^- (anions) in urine. The concentration of Na^+ and K^+ were analyzed by flame photometer [20] and the amount of Cl^- was determined by standard kit containing Chloride Reagent (Mercuric Nitrate 0.8mM/L, Nitric acid 45 mM/L, Ferric Nitrate 20mM/L and Mercuric Thiocyanate 3mM/L) from Span Diagnostics, Surat, India.

Statistical analysis

The results are expressed as mean ± standard error mean (SEM). Significance of differences between control and treated groups were determined using Student's *t*-test.

RESULTS AND DISCUSSIONS

The present finding suggests that the aqueous extract of *Withania coagulans* possess a demonstrable and potent diuretic potential. The data here found is reported for the first time that its ethnopharmacological effect is probably mediated through direct ability to increase urine volume and electrolyte excretion.

The treatment with aqueous extracts at 500 and 750 mg/kg showed dose independent diuretic effect. At 500 mg/kg, the volume of urine was increased by 79.49 % as compared to control (Table 1).

The extract at 500 mg/kg showed significant increase in Na^+, K^+ and Cl^- by 94.64 % ($P < 0.001$), 97.76 % ($P < 0.01$) and 92.72 % respectively ($P < 0.001$) (Table 2). At 750 mg/kg, the volume of urine was increased by 71.02 % as compared to control (Table 1). The excretion of Na^+, K^+ and Cl^- was found to be 80.57 % ($P < 0.001$), 90.29 % ($P < 0.01$) and 75.79 % ($P < 0.001$) respectively (Table 2).The saluretic index and $Na^+/K^+$of the standard reference drug furosemide and two doses used was found to be highly significant ($P < 0.001$) (Table 2).

Table 1: Effect of oral administration of *W. coagulans* and furosemide on urine volume

Group	Dose (mg/kg, p.o.)	Urine volume (ml/8 hr)	Diuretic index[a]
Control	-	0.12 ± 0.12	-
Furosemide	10	3.9 ± 0.12***	3.48
W. coagulans	500	3.1 ± 0.15***	2.77
W. coagulans	750	2.77 ± 0.11***	2.47

Each value represents the mean ± S.E.M. of six rats in a group.
** $P < 0.01$.
*** $P < 0.001$ when compared with the control group (student's t-test).
[a] Diuretic index = volume problem group/volume control group.

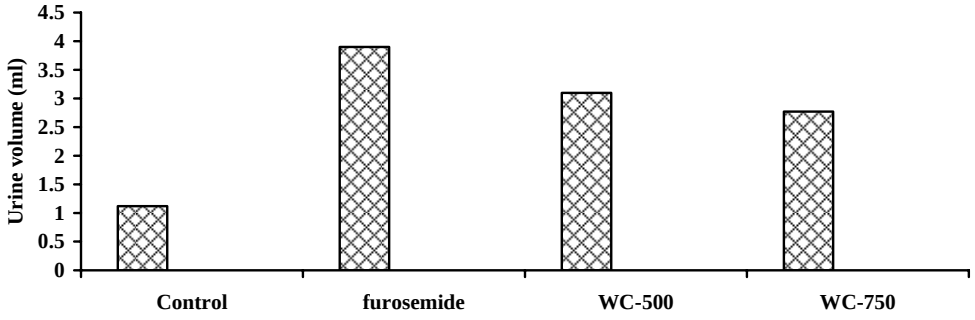

Fig. 1: Effect of oral administration of *W. coagulans* and furosemide on urinary volume

Table 2: Effect of oral administration of *W. coagulans* and furosemide on urinary electrolyte excretion

Group	Dose(mg/kg, p.o.)	Urine electrolyte concentration (mEq/L)			Saluretic index [b]			
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+/K^+
Control	-	91.84 ± 11.8	66.18 ± 7.5	90.19 ± 1.73	-	-		1.39
Furosemide	10	204.72 ± 4.95***	124.18 ± .79***	162.52 ±1.90***	2.23	1.88	1.80	1.65
W. coagulans	500	193.75 ± 11.83***	21.42 ± 0.69**	150.69 ± 1.28***	2.11	1.83	1.67	1.60
W. coagulans	750	164.95 ± 8.04***	112.13 ± .31**	123.19 ± 2.18***	1.80	1.69	1.37	1.47

Each value represents the mean ± S.E.M. of six rats in each group.
** $P < 0.01$.
*** $P < 0.001$ when compared with the control group (student's t-test).
[b] Saluretic index = mEq/L problem group/mEq/L control group.

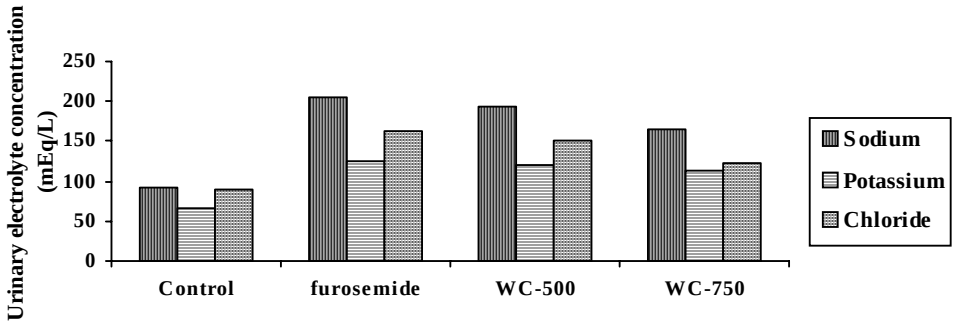

Fig. 2: Effect of oral administration of *W. coagulans* and furosemide on urinary electrolyte excretion

However, concerning electrolyte excretion and urinary volume was something different. As though 500 mg/kg dose produced diuresis with demonstrable high urine output and electrolytic excretion, the same was not observed for 750 mg/kg dose. Here the observed urinary volume and electrolytic excretion were increased as compared to control group but decreased as compared to 500 mg/kg dose. This possibly assumes that diuresis is more in 500 mg/kg treated group. This support the fact that as the concentration of *Withania coagulans* increases, the urinary volume as well as urinary excretion decreases. These could be explained through a decreased glomerular filtration rate (perhaps by renal blood flow decrease); possibly due to essential oils present in plant[16]. The *Withania coagulans* is unlikely acting as Thiazide diuretic because they increase urinary potassium level and alter urinary Na^+/K^+ output[21]. In this study Na^+ level was also increased without any significant alteration in Na^+/K^+ ratio. On the other hand, diuresis induced by *Withania coagulans* extract at 500 mg/kg dose was strong with similar intensity comparable to furosemide, accompanied by marked increase in urinary sodium and potassium levels.

Phytochemically, *Withania coagulans* fruits are shown to contain steroidal lactones, withanolides, amino acids and essential oils. However amino acids are reabsorbed in proximal convoluted tubules of nephron and can not function as diuretic. Withanolides are steroidal lactones with an ergostane skeleton found as chief chemical constituent of the plant[22]. These withanolides are more polar in nature as compared to other withania species. Finally, our data seems to indicate that this diuretic effect may be associated with the presence of active principles of polar nature where withanolides may be the main chemical protagonist of this activity.

The present investigation supports the use of *Withania coagulans* as diuretic agent in traditional folklore medicine[3]. However, the exact mechanism of action remains unelucidated. Hence further investigations are required to probe a valuable diuretic drug of indigenous medicine.

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