

Therapeutic Effect of Hydroalcoholic Extract for *Withania Coagulans* for Diuretic Activity in Rodents

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Abstract: Diuretics are used to manage fluid excess as part of a treatment strategy. *Withania coagulans* has long been utilised in traditional medicine. Including some slight modifications and by using furosemide as a standard drug, in vitro cell Lipchitz test model was used to examine a diuretic effectiveness of *Withania coagulans* fruit aqueous extract. Results demonstrated that only one unique route of oral dosage delivery of hydroalcoholic extract of fruit of *Withania Coagulans* as 500 mg/kg/b. w. and 750 mg/Kg and standard Furosemide (10 mg/kg) have enhanced the amount of urine produced as well as an increase in the amounts of sodium, potassium, and chloride ions in urine. Hydro alcoholic extract of fruit of *Withania Coagulans* 750 mg/kg produced a higher diuretic activity equivalent to that low dose extract and lower diuretic effect when comparable with furosemide (10 mg/kg) as a standard. The herb is utilised in traditional medicine for its diuretic properties. Thus, our study backs up and validates the logic for the folkloric usage of roots of Hydro alcoholic extract of fruit of *Withania Coagulans* for its diuretic activity. Withanolides isolated from ethanolic extract demonstrated antifungal, antibiotic, and anti-tumor activity. Comparing *Withania* species, withanolides are more polar in nature.

Keywords: Hydro Alcoholic Extract (*Withania Coagulans*), Furosemide, Active Diurnal, Urinary Output

1. Introduction

The Diuretics are medications that cause a rise in urine production and being employed to treat fluid-filled disorders as an example cirrhosis, heart attack, and nephritic syndrome [1]. Diuretics are used in the treatment of fluid excess, which manifests such symptoms include ascites, ankle swelling, and pulmonary edema. They also aid in fluid balancing and aid in the removal of fluid from the interstitium providing patients with vital symptom comfort and improving their living quality in relation to health [2]. Many of the used plants have demonstrated substantial diuretic activity, making plants a valuable source of pharmacological substances with potential therapeutic effects [3]. Diuresis is a crucial procedure for excreting catabolites, preserving the hydroelectrolyte balance, and getting rid of harmful compounds. There are many

illnesses that affect the human urinary tract, and traditional cures and methods are still used today. Many diuretics, including clinical settings, ethacrinic acid, thiazides, mannitol and furosemide are used. [4]. Diuretics alleviate peripheral edoema and pulmonary congestion. Orthopnea and paroxysmal nocturnal dyspnea, two symptoms of the syndrome of volume overload, can be mitigated by these medications [5]. They diminish reduced venous return to the heart due to decreased plasma volume (preload) and minimizes the strain according to the heart's requirements for oxygen and plasma volume, which lowers blood pressure. Wide ranges of phytoconstituents are present in the plants are glycosides, alkaloids and tannins, and other substances with diuretic action phenolic coumarins, triterpenoids, and so forth. Natural diuretics work by boosting urine flow as well as urinary electrolyte content. Many Indian medicinal herbs

have been documented to have exceptional diuretic effect such as:- Lepidiumsativum, Costusspeciosus, Phyla nodiflora, Tylophora indica, Tribulus terrestris, Thespesia populnea, Rungia repens, Phyllanthus fraternus, Spilanthes acmella, Mimosa pudica etc [6]. Withania coagulans has a long history of usage in traditional medicine. Many plants have a range of biological functions in the natural system of medicine. Withania Coagulans is a member of the Withania genus of the Solanaceae family [7]. Coagulans Withania Dunal, sometimes referred to as Indian cheese maker, is said to have a variety of phytochemicals and pharmacological effects. Ripe fruits have CNS depressant, emetic, sedative, anti-inflammatory, antibilious, and antiasthmatic properties; they are used to treat chronic liver problems and strangulation, according to published research. The plant produces tasty fruits that are also said to have sedative, emetic, alterative, and diuretic properties. Additionally, W. coagulans is used to treat impotence, wasting illnesses, impotence in children, handicap, and nervous weariness. Asthma, biliousness, and liver ailments are treated using its fruits. The plant's flowers can be used to treat diabetes [8]. In the fall, the root is picked and dried for later use. [9]. The plant is poisonous by nature, thus using it should be done with some caution [10]. The anti-inflammatory, hepatoprotective, immunosuppressive, anti-tumor, antibacterial, anti-hyperglycemic, free radical scavenging and central nervous system depressant properties of this plant have all been noted [11]. Withanolides isolated from ethanolic extract showed antifungal, antibiotic and anti-tumor activities properties [12]. The primary goal of this research was to evaluate the hydroalcoholic extract of Withania coagulans' diuretic properties.

2. Methodology

2.1. Collection of Plant Materials

Withania coagulans dried fruits were acquired from a local market, Jaipur. Dried Fruits of Withania coagulans authenticated by Department of Pharmacognosy (Ref. No. NU/NIP/2020/202) in Shoba Nagar, Jaipur, Rajasthan: NIMS Institute of Pharmacy, NIMS University.

2.2. Preparation of Extract

Dried Fruits of Withania coagulans were dried for removing the moisture. After the removal of sufficient amount of moisture, the fruits were coarsely powdered using grinder. Coarse powder was collected separately. Hydroalcoholic extraction was done with the help of alcohol and water in the ratio of 70:30 respectively by using Soxhlet apparatus. Then hydroalcoholic extract was concentrated by using hot water bath and a gummy mass material was obtained. These extracts were screened out for diuretics activity in albino rat.

2.3. Procurement of Animal

Albino Wistar rat (200g- 250g) either sexes were utilised for the diuretic investigation. Animals have obtained from the animal home of Nims university, Jaipur, Rajasthan. The IAEC (Institutional Animal Ethical Committee) authorised this study with the reference number NIMSUR/IAEC-01/2022/05 of NIMS University IAEC Certificate number.

2.4. Experimental Protocol Groups

Albino rats are divided into four groups; in each group contain 6 albino rats. The animals starved for eighteen hours before the experiment started. Each group received different quantity of drug. First group was normal group which is given normal saline. The second group got the same dose of saline solution (10 mg/kg body weight, i.p.) containing furosemide. The third and fourth groups were given oral administration of saline in which Withania coagulans at low doses (500 mg/kg/ b. w.) and high dose (750 mg/kg body w.t.) of extract respectively. The rats were immediately put into metabolic cages (1 in each cage), which were made specifically to segregate faeces and urine, and kept at room temperature. The total volume of urine were collected from individual animals for 8 hrs and measured. A urine sample was analyzed for the levels of K⁺, Na⁺, and Cl in urine.

Table 1. Experimental Groups.

Group	Treatment
I Group	Control group Normal saline
II Group	Standard group Normal saline + Furosemide (10 mg/kg body wt., i. p.).
III Group	Low dose extract Low doses (500 mg/kg/ b. w.) of hydroalcoholic extract of fruit of Withania coagulans + saline
IV Group	High dose extract High doses (750 mg/kg/ b. w.) of hydroalcoholic extract of fruit of Withania coagulans+ saline

3. Result and Discussion

3.1. Urine Volume of Withania Coagulans Hydro Alcoholic Extract

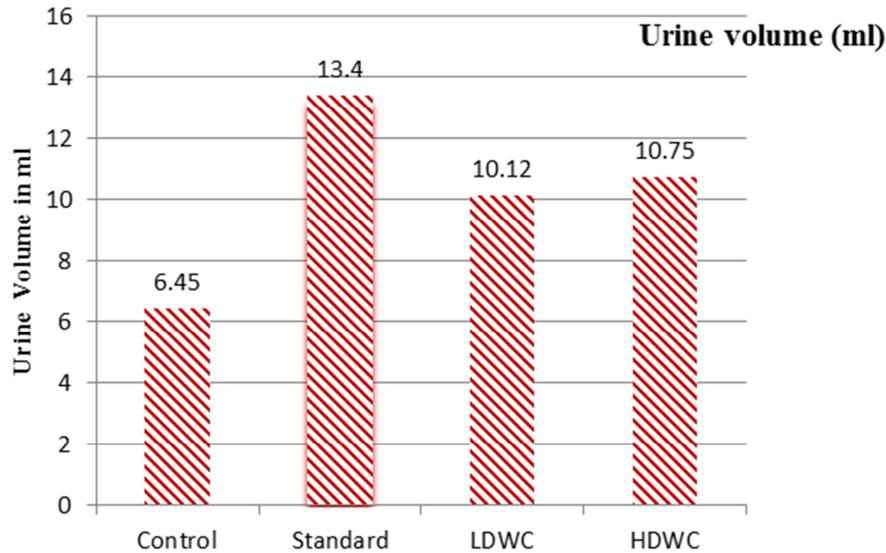
Effective extract of hydro alcoholic fruits of Withania Coagulans produced diuretic effect (Table 1) which showed little dose dependent effect. Low dose of hydro alcoholic

extract of Withania Coagulans (500mg/kg/b.w.) produce a significantly (P<0.005) diuresis (10.12±0.46 ml) compared to control (6.45±1.02 ml) group after 8 hour. Rats treated with high dose of Hydro alcoholic extract of fruits of Withania Coagulans (750mg/kg/b. w.) also showed significantly (P<0.005) increase in urine volume (10.75±0.97) when compared with control group (6.45±1.02 ml). Urine volume in standard group exhibited 13.4±1.25 ml which is higher than when compared with control group (Figure 1).

Table 2. Total urine volume content in groups.

Group	Total Urine Volume
Control group (Normal saline)	6.45±1.02
Standard (Frusemide 10 mg/kg/b. w.)	13.4±1.25**
Hydro alcoholic extract of Withania Coagulans (500mg/kg/b. w.)	10.12±0.46*
Hydro alcoholic extract of Withania Coagulans (750mg/kg/b. w.)	10.75±0.97*

Data were analysed using one-way ANOVA and the Dunnet's comparison test after being expressed as mean S. D. (n=6). *P≤ 0.05 and **P≤ 0.01 when compared to the control group, respectively.

**Figure 1.** Urine volume of hydro alcoholic extract of Withania Coagulans.

3.2. LDWC-Low Dose Withania Coagulans, HDWC-High Dose Withania Coagulans

3.2.1. Urinary Electrolyte Concentration (mmol/L) of Urine in the Hydro Alcoholic Extract of Withania Coagulans

The cumulative urine samples were collected after 8h and electrolyte content (K⁺, Na⁺, and Cl⁻) analysis (Table 2 and Figure 2). It is the presence of water soluble salts in the extracts raises the risk of interference, which would counteract the extract's secondary metabolites' inherent diuretic effects. The hydro alcoholic extract of Withania Coagulans fruits' concentration of K⁺, Na⁺ and Cl⁻ was found to be below the detection limit, according to the results (Table 2).

Low dose of hydro alcoholic Withania Coagulans fruit extract substantially (P<0.001) increase urinary sodium,

3.2.2. Urinary Electrolyte Concentration (mmol/L)

potassium and chloride excretion (Na⁺:154.76±2.85, mmol/L, K⁺: 71.85±3.05 mmol/L, Cl⁻: 124.08±2.96 mmol/L) when compared with control (Na⁺:110.04±2.09 mmol/L, K⁺: 48.08±2.85 mmol/L, Cl⁻:84.08±2.08 mmol/L).

High dose of hydro alcoholic extract of withania Coagulans also exhibited significantly (P<0.001) increase urinary sodium, potassium and chloride excretion (Na⁺:174.64±3.02 mmol/L, K⁺: 65.4±1.65 mmol/L, and Cl⁻:112.78±2.07 mmol/L) when compared with control group (Na⁺:110.04±2.09 mmol/L, K⁺:48.08±2.85 mmol/L, Cl⁻:84.08±2.08 mmol/L).

Electrolyte content (Na⁺, K⁺, and Cl⁻) in standard group are higher than extract group when compared with control group. The excretion (Na⁺:186.06±2.86, K⁺:71.85±3.05 and Cl⁻:124.08±2.96 respectively increased significantly (P<0.001) when compared with control group.

Table 3. Urinary electrolyte concentration.

Group	Na ⁺ mmol/L	K ⁺ mmol/L	Cl ⁻ mmol/L
Control group (Normal saline)	110.04±2.09	48.08±2.85	84.08±2.08
Standard (Frusemide 10 mg/kg/b. w.)	186.06±2.86***	71.85±3.05***	124.08±2.96***
Hydro alcoholic extract of Withania Coagulans (500mg/kg/b. w.)	154.76±2.85***	63.32±2.86**	98.86±1.75***
Hydro alcoholic extract of Withania Coagulans (750mg/kg/b. w.)	174.64±3.02***	65.4±1.65**	112.78±2.07***

Data were analysed using one-way ANOVA and the Dunnet's comparison test after being expressed as mean S. D. (n=6). When compared to the Control group, the following values are significant (P ≤ 0.005): *P ≤ 0.005, **P ≤ 0.01, ***P ≤ 0.001.

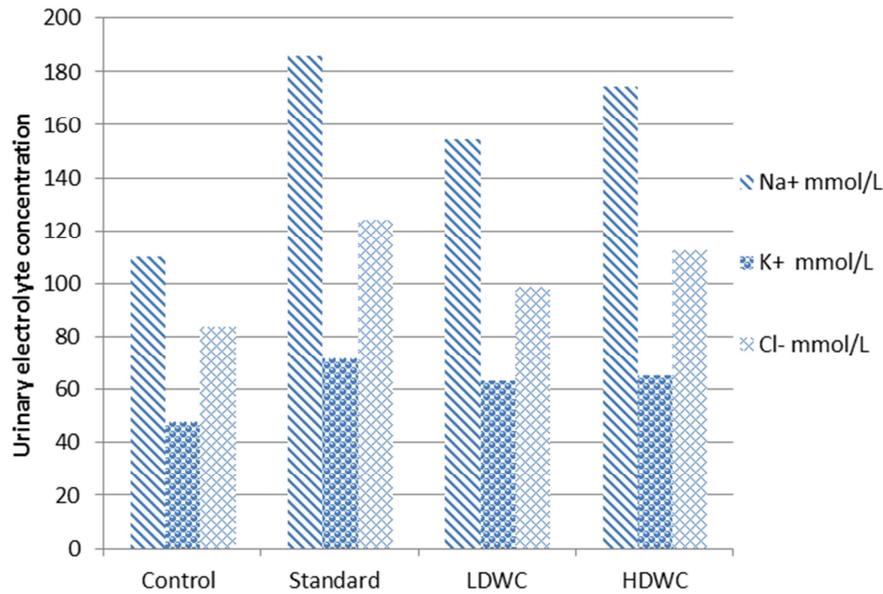


Figure 2. Urinary electrolyte concentration (mmol/L) of urine in the hydro alcoholic extract of *Withania Coagulans*.

3.3. LDWC-Low Dose *Withania Coagulans*, HDWC-High Dose *Withania Coagulans*

The results of the evaluation is due to the diuretic effects of the hydroalcoholic fruit extract from *Withania coagulans* are presented in [Table 1/Figure 1]. From the result, the alcoholic *Withania Coagulans* fruit extract contains significant diuretic compared to the control group, effect by increasing urine excretion and increasing potassium, sodium and chloride excretion. The effect of the alcoholic extract from the fruits of *Withania coagulans* shown that the effect was dosage-dependent, meaning that of the two doses examined, the larger dose had a greater impact. When compared to the typical diuretic furosemide showed that the diuretic impact seen following therapy with the liquor extraction from the fruits of *Withania Coagulans* was significant in terms of urinary excretion, sodium, potassium and chloride concentrations. Measurement of electrolyte concentration in urine showed that the alcoholic extract from the fruits of *Withania coagulans* effectively increased the electrolyte concentration in urine for each of the three ions examined (K⁺, Na⁺ and Cl⁻).

Diuretics can help with a range of illnesses, such as pulmonary edoema, nephritic syndrome, congestive heart disease, a pregnancy, hypertension, altitude sickness, premenstrual stress and so on. The current generation of diuretics is associated with various side effects.

Hyperuricemia, hypokalemia, hypotension, Hyponatremia and can all be caused by loop diuretics which can cause gout, etc. Thiazide diuretics can induce encephalopathy, erectile dysfunction hypokalemia, hyponatremia and other side effects. Diuretics have a beneficial effect in pulmonary congestion and peripheral edema [13] in CCF, as well as abrupt left ventricular failure. Diuretics reduces plasma volume and, as a result, venous return to the heart. This reduces the workload of the heart, oxygen demand, and

plasma volume, and lowers blood pressure. Therefore, in hypertensive individuals, diuretics are quite important [14]. They are used in aspirin and morphine poisoning to enhance forced diuresis (forced alkaline diuresis and forced acid diuresis). Diuretics are also useful in preventing the formation of recurrent stones. Medicinal plants and other secondary plant compounds provide a natural protective effect against disease and are an essential treatment option for some disorders. Diuretics have been demonstrated to be particularly beneficial in the treatment of mild to moderate hypertension and to increase the effectiveness of other antihypertensive medications. The current study has shown that the alcoholic extract from the roots and the alcoholic extract from the fruits of *Withania coagulans* significantly increase urinary excretion as well as urinary electrolyte excretion in a dose-dependent manner. In the present study, the alcoholic fruit extract of *Withania coagulans* was investigated for its diuretic activity. The phytochemical studies show that the fruits of *Withania Coagulans* contain resins, flavanoids, sterols, phenolic compounds, tannins, carbohydrates, and alkaloids.

Na⁺ is regarded as one of the most critical variables in primary hypertension. It is well known that increased Na⁺ intake has negative effects on arterial blood pressure [15]. In our study, oral administration of the alcoholic fruit extract of *Withania coagulans* was shown to have a significant natriuretic effect compared to the saline-treated group, especially at doses of 500 mg/kg and 750 mg/kg. However, at a dose of 750 mg/kg, the K⁺ content in urine samples also increased dramatically when the amount of However, the excretion of K⁺ in the treated groups was lower than in the control group, indicating that *Withania coagulans* has potassium-sparing capabilities. Based on these findings, it is hypothesised that *Withania coagulans*' diuretic impact is due to the blockage of epithelial sodium channels or aldosterone activity. However, future research might look at the chemical

mechanism of action.

In previous studies, phytochemicals like tannins, flavonoids, saponins, alkaloids, terpenoids, sterols, glycosides, steroids, organic acids, sesquiterpenes, amino acids, and carotenoids [16]. were detected in various plant extracts. Most of the above phytochemicals were detected in the alcoholic fruit extract of *Withania coagulans*. It has been demonstrated that the fruits of *Withania coagulans* contain essential oils, amino acids, withanolides and steroidal lactones phytochemically. Amino acids, however, remain absorbed into PCT the nephron and do not exhibit diuretic activity. Other constituents such as the steroidal lactones naming withanolides have an ergostane structure to be an active chemically-based constituents within the plant [17]. These withanolides are naturally highly polar compared to other *Withania* species. Our results suggest that the diuretic effect is related to the existence of an actively compounds that are polar in nature, and the primary chemical may be withanolides protagonists showing diuretic effect. The current research backs up the usage of *Withania coagulans* as a diuretic in traditional folk medicine. However, the precise mechanism of action is unknown. Therefore, more research is needed to identify a useful diuretic drug in traditional medicine. it is possible to declare that the detected diuretic effect is because of the mentioned phytocomponents.

4. Conclusion

Results showed that a single oral dosage of normal furosemide (10 mg/kg) and hydroalcoholic extract of *Withania Coagulans* fruit (500 mg/kg/b. w. and 750 mg/Kg) increased urine production and concentrations of sodium, potassium, and chloride ions. When compared to that low dosage extract, the hydro alcoholic extract of *Withania Coagulans*' fruit had a higher diuretic activity, while ordinary Furosemide (10 mg/kg) had a weaker diuretic impact. The plant's diuretic properties are employed in traditional medicine. Our scientific investigation identified several phytoconstituents that were previously documented for this diuretic action in our hydroalcoholic extract of *Withania Coagulans* fruit. As a result, our research backs up and explains the logic for the traditional usage of *Withania Coagulans* fruit hydroalcoholic extract from the roots for its diuretic properties.

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