The Aqueous Extract of *Withania coagulans* Fruit Partially Reverses Nicotinamide/Streptozotocin-Induced Diabetes Mellitus in Rats

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ABSTRACT *Withania coagulans* fruit has been shown to possess antihyperglycemic properties and is used in the traditional Indian system of medicine. However, there has no systematic study of its mechanism of action. In a rat model diabetes mellitus (DM) was induced by intraperitoneal injection of nicotinamide (230 mg/kg of body weight) followed by streptozotocin at 55 mg/kg of body weight. After 96 h, mildly diabetic (MD) (fasting plasma glucose [FPG] = 7–11.1 mmol/L) and severely diabetic (SD) (FPG > 11.1 mmol/L) rats were treated with aqueous extract of *W. coagulans* fruit at doses of 125, 250, and 500 mg/kg of body weight/day orally. FPG, postprandial plasma glucose (PPPG), glycosylated hemoglobin (HbA_{1c}), plasma insulin, tissue glycogen, and glucose-metabolizing enzymes were assayed at Day 30. Treatment of diabetic animals (MD and SD) with different doses of aqueous *W. coagulans* resulted in significantly decreased FPG, PPPG, and HbA_{1c} ($P < .01$), whereas serum insulin increased significantly compared with that in diabetic-untreated rats ($P < .01$). MD and SD animals treated with aqueous *W. coagulans* also showed significant increases in liver and muscle glycogen compared with diabetic-untreated animals ($P < .01$). Moreover, activities of glucokinase and phosphofructokinase were also significantly increased ($P < .01$), whereas glucose-6-phosphatase activity was significantly decreased ($P < .01$) in MD and SD groups treated with aqueous *W. coagulans* compared with diabetic-untreated groups. The most effective dose of aqueous *W. coagulans* was 250 mg/kg of body weight. These results show that the aqueous extract of *W. coagulans* fruit has significant antihyperglycemic effects, which may be through the modulation of insulin levels and related enzyme activities.

KEY WORDS: • antihyperglycemic effect • diabetes mellitus • glucose-metabolizing enzymes • *Withania coagulans*

INTRODUCTION

DIABETES MELLITUS (DM) is a chronic metabolic disorder caused by insulin deficiency or insulin resistance leading to disturbances in carbohydrate, protein, and lipid metabolism. Major chronic complications associated with DM include macrovascular complications like cardiovascular disease and stroke and microvascular complications such as retinopathy, microangiopathy, and nephropathy.¹ The worldwide prevalence of diabetes among adults (20–79 years old) was about 285 million in 2010 and is predicted to increase to 439 million by 2030.² China has the highest number of diabetics (92.4 million), and India with 50.8 million diabetics stands at number 2.^{2,3} Many pharmaceutical preparations such as sulfonylureas, metformin, α -glycosidase inhibitors, biguanides, etc., are used for treating DM.^{4,5} Plants have a long therapeutic history in the traditional healthcare system, and many herbal drugs are used for the treatment of DM. Plant extracts are thought to act on a variety of targets to exert their beneficial effects.^{6,7}

Different parts of *Withania coagulans* (Family Solanaceae) have been reported to possess a variety of biological activities.^{8,9} Some steroid-like compounds (withanolides) isolated from the roots and other parts of this plant have been shown to possess hormone-like activity.¹⁰ Withanolides isolated from aqueous extract of fruit of *W. coagulans* have cardioprotective, hepatoprotective, and anti-inflammatory activity.^{11,12} A hot aqueous extract of *W. coagulans* at a dose of 1 g/kg has been shown to lower blood glucose in streptozotocin (STZ)-induced DM in rats.¹³ Maurya *et al.*¹⁴ isolated a coagulanolide from *W. coagulans* fruits that has been shown to possess antihyperglycemic activity in experimental DM. Jaiswal *et al.*^{15,16} reported the antidiabetic effect of aqueous and ethanolic extracts of *W. coagulans* at an effective dose of 750 mg/kg of body weight/day in STZ-induced diabetic rats. Recently, Hoda *et al.*¹⁷ reported the antihyperglycemic and antihyperlipidemic effects of aqueous and chloroform extracts of *W. coagulans*, given orally at a dose of 1 g/kg for 14 days in experimental DM in rats. However, in all these reports, the doses of aqueous *W. coagulans* used are very high (i.e., 750–1000 mg/kg of body weight/day, which roughly corresponds to about 200 mg/day per rat and approximately 10,000 mg/day per human, which is very high in terms of physiological and nutritional ranges). Therefore, it is important to study

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the beneficial effects of lower doses of aqueous *W. coagulans* on glycemic control, which are easier to administer and should be relatively free of side effects. Moreover, none of the previous studies has attempted to elucidate the mechanism of action of aqueous *W. coagulans* in nicotinamide/STZ-induced DM, which is considered similar to type 2 DM.^{18,19} Therefore, the present study has been carried out with low doses of aqueous *W. coagulans* (125–500 mg/kg of body weight) prepared in cold water in nicotinamide/STZ-induced DM to explore its glucose-lowering effect and study its effect on other parameters of glucose homeostasis.

MATERIALS AND METHODS

Plant material and preparation of aqueous extract of W. coagulans

Fruit of *W. coagulans* was purchased from a local market in Delhi, India, and was identified and authenticated (voucher number NISCAIR/RHMD/Consult/-2008-09/979/10) by the National Institute of Science Communication and Information Resources, Pusa, New Delhi, India. Whole fruits of *W. coagulans* were used for the preparation of the extract. The fruits, after removal of calyx and pedicle, were soaked in distilled water and kept overnight. The next day, the extract was filtered through a filter paper/sterile muslin cloth to obtain the water extract. Freshly prepared extract was lyophilized to obtain a dry powder (yield 16% wt/wt). This sticky powder was dissolved in water and fed orally to animals by intragastric tube at different doses.

Phytochemical screening

Qualitative screening of phytochemicals in aqueous *W. coagulans* extract was performed by the standard methods of Harbone²⁰ and Ayoola *et al.*²¹

Experimental animals

Male albino Wistar rats weighing 150 g were obtained from the Central Animal House, University College of Medical Sciences, Delhi. Animals were housed in an air-conditioned room at $25 \pm 1^\circ\text{C}$, relative humidity of $50 \pm 10\%$, and 10-h light/14-h dark cycle throughout the duration of experiment. They were fed standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Induction of diabetes

Overnight fasted animals were made diabetic by intraperitoneal injection of nicotinamide (230 mg/kg of body weight) followed by freshly prepared STZ in citrate buffer (0.1 M, pH 4.5) at a dose of 55 mg/kg of body weight after 15 min.^{18,19} After 96 h of injections, when glucose levels are usually stabilized, plasma glucose was estimated. Animals having fasting plasma glucose (FPG) ≥ 7.0 mmol/L were considered as diabetic and were further categorized into two groups: mildly diabetic (MD), having FPG of 7.0–11.1 mmol/L, and severely diabetic (SD), having FPG of > 11.1 mmol/L. Forty-five percent of rats developed MD,

and 35% developed SD; no mortality has been observed in this model.^{18,19,22}

Treatment regimens

The rats were divided into 11 groups ($n=6$). Control groups were as follows: Group I, healthy control; and Group II, (a) MD and (b) SD. The treated groups were as follows: Group III, (a) MD + aqueous *W. coagulans* (125 mg/kg of body weight) and (b) SD + aqueous *W. coagulans* (125 mg/kg of body weight); Group IV, (a) MD + aqueous *W. coagulans* (250 mg/kg of body weight) and (b) SD + aqueous *W. coagulans* (250 mg/kg of body weight); Group V, (a) MD + aqueous *W. coagulans* (500 mg/kg of body weight) and (b) SD + aqueous *W. coagulans* (500 mg/kg of body weight); and Group VI, (a) MD + glibenclamide (0.5 mg/kg of body weight) and (b) SD + glibenclamide (0.5 mg/kg of body weight). Group VI served as the reference group and received a standard antidiabetic drug (glibenclamide, at a dose of 0.5 mg/kg of body weight orally).¹⁶

Collection of blood and tissues

Whole blood was collected by retro-orbital venipuncture with the help of a heparinized capillary. Blood for estimation of plasma glucose was taken in vials containing sodium fluoride and potassium oxalate and in EDTA vials for the estimation of glycosylated hemoglobin (HbA_{1c}), insulin, and enzymes. After fasting samples were collected, rats were given glucose (2 g/kg) orally using a feeding tube, and blood samples were collected after 2 h for measuring postprandial plasma glucose (PPPG).²³ Blood was centrifuged at 1300 g for 10 min to obtain plasma. FPG, PPPG, and other parameters were determined after 30 days of aqueous *W. coagulans* administration. After 30 days of treatment, animals were anesthetized by a single intraperitoneal injection of pentobarbitone at a dose of 150 mg/kg of body weight. Tissues (liver and muscle) were removed, washed with cold saline, and stored at -70°C for assays of tissue constituents and enzymes.

Estimation of biochemical parameters

FPG and PPPG were estimated by the glucose oxidase/peroxidase method using kits from Accurex (Mumbai). Insulin was assayed in plasma using enzyme-linked immunosorbent assay kits (DRG, Marburg, Germany). HbA_{1c} in blood was estimated by the ion-exchange resin method,²⁴ which is a reliable method for HbA_{1c} estimation in rats.²² Whole blood was mixed with lysing reagent containing detergent and borate, and the hemolysate was prepared and mixed with cation exchange resin. All hemoglobins were retained by the resin, and HbA_{1c} was eluted. The percentage of HbA_{1c} was determined by measuring the ratio of absorbance of the HbA_{1c} fraction and the total hemoglobin fraction at 415 nm, and results were compared with that for a standard HbA_{1c} preparation that was carried out through the test. Tissue glycogen was extracted and precipitated with ethanol,²⁵ and its content was estimated by using sulfuric acid.²⁶

Tissue was homogenized with 50% KOH followed by addition of ethanol, kept in a boiling water bath for 1 h, and left overnight at room temperature for precipitation. After centrifugation the precipitate was washed with ethanol, treated with anthrone (in 72% H₂SO₄), and kept in a boiling water bath for 15 min. Absorbance was read at 620 nm. Glucose was used as the standard, and values obtained were multiplied by 0.9 for conversion of glucose to glycogen.

Glucokinase (GK) activity was measured by the method of Porter and Chassey.²⁷ Tissue homogenate was added to assay mixture containing 1 M Tris buffer, 0.1 M MgCl₂, 0.5 M glucose, 0.01 M NADP⁺, distilled water, glucose 6-phosphate dehydrogenase, and 0.1 M ATP. Change in absorbance (ΔA) was read at 340 nm for 14 min. GK activity has been calculated by the expression ($\Delta A \times \text{total volume} \times \text{dilution factor} / (\text{sample volume} \times 6.22 \times \text{time})$). The phosphofructokinase (PFK) assay is based on the method of Racker.²⁸ Tissue homogenate was mixed with assay mixture containing 100 mM Tris-HCl (pH 8.0), 6 mM MgCl₂, 0.5 mM ATP, and 0.25 mM NADH followed by addition of enzyme solution. The absorbance was read at 340 nm for 10 min. One unit of PFK is the amount of enzyme required to convert 1 μ mol of fructose 6-phosphate to fructose 1,6-diphosphate/min. Glucose 6-phosphatase (G-6-Pase) was estimated by the method of Harper.²⁹ Tissue homogenate was incubated with glucose-6-phosphate at 37°C. After 15 min of incubation, trichloroacetic acid (10% wt/vol) solution was added, and the mixture was centrifuged to get a clear supernatant. Ammonium molybdate (2×10^{-3} M) and reducing agent (4.2×10^{-2} M) were added to the supernatant. Absorbance was read at 680 nm after 20 min. Liberated phosphate (in micromoles) in the enzymatic reaction was calculated by the expression $([\text{optical density of test mixture} - \text{optical density of control}] / \text{optical density of the standard}) \times \text{micromoles of phosphate in the standard} \times 2.2$, and units have been calculated as nano-Katals per milligram of protein.

Ethical clearance

The protocol was approved by the Institutional Ethics Committee for Animal Research, University College of Medical Sciences. Animal experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care.

RESULTS

Phytochemical screening

Phytochemical screening of aqueous *W. coagulans* showed the presence of several bioactive components in the extract (i.e., carbohydrates, glycosides, steroidal compounds, saponins, phenols, tannins, alkaloids, terpenoids, and flavanoids) (Table 1).

Effect of aqueous *W. coagulans* on plasma glucose levels in diabetic animals

As shown in Table 2, MD and SD animals showed significant ($P < .01$) increases in FPG compared with

TABLE 1. SCREENING OF PHYTOCONSTITUENTS IN AQUEOUS *W. COAGULANS*

Screen number	Test	Method	Presence*
1	Carbohydrates (reducing ketohexose)	Barfoed's/Benedict's/Seliwanoff's test	+
2	Glycosides	Borntrager/Killer-Killiani test	+
3	Steroidal compounds	Salkowski's test	+
4	Saponins	Froth test	+
5	Phenolic compounds	Ferric chloride test	+
6	Tannins	Ferric chloride/formaldehyde test	+
7	Triterpenoids	Salkowski's/Libermann's test	+
8	Alkaloids	Dragendroff's/Mayer's reagent test	+
9	Flavanoids	Acid alcohol/lead acetate/ethyl acetate	+

*Present (+) or absent in aqueous *W. coagulans*.

healthy animals. MD rats treated with three different doses of aqueous *W. coagulans* (125, 250, and 500 mg/kg of body weight) showed significant decreases (24%, 36%, and 36%, respectively) in FPG after 30 days of treatment compared with MD-untreated animals ($P < .01$). Similarly, SD rats treated with different doses of aqueous *W. coagulans* showed significant decreases (44%, 54%, and 55%, respectively) in FPG ($P < .01$) compared with SD-untreated animals. Glibenclamide-treated MD and SD rats showed significant decreases in FPG compared with the MD- and SD-untreated groups, respectively ($P < .01$), and the percentage decrease in FPG was similar to the decrease obtained with 250 mg of aqueous *W. coagulans* (i.e., 38%).

PPPG of MD and SD animals was significantly increased ($P < .01$) compared with healthy animals. MD and SD rats treated with three different doses of aqueous *W. coagulans* for 30 days showed significant decreases in PPPG compared with SD-untreated animals ($P < .01$): 42%, 54%, and 51% in MD animals and 44%, 56%, and 50% in SD animals, respectively. Glibenclamide-treated MD and SD rats showed significant decrease in PPPG in comparison with diabetic-untreated animals (54% and 60%, respectively) ($P < .01$). The percentage decrease in FPG in SD animals treated with different doses of aqueous *W. coagulans* was higher compared with MD animals at 30 days; however, no such difference was observed in percentage decrease in PPPG between MD and SD animals. As shown in Table 2, aqueous *W. coagulans* at 250 mg/kg of body weight appeared to be the most effective dose because FPG and PPPG were maximally decreased with this dose, and no further beneficial effect was apparent with the higher dose (500 mg/kg of body weight). Therefore, further experimental work was carried out with this dose.

TABLE 2. EFFECT OF DIFFERENT DOSES OF AQUEOUS *W. COAGULANS* ON FASTING AND POSTPRANDIAL PLASMA GLUCOSE OF MILDLY DIABETIC AND SEVERELY DIABETIC RATS AFTER 30 DAYS OF TREATMENT

Group	Dose (mg/kg of body weight)	FPG			PPPG		
		Day 0	Day 30	% decrease	Day 0	Day 30	% decrease
Healthy control	—	4.31 ± 0.12 ^b	4.00 ± 0.11 ^b		6.28 ± 0.19 ^b	6.17 ± 0.16 ^b	
MD							
Diabetic control	—	8.11 ± 0.27 ^d	8.10 ± 0.29 ^d		14.22 ± 0.40 ^d	14.41 ± 0.33 ^d	
Diabetic +							
aqWC	125	8.57 ± 0.27 ^d	5.87 ± 0.18 ^{ab}	28%	14.70 ± 0.43 ^d	8.30 ± 0.23 ^{ab}	42%
aqWC	250	8.32 ± 0.21 ^d	5.17 ± 0.17 ^{ab}	36%	14.3 ± 0.43 ^d	6.67 ± 0.32 ^{ab}	54%
aqWC	500	8.33 ± 0.27 ^d	5.13 ± 0.22 ^{ab}	36%	14.42 ± 0.32 ^d	7.03 ± 0.26 ^{ab}	51%
Glibenclamide	0.5	8.26 ± 0.28 ^d	5.10 ± 0.16 ^{ab}	38%	14.39 ± 0.30 ^d	6.61 ± 0.22 ^{ab}	54%
SD							
Diabetic control	—	14.32 ± 0.33 ^d	14.51 ± 0.30 ^d		20.63 ± 0.41 ^d	20.68 ± 0.40 ^d	
Diabetic +							
aqWC	125	14.15 ± 0.33 ^d	8.09 ± 0.36 ^{acd}	44%	21.50 ± 0.31 ^d	11.53 ± 0.34 ^{acd}	44%
aqWC	250	14.47 ± 0.27 ^d	6.61 ± 0.28 ^{acd}	54%	21.47 ± 0.30 ^d	9.19 ± 0.34 ^{acd}	56%
aqWC	500	13.83 ± 0.30 ^d	6.56 ± 0.32 ^{ac}	55%	21.22 ± 0.45 ^d	10.3 ± 0.37 ^{acd}	53%
Glibenclamide	0.5	14.43 ± 0.31 ^d	6.01 ± 0.26 ^{acd}	58%	21.18 ± 0.38 ^d	8.57 ± 0.22 ^{acd}	60%

Data are mean ± SEM values ($n=6$).

^a $P < .01$, Day 30 versus Day 0; ^b $P < .01$, treated MD versus MD control; ^c $P < .01$ SD treated versus SD control; ^d $P < .01$, MD and SD versus healthy control. aqWC, aqueous *W. coagulans*; FPG, fasting plasma glucose; MD, mildly diabetic; PPPG, postprandial plasma glucose; SD, severely diabetic.

Effect of aqueous *W. coagulans* on body weight

There was significant loss in body weight of diabetic animals compared with healthy animals ($P < .05$). After 30 days of treatment with aqueous *W. coagulans* with all three doses (125, 250, and 500 mg/kg), significant improvement in body weight was observed compared with diabetic-untreated animals ($P < .05$) (Table 3).

Effect of aqueous *W. coagulans* on HbA_{1c} levels

HbA_{1c} in MD and SD-untreated animals was significantly increased ($P < .01$) compared with that in healthy animals (Fig. 1A). After 30 days of treatment with aqueous *W. coagulans*, MD and SD showed significant decreases in HbA_{1c} (14% and 22%, respectively) compared with MD- and SD-untreated animals ($P < .01$). Similarly, glibenclamide-treated

MD and SD animals also showed significant decreases in HbA_{1c} ($P < .01$) compared with untreated animals after 30 days.

Effect of aqueous *W. coagulans* on serum insulin levels

Serum insulin levels in MD and SD-untreated animals were significantly decreased ($P < .01$) compared with healthy animals (Fig. 1B). After 30 days of treatment with aqueous *W. coagulans*, MD and SD rats showed significant increases ($P < .01$) in serum insulin levels compared with MD- and SD-untreated animals, suggesting an improvement in the insulin secretory status of β -cells; however, the insulin levels remained slightly lower compared with healthy controls. Results were compared with glibenclamide-treated MD and SD animals, which also showed significant

TABLE 3. COMPARISON OF EFFECT OF DIFFERENT DOSES OF AQUEOUS *W. COAGULANS* ON BODY WEIGHT OF MILDLY DIABETIC AND SEVERELY DIABETIC RATS AFTER 30 DAYS OF TREATMENT

Group	Dose (mg/kg of body weight)	Body weight (g)			
		MD		SD	
		Day 0	Day 30	Day 0	Day 30
Healthy control		154 ± 1.51	190 ± 1.84	154 ± 1.52	190 ± 4.5
Diabetic control		135.8 ± 2.38 ^d	134 ± 3.57 ^d	127 ± 1.00 ^d	131 ± 1.52 ^d
Diabetic +					
aqWC	125	136 ± 2.62 ^d	162.1 ± 2.05 ^{abd}	128 ± 2.50 ^d	153 ± 2.13 ^{acd}
aqWC	250	136 ± 2.17 ^d	162 ± 1.68 ^{abd}	128 ± 2.50 ^d	157 ± 2.50 ^{acd}
aqWC	500	134 ± 2.38 ^d	158 ± 1.68 ^{abd}	125 ± 2.25 ^d	157 ± 1.27 ^{acd}
Glibenclamide	0.5	136 ± 3.07 ^d	162 ± 1.68 ^{abd}	125 ± 2.91 ^d	158 ± 3.36 ^{acd}

Data are mean ± SEM values ($n=6$).

^a $P < .01$, Day 30 versus Day 0; ^b $P < .05$ versus MD control; ^c $P < .05$ versus SD control; ^d $P < .05$ versus healthy control.

FIG. 1. Effect of aqWC treatment on (A) glycosylated hemoglobin (HbA1c) and (B) serum insulin in diabetic rats after 30 days of treatment. Data are mean $\pm$ SEM values ($n=6$). aqWC 250, aqWC (250 mg/kg of body weight); Gliben 0.5, glibenclamide (0.5 mg/kg of body weight). ^b $P < .01$ versus MD control, ^c $P < .01$ versus SD control.

increases in serum insulin ($P < .01$) compared with MD- and SD-untreated animals.

Effect of 30 days of treatment with aqueous *W. coagulans* on glycogen content in liver and muscle

Glycogen content in liver and muscle was significantly lower ($P < .01$) in MD and SD animals compared with healthy groups (Table 4). After treatment with aqueous *W. coagulans*, glycogen content was significantly increased ($P < .01$) by 50% and 84% in liver and muscle, respectively, in MD-treated compared with MD-untreated animals. Similarly, SD-treated animals also showed significant increases ($P < .01$) in glycogen content in liver and muscle by 65% and 66%, respectively, compared with SD-untreated animals. Glibenclamide-treated MD and SD animals also showed significant increases ($P < .01$) in liver and muscle glycogen after 30 days of treatment, suggesting that the increase in insulin levels in both MD and SD animals by aqueous *W. coagulans* treatment might have led to improvement in tissue glycogen content.

TABLE 4. EFFECT OF AQUEOUS *W. COAGULANS* ON GLYCOGEN CONTENT IN LIVER AND MUSCLES IN MILDLY DIABETIC AND SEVERELY DIABETIC RATS AFTER 30 DAYS OF TREATMENT

Group	Dose (mg/kg of body weight)	Glycogen (mg/g of protein)	
		Liver	Muscles
Healthy control	—	152.7 $\pm$ 5.33 ^{bc}	87.6 $\pm$ 2.87 ^{bc}
MD			
Diabetic control	—	109.8 $\pm$ 2.05 ^d	48.3 $\pm$ 1.64 ^d
Diabetic + aqWC	250	145.0 $\pm$ 2.05 ^{bc}	85.8 $\pm$ 0.82 ^{bc}
Diabetic + glibenclamide	0.5	149.0 $\pm$ 3.69 ^{bc}	83.0 $\pm$ 3.27 ^{bc}
SD			
Diabetic control	—	98.9 $\pm$ 2.87 ^d	51.0 $\pm$ 1.64 ^d
Diabetic + aqWC	250	137.0 $\pm$ 2.87 ^{bc}	75.0 $\pm$ 2.87 ^{bc}
Diabetic + glibenclamide	0.5	138.9 $\pm$ 3.28 ^{bc}	82.0 $\pm$ 2.05 ^{bc}

Data are mean $\pm$ SEM values ($n=6$).

^b $P < .01$ versus MD control, ^c $P < .01$ versus SD control; ^d $P < .01$ versus healthy control.

Effect of aqueous *W. coagulans* on activity of carbohydrate-metabolizing enzymes in diabetic animals

The activity of GK in the liver of MD and SD animals decreased significantly ($P < .01$) compared with healthy controls. Aqueous *W. coagulans* treatment of MD and SD animals showed a significant increase ($P < .01$) in GK activity (i.e., 175% and 327%, respectively) compared with the MD- and SD-untreated groups after 30 days (Table 5). PFK activity in the liver of MD and SD was also significantly decreased ($P < .01$) compared with healthy animals. After treatment with aqueous *W. coagulans*, MD and SD animals showed significant increases ($P < .01$) in PFK activity by 29% and 88%, respectively, compared with diabetic-untreated animals. Activity of G-6-Pase in liver of MD and SD animals was significantly higher ($P < .01$) compared with healthy animals; however, aqueous *W. coagulans*-treated MD and SD animals had significantly lower activities of G-6-Pase ($P < .01$) by 138% and 123%, respectively, compared with diabetic-untreated animals. These results suggest that aqueous *W. coagulans* treatment led to an improvement in the glucose metabolism by stimulation of the glycolytic pathway and decreases in gluconeogenesis. This effect may be due to the restoration of insulin secretion/action on treatment with aqueous *W. coagulans*.

DISCUSSION

Nicotinamide followed by STZ (intraperitoneally) was used to induce experimental DM in rats in this study. STZ contains a nitroso moiety and liberates nitric oxide and free radicals, which are responsible for the breakdown of DNA strands, resulting in activation of poly(ADP-ribose) polymerase and depletion of intracellular NAD, which appear to be common factors in β -cell death, generally leading to type

TABLE 5. EFFECT OF AQUEOUS *W. COAGULANS* ON ACTIVITY OF CARBOHYDRATE-METABOLIZING ENZYMES IN LIVER OF MILDLY DIABETIC AND SEVERELY DIABETIC ANIMALS AFTER 30 DAYS OF TREATMENT

Group	Dose (mg/kg of body weight)	Activity (nKatal/mg of protein)		
		Glucokinase	Phosphofructokinase	Glucose 6-phosphatase
Healthy control	—	15.5 ± 0.68 ^{bc}	176.7 ± 4.10 ^{bc}	2.2 ± 0.21 ^{bc}
MD				
Diabetic control	—	4.0 ± 0.48 ^d	97.3 ± 4.80 ^d	6.3 ± 0.07 ^d
Diabetic + aqWC	250	11.0 ± 0.07 ^{bd}	125.0 ± 4.10 ^{bd}	2.7 ± 0.07 ^b
Diabetic + glibenclamide	0.5	11.2 ± 0.41 ^{bd}	120.0 ± 4.10 ^{bd}	3.3 ± 0.28 ^b
SD				
Diabetic control	—	1.6 ± 0.14 ^d	55.3 ± 3.90 ^d	8.2 ± 0.28 ^d
Diabetic + aqWC	250	6.8 ± 0.68 ^{cd}	103.5 ± 2.73 ^{cd}	3.7 ± 0.21 ^{cd}
Diabetic + glibenclamide	0.5	6.5 ± 0.21 ^{cd}	100.7 ± 4.10 ^{cd}	3.8 ± 0.004 ^{cd}

Data are mean ± SEM values (n = 6).

^bP < .01 versus MD control; ^cP < .01 versus SD control; ^dP < .01 versus healthy control.

1 DM. Therefore, NAD supplementation protects against β -cell damage and helps in creating a model that is similar to type 2 DM.^{18,19}

Diabetic animals showed significantly increased levels of FPG and PPPG in the present study, whereas treatment of diabetic animals with aqueous *W. coagulans* (all three doses) showed significant decreases in these parameters after 30 days. These results are comparable to those with glibenclamide treatment, which was used as a standard drug.¹⁶ Glibenclamide increases insulin secretion by inhibiting the activity of ATP-sensitive K⁺ channels of the β -cells, leading to the stimulation of Ca²⁺-dependent exocytosis of insulin from insulin-containing granules.^{30,31} Therefore, diabetic animals treated with glibenclamide showed significant increases in serum insulin levels, as did MD and SD animals treated with aqueous *W. coagulans*. The exact mechanism of action of the aqueous *W. coagulans* extract is unknown; however, it might be due to secretion of insulin from existing β -cells. During DM, the excess glucose present in the plasma reacts with hemoglobin to form HbA_{1c}, which is commonly used to assess glycemic control in individuals.³² Treatment with aqueous *W. coagulans* significantly decreased HbA_{1c}, which reflects the improvement in glycemic status as shown by decreases in FPG and PPPG.

In DM, enzymes involved in carbohydrate metabolism are significantly altered, which may lead to secondary complications of DM.^{33,34} GK, PFK, and G-6-Pase are important metabolic enzymes of glucose homeostasis. In diabetic animals, activities of GK and PFK decrease, whereas activity of G-6-Pase increases significantly. Insulin increases hepatic glycolysis by increasing the activities of GK and PFK and suppressing the biosynthesis of gluconeogenic enzymes.^{35,36} Diabetic animals treated with aqueous *W. coagulans* showed significantly increased activities of GK and PFK, whereas the activity of G-6-Pase was decreased in liver.

Glycogen is a primary intracellular storage form of glucose. Insulin promotes glycogen deposition in liver and muscle by stimulating glycogen synthase and inhibiting

glycogen phosphorylase.³⁷ The observed decrease in glycogen in liver and muscles of diabetic animals could be due to decreased glycogen synthesis or increased glycogenolysis mediated by increased activity of G-6-Pase.³⁸ However, diabetic animals treated with aqueous *W. coagulans* showed significant restoration of glycogen content in liver and muscles, thereby suggesting that this may be another probable mechanism of antidiabetic action.³⁹

Phytochemical screening of aqueous *W. coagulans* showed the presence of bioactive constituents (steroidal compounds, glycosides, tannins, phenols, triterpenoids, alkaloids, saponins, glycosides, etc.). In several studies, plant-derived flavonoids, triterpenoids, and glycosides have been reported to possess antidiabetic activity by stimulating the release of insulin from β -cells and/or modulation of enzymes of carbohydrate metabolism.^{40–42} The phenols, alkaloids, and phenolic acids have been reported to lower blood glucose levels by increasing the expression of mRNA of hepatic GK and PFK, whereas expression of gluconeogenic enzymes (*i.e.*, G-6-Pase) was decreased in liver.⁴³ Flavonoids also stimulate glucose uptake in peripheral tissues and regulate the activity and/or expression of the rate-limiting enzymes of carbohydrate metabolism.⁴⁴ In the present study, diabetic animals treated with aqueous *W. coagulans* showed improvement in glycemic status and favorable changes in the activity of glucose-metabolizing enzymes. These effects may be mediated through increases in insulin levels and/or may be due to other mechanisms related to the bioactive compounds present in aqueous *W. coagulans*.

CONCLUSIONS

Daily treatment with aqueous *W. coagulans* at 250 mg/kg of body weight for 30 days restored plasma glucose, HbA_{1c}, tissue glycogen, and glucose metabolic enzymes to near-normal ranges in both MD and SD animals. The results of this study reveal that the regular administration of aqueous *W. coagulans* extract for 30 days significantly improved glycemic status and nearly normalized plasma glucose

concentrations. Therefore, it can be concluded that aqueous *W. coagulans* extract contains active components that have antihyperglycemic effects. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of action of antidiabetic/antihyperglycemic effects of such active components from aqueous *W. coagulans*.

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AUTHOR DISCLOSURE STATEMENT

All the authors state that no competing financial interests exist.

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