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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 96h, 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), glycated hemoglobin (HbA1c), 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 HbA1c (P < 0.01), whereas serum insulin increased significantly compared with that in diabetic-untreated rats (P < 0.01). MD and SD animals treated with aqueous W. coagulans also showed significant increases in liver and muscle glycogen compared with diabetic-untreated animals (P < 0.01). Moreover, activities of glucokinase and phosphofructokinase were also significantly increased (P < 0.01), whereas glucose-6-phosphatase activity was significantly decreased (P < 0.01) 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 DM include macrovascular complications like cardiovascular and stroke and microvascular complications such as retinopathy, microangiopathy, and nephropathy.1 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.5,9 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...
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. 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-2008-09/7979/10) 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 Harborne and Ayoola.

**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 ± 1°C, relative humidity of 50 ± 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. 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.

**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).

**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 (HbA1c), 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 post-prandial plasma glucose (PPPG). Blood was centrifuged at 3500 rpm 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). HbA1c in blood was estimated by the ion-exchange resin method, which is a reliable method for HbA1c estimation in rats. Whole blood was mixed with lysing reagent containing detergent and borate, and the hemolsate was prepared and mixed with cation exchange resin. All hemoglobins were retained by the resin, and HbA1c was eluted. The percentage of HbA1c was determined by measuring the ratio of absorbance of the HbA1c fraction and the total hemoglobin fraction at 415 nm, and results were compared with that for a standard HbA1c preparation that was carried out through the test. Tissue glycogen was extracted and precipitated with ethanol and its content was estimated by using sulfuric acid.
Table 1. Screening of Phytoconstituents in Aqueous W. coagulans

<table>
<thead>
<tr>
<th>Screen number</th>
<th>Test</th>
<th>Method</th>
<th>Presence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>Barfoed’s/Benedict’s</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(reducing ketohexose)</td>
<td>Selivanoff’s test</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>Borntrager/Killer-Killiani</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Steroidal compounds</td>
<td>Salkowski’s test</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>Froth test</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phenolic compounds</td>
<td>Ferric chloride test</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>Ferric chloride/formaldehyde</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Triterpenoids</td>
<td>Salkowski’s/Liebermann’s test</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Alkaloids</td>
<td>Dragendorf’s/Mayer’s reagent</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Flavonoids</td>
<td>Acid alcohol/lead acetate/ethyl acetate</td>
<td>+</td>
</tr>
</tbody>
</table>

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

W. COAGULANS FRUIT ANTIHYPERGLYCEMIC EFFECT

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 anhydrous (97% 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 (ΔA × total volume × dilution factor)/(sample volume × 6.22 × 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), 8 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 μmol 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, tri-chloroacetic 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 × 10⁻³ 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 [(optical density of test mixture – optical density of control)/ optical density of the standard] × micromoles of phosphate in the standard × 2.2, and units have been calculated as nanomoles 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 some bioactive components in the extract (i.e., carbohydrates, glycosides, steroidal compounds, saponins, phenols, tannins, alkaloids, terpenoids, and flavonoids) (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 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg of body weight)</th>
<th>FPG</th>
<th>PPG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 30</td>
</tr>
<tr>
<td>Healthy control</td>
<td>—</td>
<td>4.31 ± 0.12a</td>
<td>4.00 ± 0.11b</td>
</tr>
<tr>
<td>MD</td>
<td>—</td>
<td>8.11 ± 0.27a</td>
<td>8.10 ± 0.29a</td>
</tr>
<tr>
<td>Diabetic +</td>
<td>aqWC 125</td>
<td>8.57 ± 0.27a</td>
<td>5.87 ± 0.18ab</td>
</tr>
<tr>
<td></td>
<td>aqWC 250</td>
<td>8.32 ± 0.21a</td>
<td>5.17 ± 0.17ab</td>
</tr>
<tr>
<td></td>
<td>aqWC 500</td>
<td>8.33 ± 0.23a</td>
<td>5.13 ± 0.22ab</td>
</tr>
<tr>
<td></td>
<td>Glibenclamide 0.5</td>
<td>8.26 ± 0.28a</td>
<td>5.10 ± 0.16ab</td>
</tr>
<tr>
<td>SD</td>
<td>—</td>
<td>14.32 ± 0.33a</td>
<td>14.51 ± 0.30a</td>
</tr>
<tr>
<td>Diabetic +</td>
<td>aqWC 125</td>
<td>14.15 ± 0.33a</td>
<td>8.09 ± 0.36abd</td>
</tr>
<tr>
<td></td>
<td>aqWC 250</td>
<td>14.47 ± 0.27a</td>
<td>6.61 ± 0.28abd</td>
</tr>
<tr>
<td></td>
<td>aqWC 500</td>
<td>13.83 ± 0.30a</td>
<td>6.56 ± 0.32acd</td>
</tr>
<tr>
<td></td>
<td>Glibenclamide 0.5</td>
<td>14.43 ± 0.31a</td>
<td>6.01 ± 0.26acd</td>
</tr>
</tbody>
</table>

Data are mean ± SEM values (n = 6).

*P < 0.01, Day 30 versus Day 0; †P < 0.01, treated MD versus MD control; ‡P < 0.01, MD and SD versus healthy control.

aqWC, aqueous W. coagulans; FPG, fasting plasma glucose; MD, mildly diabetic; PPG, 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 < 0.05). 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 < 0.05) (Table 3).

Effect of aqueous W. coagulans on HbA1c levels

HbA1c in MD and SD-untreated animals was significantly increased (P < 0.01) compared with that in healthy animals (Fig. 1A). After 30 days of treatment with aqueous W. coagulans, MD and SD showed significant decreases in HbA1c (14% and 22%, respectively) compared with MD- and SD-untreated animals (P < 0.01). Similarly, glibenclamide-treated MD and SD animals also showed significant decreases in HbA1c (P < 0.01) 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 < 0.01) compared with healthy animals (Fig. 1B). After 30 days of treatment with aqueous W. coagulans, MD and SD rats showed significant increases (P < 0.01) 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 increases.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg of body weight)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Healthy control</td>
<td>—</td>
<td>154 ± 1.51</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>—</td>
<td>135.8 ± 2.38d</td>
</tr>
<tr>
<td>Diabetic +</td>
<td>aqWC 125</td>
<td>136 ± 2.62d</td>
</tr>
<tr>
<td></td>
<td>aqWC 250</td>
<td>136 ± 2.17d</td>
</tr>
<tr>
<td></td>
<td>aqWC 500</td>
<td>134 ± 3.38d</td>
</tr>
<tr>
<td></td>
<td>Glibenclamide 0.5</td>
<td>136 ± 3.07d</td>
</tr>
</tbody>
</table>

Data are mean ± SEM values (n = 6).

*P < 0.05 versus MD control; †P < 0.05 versus SD control; ‡P < 0.05 versus healthy 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**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg of body weight)</th>
<th>Glycogen (mg/g of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Healthy control</td>
<td>—</td>
<td>152.7 ± 5.33$^{bc}$</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>—</td>
<td>109.8 ± 2.05$^{d}$</td>
</tr>
<tr>
<td>Diabetic + aqWC 250</td>
<td>145.0 ± 2.05$^{bc}$</td>
<td>85.8 ± 0.82$^{bc}$</td>
</tr>
<tr>
<td>Diabetic + glibenclamide 0.5</td>
<td>149.0 ± 3.69$^{bc}$</td>
<td>83.0 ± 3.27$^{bc}$</td>
</tr>
<tr>
<td>SD</td>
<td>—</td>
<td>98.9 ± 2.87$^{d}$</td>
</tr>
<tr>
<td>Diabetic + aqWC 250</td>
<td>137.0 ± 2.87$^{bc}$</td>
<td>75.0 ± 2.87$^{bc}$</td>
</tr>
<tr>
<td>Diabetic + glibenclamide 0.5</td>
<td>138.9 ± 3.28$^{bc}$</td>
<td>82.0 ± 2.05$^{bc}$</td>
</tr>
</tbody>
</table>

Data are mean ± SEM values ($n=6$).

$^{a}P < .01$ versus MD control, $^{b}P < .01$ versus SD control; $^{c}P < .01$ 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 ± SEM values ($n=6$). aqWC 250, aqWC (250 mg/kg of body weight); Gliben 0.5, glibenclamide (0.5 mg/kg of body weight). $^{a}P < .01$ versus MD control, $^{b}P < .01$ versus SD control.

**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
Healthy control — 15.5
in individuals.\(^{32}\) Treatment with aqueous Glibenclamide increases insulin secretion by inhibiting the type 2 DM.\(^{18,19}\)

Therefore, NAD supplementation protects against SD DM.\(^{33,34}\) GK, PFK, and suppressing the biosynthesis of gluconeogenic enzymes.\(^{35,36}\) Diabetic animals treated with aqueous W. coagulans 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 \(\beta\)-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 glycan 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 glycogenesis 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 \(\beta\)-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 glycan 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 levels.

### 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

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

Data are mean ± SEM values (n=6).

\(^{a}\)P < 0.01 versus MD control; \(^{b}\)P < 0.01 versus healthy control; \(^{c}\)P < 0.01 versus SD control; \(^{d}\)P < 0.01 versus healthy control.
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*.

**ACKNOWLEDGMENTS**

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**AUTHOR DISCLOSURE STATEMENT**

All the authors state that no competing financial interests exist.

**REFERENCES**


Ameliorative effect of *Withania coagulans* on dyslipidemia and oxidative stress in nicotinamide–streptozotocin induced diabetes mellitus

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**Abstract**

Present study aims to evaluate the effect of *Withania coagulans* fruit (aqWC) on diabetic-dyslipidemia and antioxidant/oxidant status in DM. Diabetic animals were treated with aqWC at a dose of 250 mg/kg bw for 30 days. Lipid profile, MDA, GSH, SOD, FRAP, HMG CoA reductase and acetyl CoA carboxylase activities were estimated in blood and tissues. Total cholesterol, TAG and LDL were significantly elevated whereas HDL was decreased in diabetic animals (*p* < 0.05), simultaneously the lipid content and HMG CoA reductase activities were also increased, whereas acetyl CoA carboxylase activity decreased significantly in tissues of diabetic animals. MDA was increased and antioxidants such as SOD, GSH and FRAP decreased significantly in DM (*p* < 0.05). Oral administration of aqWC to diabetic animals produced significant improvement in serum lipid profile and tissue lipid content. Activity of HMG CoA reductase decreased, whereas acetyl CoA carboxylase activity increased significantly in tissues after aqWC treatment. Administration of aqWC to diabetic animals also showed significant increase in antioxidant levels i.e., GSH, SOD, FRAP and reduced level of MDA in blood and tissue homogenates as compared to diabetic controls (*p* < 0.05). These results suggest that aqWC treatment improved lipid profile and decreased oxidative stress in diabetes mellitus.

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1. Introduction

Hyperglycemia and characteristic dyslipidemia of diabetes mellitus (DM) along with increased oxidative stress leading to endothelial dysfunction have been implicated as early events in the pathogenesis of atherothrombotic macrovascular disease (Nathan et al., 1997). It is increasingly recognized that insulin deficiency contributes to the characteristic dyslipidemia associated with diabetes mellitus, and is a major risk factor for macrovascular complications (Adiels et al., 2006).

Several medicinal plants such as *Momordica charantia*, *Azadirachta indica*, *Gymnema sylvestre*, *Pterocarpus marsupium*, *Coccinia indica*, *Trigonella foenum graecum*, *Allium sativum*, *Ocimum sanctum* etc. have been reported for the management of DM and its complications. Fruit of *Withania coagulans* (Family: Solanaceae) have been reported to possess a variety of biological activities (Kirtikar and Basu, 1993) and its ethanopharmacological applications are well known (Chadha, 1976). Anti-hyperglycemic effects of fruit extract of *W.coagulans* in experimental diabetes mellitus using high doses have been reported in few studies (Hemlatha et al., 2004; Hoda et al., 2010; Jaiswal et al., 2009, 2010). Hemalatha et al. (2006) have also reported the anti-hyperlipidemic effect of aqueous extract of *W.coagulans* at a dose of 1000 mg/kg bw for 7 weeks in high fat diet induced hyperlipidemic rats. Whereas, Saxena (2010) showed the hypolipidemic activity of aqueous extract of fruit of *W.coagulans* at a dose of 1000 mg/kg bw for 28 days in streptozotocin induced diabetes. Thus, in all these reports, the doses of *W.coagulans* were purchased from the local market of Delhi and were identified and authenticated by National Institute of Science Communication and Information Resources, Pusa, New Delhi (Voucher Number, NISCAIR/RHMD/Con-

**Abbreviations:** ACC, acetyl CoA carboxylase; aqWC, aqueous extract of *Withania coagulans*; bw, body weight; DM, diabetes mellitus; FPG, fasting plasma glucose; FRAP, ferric reducing ability of plasma; g, gram; HMGCR, HMG CoA reductase; LDL, low density lipoprotein; MDA, malondialdehyde; mg, milligram; NAD, nicotinamide; GSH, reduced glutathione; SD, standard deviation; SOD, superoxide dismutase; TC, total cholesterol; TAG, triacylglycerol.

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Animals were fed with a standard laboratory diet and water (12 h) and dark (12 h) cycles were maintained throughout experimental period. At temperature of 22 ± 2°C. 2.2. Induction of diabetes in rats Male wistar rats weighing 150 ± 10 g were housed in an air-conditioned room at temperature of 22 ± 2°C with relative humidity 55 ± 5 units. Standard light (12 h) and dark (12 h) cycles were maintained. Before experimental period, animals were fed with a standard laboratory diet and water ad libitum (Hundastan Lever Ltd., Mumbai, India). Ethical clearance was obtained from Institutional Animal Ethics Committee of Animal Research (IAEC-AR) at University College of Medical Sciences and GTB Hospital and experiments were carried out as per the guidelines of the committee. Overnight fasted animals were made diabetic by intraperitoneal injection of nicotinamide (230 mg/kg) followed by freshly prepared streptozotocin in citrate buffer (0.1 M, pH 4.5) at a dose of 55 mg/kg bw after 15 min (Masiello et al., 1998). After 96 h of induction when blood glucose is usually stabilized, FPG was determined and rats having FPG >126 mg/dl were designated as having diabetes mellitus and were used in this experiment. No mortality has been observed in this model.

2.3. Experimental design

In our previous study, three different doses of aqueous extract of W.coogulums (aqWC) i.e., 125, 250 and 500 mg/kg bw were administered for 30 days which produced the significant glucose lowering effect in diabetic animals. However, 250 mg/kg bw was found to be most effective doses (MED) (Shukla et al., 2012). Therefore, the present study was carried out with this dose.

The rats were divided into 4 groups (n = 6) as follows:

Group-I: Healthy control, Group-II: Diabetic control (Untreated), Group-III: Diabetic + aqWC (250 mg/kg bw) and Group-IV: Diabetic + Glibenclamide (0.5 mg/kg bw).

2.4. Collection of blood and tissues

Biochemical parameters in blood and tissues were determined after 30 days of aqWC treatment, whole blood (about 1.0 ml) was collected by retro-orbital venipuncture in EDTA vials. Blood was centrifuged at 1300 x g for 10 min to obtain plasma. Animal were anesthetized by single i.p. injection of pentobarbitone at a dose of 150 mg/kg bw and tissues (liver, heart and muscle) were removed, washed with cold saline and stored at –70°C till further use for tissue constituents and enzyme assays.

2.5. Biochemical parameters

Glucose estimation was done in plasma by glucose oxidase/peroxidase method using kits (Accurex Biomedical Pvt Ltd.). Total cholesterol (TC), triacylglycerol (TAG) and HDL-cholesterol (HDL-C) were estimated in fasting serum samples by using commercially available kits (Accurex Biomedical Pvt Ltd., Mumbai, India) and LDL-C was calculated by Friedwald's and Frederickson’s Equation. HMG-CoA reductase activity (HMGCaR) was estimated by the method of Rao and Ramakrishnan (1975). HMG-CoA and mevalonate levels in liver, heart and muscle homogenates were estimated by colorimetry and the ratio of two was taken as an index of activity of enzyme, decreased ratio indicated increased activity and vice versa. Acetyl CoA carboxylase activity (ACC) in liver, heart and muscle was estimated by the methods of Numa (1960) and Nakamshi and Numa (1970). Estimation of reduced glutathione (GSH) in whole blood and tissue homogenates was carried out by the method of Beutler et al. (1963) and Ellman (1959) respectively by using dithio-nitrobenzene (DTNB). The extent of lipid peroxidation (MDA) in serum and tissue homogenates was estimated by measuring the thiobarbituric acid reactive substance (TBARS) as described by of Satoh (1978) and Wills (1966) respectively. Activity of superoxide dismutase (SOD) was measured in erythrocytes and tissue homogenates by the method of Marklund and Marklund (1974) as modified by Nandi and Chatterjee (1988). Total anti-oxidant content of plasma was determined by measuring the ability to reduce Fe2+ (FRAP), which was estimated by the method of Benzie and Strain (1999). Total lipids from tissues were extracted and estimated by the method of Folch et al. (1957).

2.6. Statistical Analysis

Data were expressed as mean ± SD. Total 6 animals were included in each group and experiments were performed in duplicate. The data were analyzed by repeated analysis of ANOVA followed by Turkey's test using SPSS 17 software. The significance of results were considered at p < 0.05.

3. Results

3.1. Effect of aqWC on plasma glucose levels

The antidiabetic and anti-hyperglycemic effect of aqueous extract of W.coogulums has already been reported in our previous study. However in brief, the diabetic animals were divided and fed three different doses of aqWC i.e., 125, 250 and 500 mg/kg bw for 30 days. Significant anti-hyperglycemic effect was observed with all the three doses however, 250 mg/kg bw showed maximum effect on fasting and postprandial plasma glucose of diabetic animals (p < 0.05) and no extra benefit was observed with higher dose. Therefore, further experimental work was carried out with this dose (Shukla et al., 2012).

3.2. Effect of aqWC on serum lipid profile

Total cholesterol, TAG and LDL-C significantly increased in diabetic animals as compared to healthy controls. Diabetic animals treated with aqWC showed significant decrease in TC, TAG, and LDL-C levels as compared to diabetic controls. HDL-C levels were lower in diabetic animals and treatment with aqWC increased HDL-C levels significantly. Thus, treatment with aqWC improved the lipid profile parameters of diabetic animals (p < 0.05). The results were compared with standard drug i.e., glibenclamide treated-diabetic animals, which also showed significant improvement in lipid profile (Table 1).

3.3. Effect of aqWC on oxidant/antioxidant levels in blood

Activities of HMGCaR and ACC were assayed in liver, heart and muscle tissue homogenates. HMGCaR activity was significantly increased in diabetic animals as the ratio of HMG-CoA/mevalonate decreased significantly as compared to healthy animals (p < 0.05). However, diabetic animals treated with aqWC for 30 days showed decrease in the activity of HMGCaR as compared to diabetic animals. Acetyl CoA carboxylase activity was significantly decreased in diabetic animals as compared to healthy animals. However, diabetic animals treated with aqWC for 30 days showed significantly increased ACC activity as compared to diabetic controls (p < 0.05) (Table 2).

3.4. Effect of aqWC on total lipid content extracted from tissues

Total lipid contents of liver, heart and muscle tissue were significantly higher in diabetic animals as compared to healthy animals (p < 0.05). However, treatment of diabetic animals with aqWC showed significantly reduced lipid content in these tissues as compared to diabetic animals (p < 0.05). Glibenclamide treated diabetic animals also showed significant decrease in total lipid content in liver, heart and muscle tissue as compared to diabetic controls (Fig 1).

3.5. Effect of aqWC on oxidant/antioxidant levels in blood

In diabetic animals, the MDA levels in serum were significantly increased as compared to healthy controls (p < 0.05), however, aqWC treatment for 30 days showed significantly reduced MDA as compared to diabetic controls (p < 0.05). Glibenclamide treated diabetic animals also showed significant decrease in MDA levels as compared to diabetic controls. Reduced glutathione (GSH) and SOD activity in diabetic animals were significantly decreased as compared to healthy controls (p < 0.05). Whereas, aqWC treated and glibenclamide treated diabetic animals had significantly increased levels of GSH and SOD activity (p < 0.05). FRAP, a measure of total antioxidant capacity in plasma, it was significantly decreased in diabetic controls as compared to healthy controls.
3.6. Effect of aqWC on oxidant/antioxidant levels in tissue homogenates

In diabetic animals, the GSH and SOD reduced whereas MDA increased significantly \((p < 0.05)\) in liver, heart and muscle as compared to healthy animals \((p < 0.05)\). However, treatment with aqWC showed significant improvement in antioxidant status as shown by increased levels of GSH and SOD activity, whereas MDA decreased in Group III \((p < 0.05)\) (Fig 2).

4. Discussion

Diabetes mellitus is a metabolic disorder due to decreased insulin secretion or insulin resistance, with common feature of hyperglycemia. In the present study, diabetes was induced by single i.p. injection of nicotinamide followed by streptozotocin. Streptozotocin generates free radicals which break the DNA strands, resulting in the activation of the PARP and depletion of intracellular NAD, which appear to be common factors in \(\beta\)-cell death, generally leading to type I diabetes. Therefore, NAD supplementation protects against \(\beta\)-cells damage and helps in creating a model which is quite similar to type 2 DM (Masiello et al., 1998). In our earlier study we have reported that treatment of diabetic animals with aqWC for 30 days showed significant decrease in fasting and postprandial glucose as well as modulated the activities of enzymes of glucose homeostasis (Shukla et al., 2012). These results are comparable

### Table 1
Effect of aqWC treatment on lipid profile in different groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol (TC) (mg/dl)</th>
<th>Triglyceride (TAG) (mg/dl)</th>
<th>High density lipoprotein (HDL) (mg/dl)</th>
<th>Low density lipoprotein (LDL) (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>72.2 ± 9.8</td>
<td>97.4 ± 4.4</td>
<td>33.9 ± 2.3</td>
<td>18.8 ± 6.4</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>126.4 ± 7.4</td>
<td>153 ± 8.6</td>
<td>23.1 ± 0.6</td>
<td>72.0 ± 5.0</td>
</tr>
<tr>
<td>Diabetic + aqWC (250 mg/kg bw)</td>
<td>85.4 ± 7.2^a</td>
<td>96.6 ± 2.7^b</td>
<td>30.2 ± 1.4^b</td>
<td>36.0 ± 5.3^h</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (0.5 mg/kg bw)</td>
<td>93.2 ± 6.0^h,a</td>
<td>101.6 ± 5.5^b</td>
<td>29.3 ± 1.3^b</td>
<td>43.6 ± 3.6^e</td>
</tr>
</tbody>
</table>

\(^a p < 0.05\) vs. healthy control. 
\(^b p < 0.05\) vs. diabetic control, \(n = 6\).

### Table 2
Effect of aqWC treatment on lipid metabolizing enzymes in liver, heart and muscle tissue.

<table>
<thead>
<tr>
<th>Group</th>
<th>HMGCoA/mevalonate ratio</th>
<th>Acetyl CoA carboxylase (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Heart</td>
</tr>
<tr>
<td>Healthy control</td>
<td>3.72 ± 0.4</td>
<td>2.47 ± 0.2</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>2.45 ± 0.2^a</td>
<td>1.08 ± 0.2^a</td>
</tr>
<tr>
<td>Diabetic + aqWC (250 mg/kg bw)</td>
<td>3.12 ± 0.4^a</td>
<td>2.30 ± 0.3^b</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (0.5 mg/kg bw)</td>
<td>2.95 ± 0.5^h,a</td>
<td>1.94 ± 0.2^b,a</td>
</tr>
</tbody>
</table>

\(^a p < 0.05\) vs. healthy control. 
\(^b p < 0.05\) vs. diabetic control, \(n = 6\).

\(c\) The activity of HMG CoA reductase is expressed by the ratio of HMG CoA/Mevalonate which is inversely proportional to its activity.

### Table 3
Effect of aqWC treatment on Lipid peroxidation, reduced glutathione, superoxide dismutase and ferric reducing ability of plasma.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma</th>
<th>Hemolysate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lipid peroxidation (MDA) (nmol/ml)</td>
<td>Ferric reducing ability of plasma (FRAP) (mmol/L)</td>
</tr>
<tr>
<td>Healthy control</td>
<td>1.18 ± 0.3</td>
<td>2.85 ± 0.74</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>5.65 ± 0.7^a</td>
<td>1.02 ± 0.25^a</td>
</tr>
<tr>
<td>Diabetic + aqWC (250 mg/kg bw)</td>
<td>2.72 ± 0.8^h,a</td>
<td>1.96 ± 0.30^h,a</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (0.5 mg/kg bw)</td>
<td>3.09 ± 0.6^h,a</td>
<td>1.74 ± 0.44^h,a</td>
</tr>
</tbody>
</table>

\(^a p < 0.05\) vs. healthy control. 
\(^b p < 0.05\) vs. diabetic control, \(n = 6\).
with glibenclamide treatment which also showed significant decrease in fasting and postprandial plasma glucose levels.

In Diabetes mellitus, the lipid metabolism is altered and there is increased mobilization of free fatty acids from muscle and fat deposition in tissues like liver and heart (Bloomgarden, 2003). The typical dyslipidemia of DM is characterized by increased triacylglycerol (TAG) and decreased high density lipoprotein (HDL), in addition to hypercholesterolemia which is a major risk factor for atherosclerosis and cardiovascular disease (Mooradian, 2009). Insulin affects many sites of lipid metabolism and regulates cholesterol and fatty acid biosynthesis by regulating the lipid metabolizing enzymes (Murray et al., 2006). The aqWC treated diabetic animals showed significantly decreased TC, TAG and LDL-C levels and increased HDL-C, which may help to reduce the risk of diabetes associated complications. Treatment with aqWC also showed significant decrease in lipid content in liver, heart and muscle as compared to diabetic animals.

Acetyl CoA carboxylase plays an essential role in regulating fatty acid biosynthesis, when a cell or organ has more than enough metabolic fuel to meet its energy needs, the excess is generally converted to fatty acids and stored as lipids such as TAG (Murray et al., 2006). The reaction catalyzed by acetyl-CoA carboxylase is the rate-limiting step and important site of regulation of fatty acid biosynthesis. Insulin stimulates fatty acid synthesis by activating this enzyme (Witters et al., 1988). In diabetes mellitus, the activity of ACC decreased significantly, whereas after treatment with aqWC, the activity of ACC was significantly increased in treated-diabetic animals. That effect might be due to overall improvement in glycemic control after aqWC treatment in diabetic animals.

HMGCoA reductase (HMGCR) is a polytropic, transmembrane protein and an important regulatory enzyme of cholesterol biosynthesis. It stimulates the production of mevalonic acid from HMGCoA. In DM, the activity of HMGCR is increased due to high plasma glucose levels. Elevated plasma glucose and insulin levels promotes the activity of HMGCR in tissues but several studies have reported that moderate insulin deficiency or plasma glucose <360 mg/dL do not have any significant effect on the activity of HMGCR (Young et al., 1982). HMGCR activity was decreased in aqWC treated-diabetic animals which may be due to decreased availability of substrate i.e., acetyl CoA, which may be diverted towards fatty acid biosynthesis and ultimately decrease cholesterol biosynthesis.

In our previous study, we reported that aqWC has bioactive plant metabolites i.e., alkaloids, flavonoids, terpenoids, saponins, glycosides, steroidal compounds, saponins, phenols and tannins. These phytochemicals are potential antioxidants and free radical scavengers (Kusirisin et al., 2009; Georgetti et al., 2003). Oxidative stress results from imbalance between the production of free radicals and antioxidant defense mechanisms and is an important causative factor in several chronic diseases viz diabetes and associated complications (Halliwell, 1994). Lipid peroxidation is a marker of cellular oxidative damage initiated by reactive oxygen species (Memisog˘ullari et al., 2003). The increased level of lipid peroxidation induces oxidative damage by increasing peroxyl radicals and hydroxyl radicals. Flavonoids present in aqWC are effective in reducing lipid peroxidation and may enhance the antioxidant enzyme activity resulting the decreased level of MDA. This antioxidant potency of flavonoids could be due to arrangement of hydroxyl groups on benzene ring (Van Acker et al., 1996). Hyperglycemia can induce oxidative stress through advanced glycation end product (AGEs) formation and increased polyol and hexosamine pathway (Chakravarty et al., 1998). AGEs produce ROS and superoxide and the subsequent increase in oxidative stress may lead to endothelial dysfunction and ultimately cardiovascular disease (CVD) through several different mechanisms. (Creager et al., 2003) Superoxide dismutase (SOD) converts superoxide to hydrogen peroxide (H₂O₂) which is then transformed into water. Hydrogen peroxide can also give rise to hydroxyl radicals in the cells. Thus the removal of H₂O₂ is very important for antioxidant defense in cell or food systems. H₂O₂ can cross membranes and may oxidize a number of compounds. AqWC treated diabetic animals showed improved SOD activity which may be attributed to the antioxidant present in aqWC, but importantly due to decrease glucose levels (Kusirisin et al., 2009). Reduced glutathione...
(GSH) is essential to maintain the structural and functional integrity of erythrocytes. During hyperglycemia, ROS bind to receptors that promote oxidative stress and generate intracellular oxidants (Hofmann et al., 1999). An increased polyol pathway flux during hyperglycemia is due to increase in aldose reductase (AR) activity in tissues which reduces glucose to sorbitol by consuming NADPH. Aldose reductase is being reported to metabolize GSH-lipid derived aldehyde adducts which results in decrease in GSH and subsequently increases oxidative stress (Srivastava et al., 1998; Bhatnagar and Srivastava, 1992). In DM, altered activities of these enzymes and reduced level of GSH have been observed which affect the ability to defend against oxidative stress (Maritim et al., 2003). The diabetic animals treated with aqWC showed significantly increased levels of GSH in blood and tissue homogenates. FRAP is a measure of the total antioxidant capacity, based on the reduction of ferrous ions by the effect of the reducing power of plasma constituents, and contributed by low molecular weight antioxidants of hydrophilic and/or hydrophobic nature. FRAP is said to give better biologically relevant information than provided by individual antioxidant measurements and may describe the dynamic equilibrium between pro-oxidants and antioxidants in the plasma (Benzie and Strain, 1999). Since in the diabetic state, FRAP activity has been decreased, whereas, it increased after aqWC treatment in DM may be due to modulatory effect of aqWC treatment.

5. Conclusions

It is evident from data that aqueous extract of fruit of W. coagulans (aqWC) possesses salutary effects on dyslipidemia and oxidative stress in diabetes mellitus. Moreover, it modulated the enzymes of lipid metabolism. However, studies to identify the active principle involved in these effects in diabetes mellitus are underway in our laboratory.

6. Conflicts of Interests

The author(s) declare that they have no conflicts of interests.

Acknowledgement

The authors are grateful to Indian Council of Medical Research, Delhi, India for providing financial assistance for this study (Ref.: PI: Jasvidner K. Gambhir, 59/33/2005/BMS/TRM).

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