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ANTIDIABETIC EFFECT OF *WITHANIA COAGULANS* IN EXPERIMENTAL RATS

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ABSTRACT

The present study defines the systematic evaluation and the role of minerals in glycemic potential of aqueous extract of Withania coagulans fruits in order to develop an effective and safe alternative treatment for diabetes mellitus. Laser Induced Breakdown Spectroscopy was used for glycemic element detection. The study is based on the results of lowering in blood glucose levels of normal, sub, mild and severely diabetic rats assessed during fasting blood glucose, glucose tolerance test and post prandial glucose studies. The dose of 1000mg/kg was identified as the most effective dose, which reduces the Fasting Blood Glucose level maximum by 33.2% at 4h in normal rats during fasting blood glucose studies. Glucose tolerance test studies of normal, sub and mild diabetic rats showed the maximum reduction of 15.7, 28.9 and 37.8% at 3h respectively. Long-term study in case of severely diabetic rats showed reduction of 52.9 and 54.1% in Fasting Blood Glucose and Post Prandial Glucose levels respectively after 30 days of treatment. The present study, besides confirming hypoglycemic and antidiabetic activities of aqueous extract of W. coagulans, helps in identifying the role of trace minerals like Mg & Ca responsible for antidiabetic potential of this potent indigenous shrub.

KEY WORDS

Antidiabetic, Diabetes, Hypoglycemic, Indian Cheese maker, Withania coagulans.

INTRODUCTION

In the natural system of medicine many plants have been claimed to be useful for the treatment of diabetes mellitus (1). There are two species of *Withania*, a small genus of shrubs distributed in east of the Mediterranean region extending to South Asia are found in India (2). *Withania coagulans* Dunal (family: Solanaceae) commonly known as Indian cheese maker, was selected for the present study. It is a rigid, gray under shrub, 60-120cm high occurring in drier parts of India. In Northern India traditional healers use dry fruits of *W. coagulans* for the treatment of diabetic patients though its antihyperglycemic activity has not been evaluated systematically. It is well known for its ethnopharmacological applications (3). The fruits possess milk-coagulating property due to the presence of an enzyme, Withanin. A steroidal lactone, withanolide isolated from the aqueous extract of fruits of *W. coagulans*, has cardiovascular effect. Alcoholic extract of *W. coagulans* has shown antibacterial and antihelmintic activities (4).

The hot aqueous extract of *W. coagulans* fruits has been shown to exert hepatoprotective, antiinflammatory and antidiabetic effects (5,6). The same hot aqueous extract of *W. coagulans* fruits has also increased the glucose utilization in isolated rat hemidiaphragm cells (7). These observations prompted us to evaluate systematically glycemic potential of aqueous extract of *W. coagulans* prepared at room temperature (r.t.) keeping in mind that hot extraction may reduce its activity. Hence, the present study deals with the assessment of hypoglycemic and antidiabetic activities of aqueous r.t. extract of *W. coagulans* in normal as well as streptozotocin (STZ) induced sub, mild and severely diabetic rats, along with its glycemic mineral identification responsible for its glycemic potential. The study gives a complete scientific view of antidiabetic potential of *W. coagulans* extract and the data eventually contributes to evidence based evaluation of

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this traditional medicine with special reference to its glycemic activities.

MATERIALS AND METHODS

Plant material: Dried fruits of *Withania coagulans* Dunal (family: Solanaceae) were purchased from the local market of Allahabad, India and identified by Dr S.L. Bondya, Taxonomist, Botanical Survey of India, Allahabad, India. A voucher specimen no. (AC–422/07) has been submitted. The whole fruits (5kg) were mechanically crushed and extracted with distilled water at room temperature up to 48h. The extract was filtered and concentrated in rotatory evaporator under reduced pressure to obtain semisolid material, which was then lyophilized to get a powder (yield: 13.8% w/w). The lyophilized powder was dissolved in distilled water (DW) and used for experimental work.

Experimental animals: Male albino Wistar rats of same age group and body weight 150-200g were selected for all the experiments. Rats obtained from National Institute of Communicable Disease (NICD) New Delhi, India, were housed in polypropylene cages at an ambient temperature of 25-30º C and 45-55% relative humidity with a 12h each of dark and light cycle. Rats were fed pellet diet (Golden feed, New Delhi India) and water *ad libitum*. The Institutional Ethical Committee has approved the study.

Induction of diabetes: Diabetes was induced by a single intraperitonial injection of freshly prepared STZ (55mg/kg bw) in 0.1m citrate buffer (pH 4.5) to a group of overnight fasted rats. After 3 days of STZ administration, depending upon their blood glucose levels the rats were divided into three groups: (I) Sub diabetic rats with Fasting Blood Glucose (FBG) 80-120mg/dl and Post Prandial Glucose (PPG) more than 210mg/dl; (II) Mild diabetic rats with FBG 120-250mg/dl and PPG more than 350mg/dl and (III) Severely diabetic rats with FBG 250mg/dl or more and PPG more than 350mg/dl.

Estimation : Blood glucose level (BGL) was estimated by Glucose Oxidase method (8) using standard kit of Bayer Diagnostics India Limited. Urine Sugar was detected by reagent based Uristix from Bayer Diagnostics.

Experimental Design: Initial screening of the aqueous extract for identifying its most effective dose was done with a range of variable doses in normal as well as sub and mild diabetic rats by conducting FBG and Glucose Tolerance Test **(**GTT) studies. The antidiabetic effect of the extract was also assessed in Severely Diabetic (SD) models by treating them with the identified most effective dose daily up to 30 days.

FBG based study of normal rats: Five groups of six rats each fasted overnight were used in the experiment. Group I served as untreated control received vehicle (distilled water only). Rats of group II, III, IV and V received variable doses of 500, 750, 1000 and 1250mg/kg respectively, of aqueous fruit extract suspended in distilled water. FBG was taken initially and then blood samples were collected from tail vein at 2, 4 and 6h after giving the extract.

GTT based study of normal rats: The aqueous extract was given orally to a different group of normal rats which was divided and treated on the same pattern as above. Effect on FBG was assessed at 90 minutes and this BGL value was considered as '0' h value for GTT. The rats were then orally administrated with 2g/kg of glucose and their glucose tolerance was studied up to 3h at regular intervals of 1h each.

GTT based study of sub diabetic rats: Overnight fasted rats were divided into six groups of six rats each. Group I served as control, received vehicle (distilled water only), whereas variable doses of 500, 750, 1000 and 1250mg/kg of extract were given orally to groups II, III, IV and V respectively. Group VI received a dose of 2.5mg/kg of a known antidiabetic drug Glipizide as reference drug. FBG was checked initially and then after 90 minutes of treatment BGL was taken, considered as '0' h value. Glucose was given then orally at the dose 2g/kg to all the groups. BGL was further checked up to three hours at regular intervals of 1h each, considered as 1, 2 and 3h values.

GTT based study of mild diabetic rats: The rats fasted over night were divided into six groups of six rats each. Group I to VI received the same treatment as mentioned for the subdiabetic rats. The values of FBG and BGL were also assessed at the same interval of times as in case of subdiabetic models.

FBG and PPG based long-term study of severely diabetic rats : Three groups of six rats each were used in the experiment. Group I served as SD control received vehicle (distilled water only), group III served as positive control received glipizide at a dose of 2.5mg/kg as a reference drug and group II received extract at a dose of 1000mg/kg. All the groups were treated once a day up to 30 days. Blood and urine samples were collected at the beginning and then weekly up to 30 days and levels of FBG, PPG and urine sugar were assessed. Body weight was taken weekly.

Median LD_{50} experiment : Two groups of rats of both sex (6 animals per group, 3 females and 3 males), weighing about 150–200g were orally administered with a dose of ten times the effective dose of aqueous extract of *W. coagulans.* The rats were then observed for gross behavioral, neurologic, autonomic and toxic effects continuously. Food consumption, faeces and urine were also examined at 2h and then at 6h intervals for 24h.

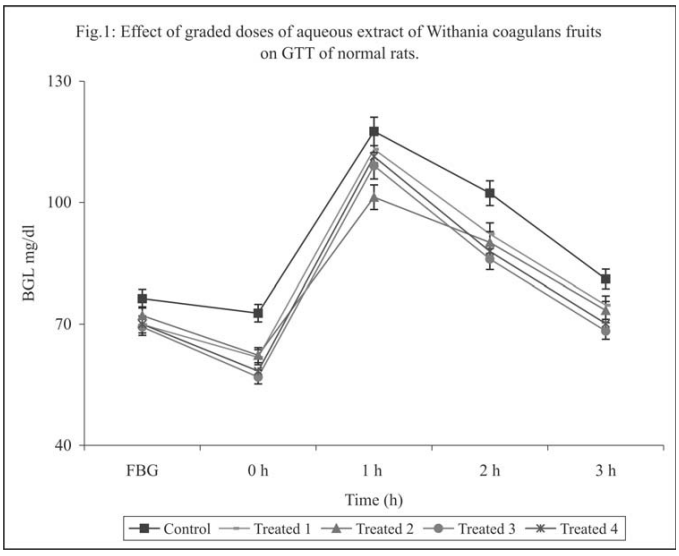
Statistical analysis: Data was expressed as mean±SD. Two-way analysis of variance (ANOVA) was performed using Graph Pad Prism 4.00 for Windows (Graph Pad Software, San Diego CA, USA). Differences were considered to be significant when $p<0.05$.

RESULTS

Analysis of minerals: Since, according to the Boltzmann Distribution Law, intensity is directly related to its concentration (9) therefore, the high concentration of Mg and Ca with respect to other minerals present in the extract can minor minerals present in the extract can define their role in diabetes. Moreover, the fact that higher concentration of Mg is responsible for diabetes management had already been established (10).

Effect of aqueous extract on FBG of normal rats: Table 1 describes the hypoglycemic effect of a single oral administration of variable doses of 500, 750, 1000 and 1250mg/kg of aqueous extract of *W. coagulans* fruits on FBG of normal rats. Rats treated with 1000mg/kg showed a maximum fall of 33.2% in FBG after 4h of oral administration, whereas fall of 23.5, 29.6 and 32.8% was observed with the doses of 500, 750 and 1250mg/kg respectively.

Effect of aqueous extract on GTT of normal rats: Figure 1 depicts the hypoglycemic effect of a single oral administration of doses of 500, 750, 1000 and 1250mg/kg of GTT of normal rats. The dose of 1000mg/kg produced a maximum fall of 15.7% in normal rats after 3h of glucose administration, whereas fall of 7.7, 10.8 and 13.5% was observed with the doses of 500, 750 and 1250mg/kg, respectively.



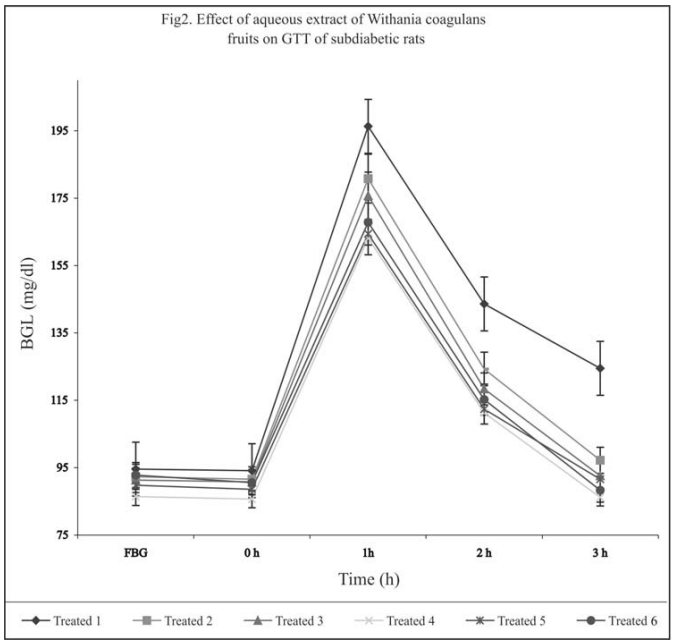
Control- DW, Treated 1-500 mg/kg, Treated 2 - 750 mg/kg ($P<0.01$), Treated 3 -1000 mg/kg, Treated 4 - 1250 mg/kg

Effect of aqueous extract on GTT of sub diabetic rats: Figure 2 demonstrates the antidiabetic effect of a single oral administration of variable doses of aqueous extract of *W. coagulans* fruits on BGL of sub diabetic rats during GTT studies. The fall of 21.9, 23.8, 28.9 and 27.6% was observed after 3 h of glucose administration with the doses of 500, 750, 1000 and 1250mg/kg respectively. Hence the dose of 1000mg/kg associated with maximum fall was identified as the most effective dose. Moreover glipizide, the reference drug, produced a fall of 29.1% at 3h.

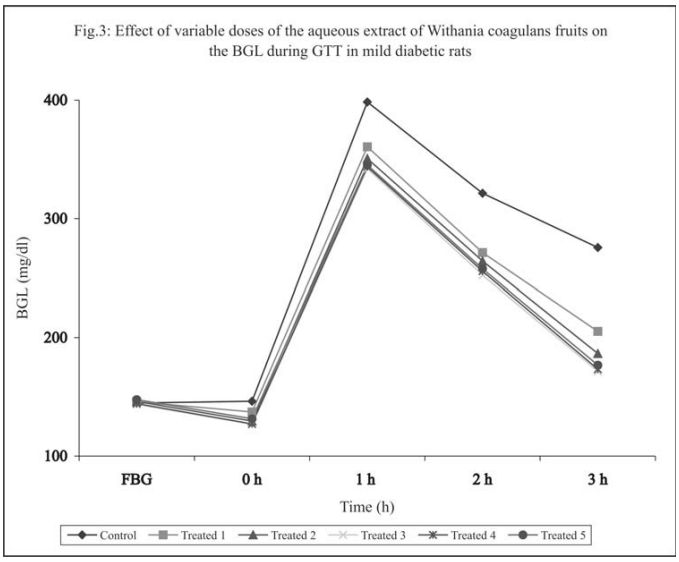
Table 1: Effect of graded doses of aqueous extract of *Withania coagulans* fruits on FBG of normal rats (mean ± SD)

		Blood glucose levels (mg/dl)			
	Pretreatment		Post treatment (h)		
Experimental Groups	Treatment (mg/kg bw)	FBG	2	4	6
Control	DW	73.2 ±4.8	72.6 ± 4.2	72.1 ± 3.6	72.9 ± 3.9
Fruit extract	500	74.7 ± 4.1	64.2 ± 2.7*	57.2 ± 5.1*	68.7 ± 4.3
Fruit extract	750	71.0 ± 3.6	59.4 ± 3.9*	49.9 ± 3.8*	64.4 ± 3.6*
Fruit extract	1000	78.7 ± 3.2	64.2 ± 4.3*	52.6 ± 2.8*	69.3 ± 4.9*
Fruit extract	1250	77.6 ± 3.8	63.1 ± 4.2*	52.1 ± 4.8 *	69.8 ± 3.7

* $P < 0.05$ as compared with control



Control- Distilled water, Treated 1-500 mg/kg, Treated 2 - 750 mg/kg (P<0.01), Treated 3 -1000 mg/kg, Treated 4 - 1250 mg/kg, Treated 5- Glipizide (2.5 mg/kg)



Treated 1-500 mg/kg, Treated 2 - 750 mg/kg (P<0.01), Treated 3 -1000 mg/kg, Treated 4 - 1250 mg/kg, Treated 5 – Glipizide (2.5 mg/kg)

Effect of aqueous extract on GTT of mild diabetic rats: Figure 3 depicts the effect of variable doses of 500, 750, 1000 and 1250mg/kg of aqueous extract of *W. coagulans* fruits on glucose tolerance of mild diabetic rats. The fall observed after 3 h of glucose administration was 25.5, 32.4, 37.8 and 37.2% with the doses of 500, 750, 1000 and 1250mg/kg respectively and the dose of 1000mg/kg was further confirmed as the most effective dose for assessing the antidiabetic potential of aqueous extract in SD models. The reference drug glipizide

Table 2: Effect of the most effective dose of aqueous extract of *Withania coagulans fruits* on FBG, PPG, urine sugar and body weight of Severely Diabetic rats (mean ± SD)

Group	0 Day	7 Day	15 Day	22Day	30 Day
FBG (mg/dl)					
Group 1	330.06±4.5	336.64±4.1	340.88±5.3	348.90±6.3	352.45±4.8
Group 2	335.70±5.3	272.51±6.3	235.81±6.6*	198.6±5.9*	170.4±6.7*
Group 3	340.86±6.4	282.35±5.8*	235.85±5.3*	185.78±6.8**	160.49±7.3**
PPG (mg/dl)					
Group 1	445.94±5.1	469.49±6.3	470.62±4.9	482.48±3.2	492.19±6.4
Group 2	432.61±7.3	374.58±5.9	312.43±6.4	265.56±6.9*	215.42±7.2**
Group 3	428.75±6.3	388.22±7.2	308.92±6.4*	258.35±5.9**	196.70±7.4**
Body Weight (mg/dl)					
Group 1	140.5±6.4	140.1±5.7	137.3±6.2	134.5±7.3	128.7±4.9
Group 2	154.5±3.2	156.8±4.3	164.2±6.4	167.8±5.3	174.5±7.2
Group 3	156.8±4.2	160.3±4.3	166.4±7.1*	172.5±5.8**	181.8±4.9**
Urine Sugar (u/dl)					
Group 1	+4	+4	+4	+4	+4
Group 2	+4	+4	+3	+3	+2*
Group 3	+4	+3	+2	+1*	Nil*

* P < 0.05 as compared with control; * * P < 0.01 as compared with control

Group 1: SD control; Group 2: SD glipizide treated (2.5 mg/kg); Group 3: SD extract treated (1000 mg/kg)

produced a fall of 35.9% at 3h. These results help in depicting the dose of 1000mg/kg as the most effective dose for the study of SD models.

Effect of aqueous extract on FBG, PPG, urine sugar and body weight of SD rats: Table 2 mentions the impact of the most effective dose of the extract on FBG, PPG, urine sugar and body weight of SD rats. The dose of 1000mg/kg reduced the FBG levels by 17.2, 30.8, 45.5 and 52.9% on 7^{th}, 15^{th}, 22^{nd} and 30^{th} day of treatment respectively. The regular fall of 9.5, 28.0, 39.7 and 54.1% was noticed in PPG levels, on the same days. Glipizide reduced the FBG levels by 18.8, 29.8, 40.8 and 49.2% on 7^{th}, 15^{th}, 22^{nd} and 30^{th} day of treatment respectively. The regular fall of 13.4, 27.8, 38.6 and 50.2% was noticed in PPG levels on the same days. In case of urine sugar, significant decrease from + 4 to Nil was observed on 30^{th} day of extract treatment. Whereas in case of glipizide treatment it decreased from +4 to +2 only. Moreover, there was a significant increase in body weight of about 25g after 30 days of extract treatment of rats of group II. However increase in weight of glipizide treated rats only upto 20g. Enhanced levels of FBG and PPG along with body weight loss were observed in case of severely diabetic control whereas there was no change in urine sugar levels.

LD_{50} : The behavior of the treated rats appeared normal. No toxic effect was reported up to 10 times of the effective dose of the aqueous extract, as there was no death in any of these groups.

DISCUSSION

In Ayurvedic and indigenous folk medicine system, the hypoglycemic plants have been used mostly in their natural forms, consisting of both inorganic and organic constituents of the concerned herbs. It is important to note that the inorganic part of medicinal plants containing mainly mineral, plays a contributory role in enhancing hypoglycemic activity (10-11) and their indirect role in diabetes management is increasingly recognized (12).

The identified hypoglycemic and antidiabetic potential of fruits of *W. coagulans* may to be due to the significant presence of Mg (13) and Ca (14) in the extract. Since, Boltzmann distribution law states that intensity is proportional to the concentration. The concentration of Mg and Ca was found much higher than the other inorganic minerals. It has been already reported that the higher concentration of Mg and lower concentration of K plays a vital role in diabetes management (15,16). Hence the significant antidiabetic potential of *W. coagulans* could be due to the high concentration of Mg along with Ca. As Ca^{2+} ion activates insulin gene expression via CREB (Calcium Responsive Element Binding protein) responsible for exocytosis of stored insulin (17)

The fruits of *W. coagulans* are used in Ayurvedic and Unani systems of medicine. The hot aqueous extract of *W. coagulans* has been reported to possess hypoglycemic effect in normal and severely diabetic animals after seven days of treatment. So the present study was carried out to evaluate the glycemic effect of extract, prepared at room temperature in various diabetic modes. It is expected that the extract prepared at room temperature (r.t.) will retain its activity more than that of hot extract. The present study is different from the existing one as it dealt with the different diabetic models and different experimental assays.

The study deals with the assessment of hypoglycemic and antidiabetic effect of variable doses of r.t. extract in normal, sub and mild diabetic rats based on FBG and GTT studies. The maximum reduction was observed with the dose of 1000mg/kg, identified as the most effective dose. Antidiabetic effect was further confirmed by long term study of thirty days of severely diabetic rats based on FBG and PPG studies. The long term study was carried out only with the maximum effective dose. The controlled metabolic disturbances after thirty days of extract administration were shown by near normalization of FBG and reduction in PPG levels. The reduction in urine sugar and gain in body weight are additional evidences of its antidiabetic potential.

STZ- induced diabetes is characterized by severe loss in body weight of untreated rats. The characteristic loss of body weight is due to increased muscle wasting in diabetes (18). When aqueous extract of fruits was administered to diabetic rats, the weight gain seems to be as a result of its ability to reduce hyperglycemia in a short period of one month. Since *in vitro* study on isolated rat hemidiaphragm indicated that hot aqueous extract of *W. coagulans* fruits reduces BGL by increasing the peripheral glucose utilization (7). Therefore the present *in vivo* study also suggests that the aqueous r.t. extract of *W. coagulans* might be reducing the raised blood glucose level in the same mechanism.

The phytochemical studies reported earlier indicate that the alkaloids and steroids isolated from other plant sources are responsible for their hypoglycemic activity (19). However, a number of steroidal lactones have also been reported from whole plant extract of *W. coagulans* (20-21). Aqueous extract of its fruits has yielded 2 compounds 3β, 14α, 17 β, 20 α- tetra

hydroxyl-1-oxo-20S, 22 R-with a-5,24-dienolide and ergosa-5,25-diene-3 β, 24 ε- diol (22). From ethyl acetate extract of its fruits a steroidal lactone (withanolide) and sitosterol β-D glucosides were isolated (23). Steroidal lactones isolated from *Momordica charantia* fruits are responsible for its hypoglycemic effect (24).

Thus, the significant antidiabetic activity of W. coagulans observed in the present study may be attributed to the presence of steroidal lactones in addition to glycemic minerals. Since the extract is effective against STZ induced SD rats therefore it suggests that it will be helpful for type II diabetic patients in controlling their BGL. Further investigations are underway to elucidate its impact on hyperlipidemia caused by diabetes and mechanism of action responsible for its antidiabetic effect.

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