Research Article

Diuretic activity of methanolic extract of the whole plant of *Withania somnifera* in rats

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Abstract

Objective: The objective of the present study is to evaluate the diuretic activity of the methanolic extract of *Withania Somnifera* whole plant extract in rats. **Methods:** Rats were randomly divided into five groups each comprising six rats. Group I served as negative control and received distilled water 10 ml/kg, Group II served as a positive control and was given a standard drug 10mg/kg hydrochlorothiazide, Group III, IV and V were test groups and received 100, 200 and 400 mg/kg of methanolic extract of *Withania Somnifera*, respectively. Urine output was collected up to 24 hr and analyzed for electrolytes. **Results:** The methanolic extract of *Withania Somnifera* increased diuresis significantly at the doses of 200 and 400 mg/kg ($p<0.01$). Regarding electrolyte excretion, 400 mg/kg of methanolic extract had increased Na^+, K^+, Cl^+ ($p<0.01$), when compared with the standard. Phytochemical analysis revealed the presence of secondary metabolites like alkaloids, flavonoids, tannins, terpenoids, flavonoids and saponins, which could be the responsible components for the diuretic activity. **Conclusion:** The results of the present study indicated that the plant is enriched with significant diuretic activity providing evidence for its traditional claim. The increased diuresis effects of the crude extracts may be attributable for presence of increased phytoconstitutents.

***Keywords*:** *Withania somnifera,* electrolyte excretion, flavonoids, diuresis

Introduction

Diuretics are the drug that increases the rate of urine formation together with natriuresis. Diuretics are used to adjust the volume and composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis (Doucet et al., 2007). Most diuretics exert their action by decreasing renal tubular sodium reabsorption, thereby reducing the luminal-cellular osmotic gradient, which limits water reabsorption and results in a diuresis.

Drug induced diuresis is beneficial in many life -threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxaemia (Vanamala et al., 2012). These also play an important role in hypertensive patients, pulmonary congestion, this decreases cardiac work load, oxygen demand, plasma volume, thus decreasing blood pressure & also treat the acute and chronic renal failure, hypercalciuria, cirrhosis of liver (Kumar et al., 2010). Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue, and weakness.

Withania Somnifera, belonging to family Solanaceae, is an Ayurvedic herb also known as Indian winter cherry and Indian ginseng that has been traditionally known since ancient times in India for its numerous beneficial health activities. It is one of the most important herbs in Ayurveda, which has been used for stress management, energy elevation and improving cognitive health (Pratte et al., 2014) and to lower inflammation, blood sugar levels, cortisol, anxiety, and depression (Montalvan et al., 2015). To date, this medicinal plant has been found to have anti-epileptic, anti-inflammatory, anti-arthritic, anti-depressant, anti-coagulant, anti-oxidant, anti-diabetic, anti-pyretic efficacies along with palliative effects such as analgesic, rejuvenating, regenerating and growth-promoting effects (Subbaraju et al., 2006).

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Materials and methods

Plant Material

The whole plant of *Withania somnifera* was collected from kompally, Secunderabad, Telangana in the month of April. The plant was cleaned and air-dried at room temperature for one week and ground into a fine powder. Powdered samples were collected and stored in air- and water-proof containers protected from direct sunlight and heat until required for extraction.

Preparation of plant extract

The whole plant of *Withania somnifera* was isolated, chopped into small pieces and air dried under shade at room temperature for 7-10 days. The dried plant was powdered. This powder was used for methanolic extract. The dried powdered plant of *Withania somnifera* (250 g) were extracted with 500ml of methanol by maceration. The powdered plant is placed in a stoppered container with the methanol and allowed to stand at room temperature for a period of at least 5 days with frequent agitation until the soluble matter has dissolved. On the 5th day, the macerated mixture was filtered and subjected to steam distillation. The residue obtained was dried and preserved in a air tight container. The extract was named as Methanolic extract of *Withania somnifera* (MEWS).

Experimental animals

The Wister albino rats of either sex weighing between 150 and 200 grams were procured from albino chemical labs, ghattkesar, Hyderabad. The animals were maintained in a well ventilated room with12:12 hour light/dark cycle in polypropylene cages. Animals were feed with standard pellet diet and drinking water ad libitum. All animals were allowed acclimatize to the laboratory environment for at least one week before the experimental session. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Malla Reddy Institute of Pharmaceutical Sciences, Secunderabad (Reg. No:1662/PO/Re/S/12/CPCSEA).

Preliminary Phytochemical Screening

The preliminary phytochemical analysis of methanolic extract of *Withania somnifera* was performed for Alkaloids, Saponins, Tannins, Steroids and triterpenoids Flavonoids, Phenols, Glycosides, Carbohydrates according to published standard methods (Kokate, 1996).

Experimental Procedure

The diuretic activity of Methanolic extract of whole plant of *Withania Somnifera* in albino rats was studied by the Lipschitz Test (Lipschitz, 1943; Jayasree and Kiran, 2011; Dubey et al., 2010). Male Albino rats were divided into 5 groups of 6 rats in each. The group I serves as normal control received vehicle (normal saline 10 ml/kg b.wt). The group II received HCTZ (10 mg/kg, p.o) in vehicle. The groups III, IV, V were treated with 100, 200, and 400mg/kg doses of methanolic extract of whole plant of *Withania Somnifera* in vehicle and immediately after the extract treatment all the rats were hydrated with saline (10 ml/kg) and placed in the metabolic cages (2 per cage), specially designed to separate urine and faeces. The total volume of urine collected for 24 hr was measured at the end. During this period no food and water was made available to animals. Various parameters like total urine volume and concentration of sodium, potassium and chloride in the urine were measured and estimated respectively.

Statistical analysis

The results obtained were expressed as mean ± SEM. Statistical analysis was performed using a one-way analysis of variance (ANOVA). Data was considered statistically significant at $p < 0.05$. When data was found to be very ($p<0.01$) or highly ($p< 0.001$) significant, this was indicated in the results. All statistical analyses were performed using Graph Pad prism 8 software.

Results

Preliminary Phytochemical Screening

The Methanolic Extract of whole plant of *Withania somnifera* was subjected to qualitative phytochemical tests to identify the phytoconstituents and it revealed the presence of carbohydrates, alkaloids, flavonoids, sterols, phenolic compounds, tannins.

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Table 1. Preliminary phytochemical screening of Methanolic extract of *Withania somnifera*

Test	Methanolic extract of *Withania somnifera*
Alkaloids	
Mayer's test	+
Wagner's test	+
Hager's test	+
Dragendorff's test	+
Carbohydrates	+
Tannins	+
Flavonoids	++
Steroids & Terpenoids	+
Glycosides	_
Saponins	+
Phenols	+

+ Indicates presence; -- Indicates absence

The results obtained with evaluation of diuretic activity of Methanolic Extract of whole plant of *Withania somnifera* was shown in (Table 2 and 3; Figure 1 and 2). From the results it can be observed that MEWS has shown a significant diuretic activity by increasing urinary output and increased excretion of sodium, potassium, chloride when compared to control. The effect of MEWS was found to be dose dependent, i.e., among the three doses studied, higher dose produced more effect. A comparison was made with the standard diuretic drug HCTZ, the diuretic effect observed after treatment with MEWS was found to be significant in terms of urinary output, sodium, potassium, chloride concentrations. Determination of urinary electrolyte concentration revealed that MEWS was effective in increasing urinary electrolyte concentrations for all the three ions tested (Na^+, K^+, Cl^-).

Table 2. Effect of methanolic extract of *Withania somnifera* (MEWS) on urine volume in hydrated rat model in albino rats

S. No.	Groups	Total Urine Volume (ml/kg/24 hr)
1	Control (10 ml/Kg b. wt)	10.10 ± 1.40
2	Standard (HCTZ 10 mg/kg b.wt)	41.50 ± 1.30**
3	MEWS (100 mg/kg b.wt)	20.63± 0.92*
4	MEWS (200 mg/kg b.wt)	25.48±1.45**
5	MEWS (400 mg/kg b.wt)	34.69±1.82**

Values expressed as mean ± S.E.M., n=6, Significance at p<0.05*, p<0.01**, Compared with control group (One Way ANOVA followed by Dunnetts 't' test).
MEWS: Methanolic extract of *Withania somnifera*

Table 3. Effect of Methanolic extract of *Withania Somnifera* (MEWS) on electrolyte concentration in hydrated rat model in albino rats

S. No	Groups	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
1	Control (10 ml/Kg b. wt)	105.20 ± 1.50	45.89±2.30	75.68±1.46
2	Standard (HCTZ 10 mg/kg b.wt)	172.46± 2.74**	73.30±1.80**	115.90±1.31**
3	MEWS (100 mg/kg b.wt)	119.63±2.50*	56.50±1.29*	85.65±1.68*
4	MEWS (200 mg/kg b.wt)	143.37±2.17**	61.28±1.64**	93.71±1.83**
5	MEWS (400 mg/kg b.wt)	166.19±2.58**	70.70±1.95**	109.67±1.76**

Values expressed as mean ± S.E.M., n=6, Significance at p<0.05*, p<0.01**, Compared with control group (One Way ANOVA followed by Dunnetts 't' test);
MEWS: Methanolic extract of *Withania somnifera*

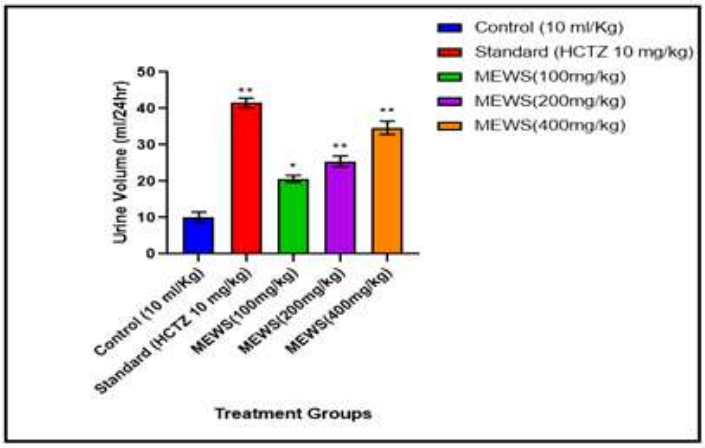

Figure 1. Effect of methanolic extract of *Withania somnifera* on urine volume in albino rats. Values expressed as mean ± S.E.M., n=6, Significance at p<0.05*; p<0.01**; Compared with control group (One Way ANOVA followed by Dunnetts 't' test). MEWS: Methanolic extract of *Withania Somnifera*

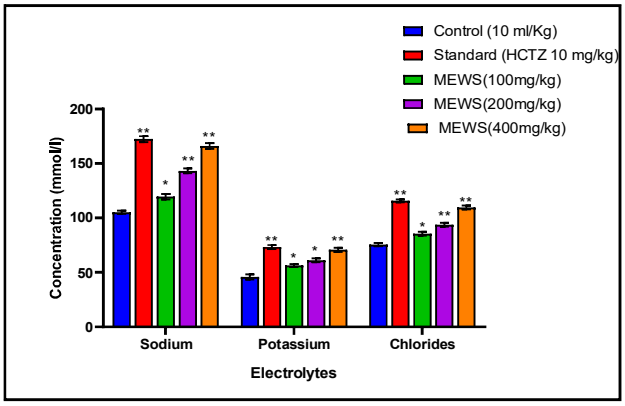

Figure 2. Effect of methanolic extract of *Withania somnifera* on Urinary Sodium, Potassium, Chloride (mmol/L) Ions concentration in albino rats. Values expressed as mean ± S.E.M., n=6, Significance at p<0.05*; p<0.01**; Compared with control group (One Way ANOVA followed by Dunnetts 't' test). MEWS: Methanolic extract of *Withania somnifera*

Discussion

The present study revealed that Methanolic extract of whole plant of *Withania somnifera* significantly increased the urinary out put, as well as the elimination of urinary electrolytes in a dose dependant manner.

The phytochemical studies reveals that the Methanolic Extract of whole plant of *Withania somnifera* contains flavanoids, alkaloids, carbohydrates, sterols, phenolic compounds, tannins, resins (Khandelwal KR, 2000). When tested for diuretic activity, the phytoconstituents increased urine excretion in the rats. Increase in the urinary volume was also accompanied by an increase in the Na+, K+ excretion similar to the standard diuretic hydrochlorthiazide, suggesting that phytoconstituents induced diuresis is caused by its saluretic effect. Earlier studies reported phytochemical substances like flavonoids, saponins, steroids, carbohydrates, tannins, phenolic compounds, terpenoids (Ancy et al., 2013), alkaloids (Patel et al., 2011), glycosides (Kumarasamyraja et al., 2011), sterols (Kumar et al., 2012) in different plant extracts. Methanolic Extract of whole plant of *Withania somnifera* was identified with most of these plant phytochemical substances mentioned above. Hence it can be reported that the observed diuretic activity is due to these above phytoconstituents.

Conclusion

In congruence with the results, it was initially consider that the presence of phytoconstituents such as flavonoids, alkaloids, tannins, saponins could be responsible for the diuretic activity. The results of the diuretic activities were found to be very significant. so the studies proves that methanolic extract of *withania somnifera* exhibited a significant diuretic activity.

It can be concluded that methanolic extract of *withania somnifera* possesses diuretic activity and may be due to presence of flavonoids, alkaloids, tannins, saponins present in it, which seems to support the use of this plant in traditional medicine.

Conflict of interest

The authors declare that they have no conflict of interest.

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