STUDY OF ANTIHYPERGLYCEMIC, ANTIHYPERLIPIDEMIC AND ANTIOXIDANT ACTIVITIES OF *WITHANIA COAGULANS* FRUITS IN STREPTOZOTOCIN INDUCED NON-INSULIN DEPENDENT DIABETES MELLITUS IN RATS

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ABSTRACT

Withania coagulans Dunal (fam. Solanaceae) is an indigenous plant used traditionally for the treatment of diabetes and associated complications. However, the mechanistic approach of antihyperglycemic potential of the plant is not scientifically validated. The present study was undertaken to investigate the antihyperglycemic and antihyperlipidemic potential of aqueous extract of *W. coagulans* fruits (AEWC) in streptozotocin-induced non-insulin dependent diabetes mellitus (NIDDM) in rats and to focus on its possible mechanism of action. NIDDM was induced by single intraperitoneal injection of STZ (65 mg kg^{-1} i.p.) in male Wistar rats. Animals were divided in five groups (n=6) and treated with AEWC (250 mg kg^{-1} and 500 mg kg^{-1}). Glibenclamide (4 mg kg^{-1}) was used as a standard drug. At the end of 4 weeks, fasting blood glucose, glycosylated haemoglobin, serum insulin, lipid profile, glucose uptake from rat hemidiaphragms, liver glycogen, serum aspartate transaminase (AST), serum alanine transaminase (ALP) and serum alkaline phosphatase (ALP) were determined. Antioxidant enzymes of liver including superoxide dismutase and catalase were evaluated. Histopathology of pancreas was studied. The results of our study demonstrate antihyperglycemic potential of AEWC because of improvement in the lipid metabolism and by relieving the oxidative stress as well.

Keywords: Streptozotocin, *Withania coagulans,* Hyperglycemia, Hyperlipidemia, Oxidative stress

INTRODUCTION

The aging population, consumption of calorie-rich diet, obesity and sedentary life styles have led to a tremendous increase in the number of diabetic individuals worldwide. The number of people around the world suffering from diabetes has sky rockeed in the last two decades from 30-230 million[1]. It has been estimated that India, considered as the diabetic capital of the world, would continue to lead even in 2030[2].

Currently available therapies, for diabetes mellitus includes insulin and various oral antidiabetic agents. In spite of the presence of known antidiabetic medicines in the pharmaceutical market, plant drugs and herbal formulations are frequently considered to be less toxic and show lesser side effects than synthetic ones[3-6]. Moreover, providing modern medical healthcare across the world especially in developing countries is still a far reaching goal due to economic constraints. The traditional medicines, when verified scientifically, provided a number of promising drugs for new antidiabetic agents. Still, there are a number of plants to be scientifically verified which have already been proved ethno-pharmacologically in their crude form for the treatment of diabetes by the native people.

Withania coagulans Dunal, belonging to family Solanaceae, is one of such plant used traditionally for management of diabetes. It is well known in the indigenous system of medicine for the treatment of ulcers, rheumatism and dropsy[7]. Fruits of *W. coagulans* have been shown to exert hepatoprotective and anti-inflammatory activity[8]. Fruit extract has been reported to possess *in vitro* antioxidant potential[9]. Based on its traditional use, we have earlier studied the preliminary hypoglycemic activity of *W. coagulans* fruits[10]. The present study is continuation of our earlier work with an objective to explore

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the antihyperglycemic and antihyperlipidemic activity of aqueous extract of *W. coagulans* fruits and to focus on the possible mechanism of action.

MATERIALS AND METHODS

Plant materials

Fruits of *W. coagulans* were collected from hilly regions of Trimbakeshwar, Nasik in the month of March 2017. It was authenticated by from Dr. A.M. Mujumdar, Head, Plant Sciences Division, Agharkar Research Institute, Pune The fruits were dried in shade, ground to a coarse powder by using a dry grinder and stored in an air-tight container for further use.

Preparation of aqueous extract of *W. coagulans* fruits (AEWC)

It was prepared by the method of maceration. 250 g powdered material was put in 1000 mL of water for 48 h with intermittent stirring and it was filtered. The supernatant was collected and evaporated to dryness under reduced pressure in a rotary evaporator.

Phytochemical analysis

Preliminary phytochemical analysis of AEWC was performed to detect presence of various phytoconstituents present in the extract.

Acute toxicity study

Acute toxicity study was performed according to Organization of Economic Co-Operation and Development (OECD) guideline no. 423 -Acute toxic class method[11].

Animals

Male Wistar rats weighing between 150-180 g were used for the study. The rats were procured from National Institute of Biosciences, Pune. They were caged in standard polypropylene cages of size 38 cm x 33 cm x 10 cm with stainless steel coverlids. The animals were fed on a standard pellet diet (Pranav Agro Industries, Pune, India) and water was freely available. The animals were maintained in a controlled environment (12 h light/dark cycle) and temperature (30±2 °C). Before every experiment, rats were kept fasting for 18-24 h and water was allowed *ad libitum*. All the experimental procedures were carried out in accordance with IAEC as per guidelines of CPCSEA (Reg. No. 884/ac/05/CPCSEA). The preparations (extracts, standard drugs, and vehicle) were administered orally with feeding needle and syringe directly into the stomach.

Drugs and chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Glibenclamide was procured from Aventis Pharma Ltd (India). All other chemicals were procured from local sources and were of analytical grade.

Induction of diabetes

Streptozotocin was dissolved in ice cold citrophosphate buffer (pH 4.3) immediately before use and was administered intraperitoneally (65 mg kg^{-1}) to overnight fasted rats. In order to avoid the mortality associated with STZ-induced hypoglycemia, 5 % glucose solution was given for 24 h to STZ-treated animals[12]. The fasting blood glucose was estimated after 72 h of STZ administration to confirm the diabetic state. Rats showing fasting blood glucose more than 200 mg dL^{-1} were considered diabetic and used for the study[13].

Experimental groups

Group I: Normal control where rats received carboxymethyl cellulose suspension (0.1 %) for 28 days.

Group II: Diabetic control where rats received single dose of STZ (65 mg kg^{-1}, i.p.).

Group III: Diabetic rats received AEWC (250 mg kg^{-1}) orally for 28 days.

Group IV: Diabetic rats received AEWC (500 mg kg^{-1}) orally for 28 days.

Group V: Diabetic rats received glibenclamide (4 mg kg^{-1}) orally for 28 days.

EVALUATION PARAMETERS

Fasting blood glucose

Fasting blood glucose was determined on 0, 7th, 14th, 21st, and 28th day of the study period using a glucometer (Accu Check, Germany).

Lipid profile

Serum lipid profile including parameters like total cholesterol, HDL-cholesterol and triglycerides were evaluated using commercially available kits (Span Diagnostics) and autoanalyser (Erba Chem). VLDL-cholesterol and LDL-cholesterol were calculated using the Friedewald's formula[14].

Liver antioxidant activity

Superoxide dismutase (SOD) activity was measured according to method of Marklund[15]. Assay mixture includes

2.95 mL Tris-HCl buffer, 25 μL of pyrogallol and 0.05 mL of tissue homogenate in total volume of 3 mL. The difference between the optical densities obtained at 1.30 and 3.30 min was estimated and expressed as U mg^{-1} protein. Catalase (CAT) was measured according to method described by Clairborne[16]. The catalase activity in the liver homogenate was estimated using quantitative spectrophotometric technique developed for routine studies of catalase kinetics following the breakdown of hydrogen peroxide into water and oxygen.

Plasma insulin

Insulin concentrations were determined through a radio immunoassay procedure, using insulin kit (Coral, India) according to manufacturer's instructions.

Estimation of glucose uptake by isolated rat diaphragm

The hemidiaphragms of rats were placed in two small tubes containing 2 mL of tyrode solution with 2 % glucose and incubated for 30 min at 37±0.2 ˚C with appropriate aeration. Glucose uptake by the hemidiaphragm was calculated as the difference between the initial and final glucose content in the incubation medium[17].

Glycosylated hemoglobin

Glycosylated hemoglobin was determined in heparinised whole blood by ion exchange resin method[18].

Liver glycogen estimation

Liver of individual animal was homogenized in 5 % w/V trichloroacetic acid and its glycogen content was estimated by the method of Carrol[19].

Evaluation of liver function parameters

Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphates (ALP) were analysed by using commercial kits (Crest Biosystems, Goa, India).

Histopathology study

Rats were sacrificed and pancreas was removed. Small slices of pancreas were fixed in 10 % formalin for 4-5 h and then processed by the paraffin technique. Sections of 5 μm thickness were cut and stained by hematoxylin and eosin (H&E) for histopathological study[20].

Statistical analysis

The results are presented as Mean ± S.E.M of 6 rats per group. Data was analyzed using one-way analysis of variance followed by Dunnett's multiple comparison test for analysis of biochemical data using software GraphPad Instat (Version-3). Values were considered statistically significant at $p<0.05$.

RESULTS

Preliminary phytochemical analysis of AEWC showed presence of flavonoids, alkaloids, saponins and tannins.

As shown in Table I, diabetic control group showed increase in blood glucose level as compared to normal rats. Oral administration of AEWC (250 and 500 mg kg^{-1}) for 28 days showed dose dependent decrease in blood glucose level in diabetic rats.

As shown in Table II, diabetic control group showed significant increase in total cholesterol, triglycerides, LDL-CH and VLDL-CH while there was significant decrease in HDL cholesterol in comparison to normal control. Treatment of diabetic rats with AEWC (250 and 500 mg kg^{-1}) for 28 days showed significant reduction in elevated serum T-CH, TG, LDL-CH and VLDL-CH. HDL cholesterol was improved after administration of AEWC (500 mg kg^{-1}).

Diabetic control group showed significant decrease in liver glycogen, serum insulin and glucose uptake by rat diaphragm while there was significant increase in glycosylated haemoglobin as compared to normal control as shown in Table III. Treatment of diabetic rats with AEWC (250 and 500 mg kg^{-1}) for 28 days showed significant improvement in liver glycogen, serum insulin and glucose uptake by rat diaphragm while there was significant decrease, in glycosylated haemoglobin.

Diabetic control group showed significant decrease in liver SOD and liver CAT enzymes as compared to normal control. Administration of AEWC (250 and 500 mg kg^{-1}) for 28 days showed significant improvement in liver SOD and catalase, as shown in Table IV.

Serum AST, ALT and ALP levels were significantly increased while total proteins were significantly decreased in diabetic control group in comparison of normal control as shown in Table V. Treatment of diabetic rats with AEWC (250 mg kg^{-1} and 500 mg kg^{-1}) for 28 days showed significant reduction in serum ALP levels. Serum ALT and AST levels were reduced by oral administration of higher dose of AEWC (500 mg kg^{-1}). There was significant improvement in total proteins in diabetic rats treated for 28 days with AEWC (250 and 500 mg kg^{-1}).

Body weight of rats in diabetic control group was significantly decreased at the end of 28 days as compared

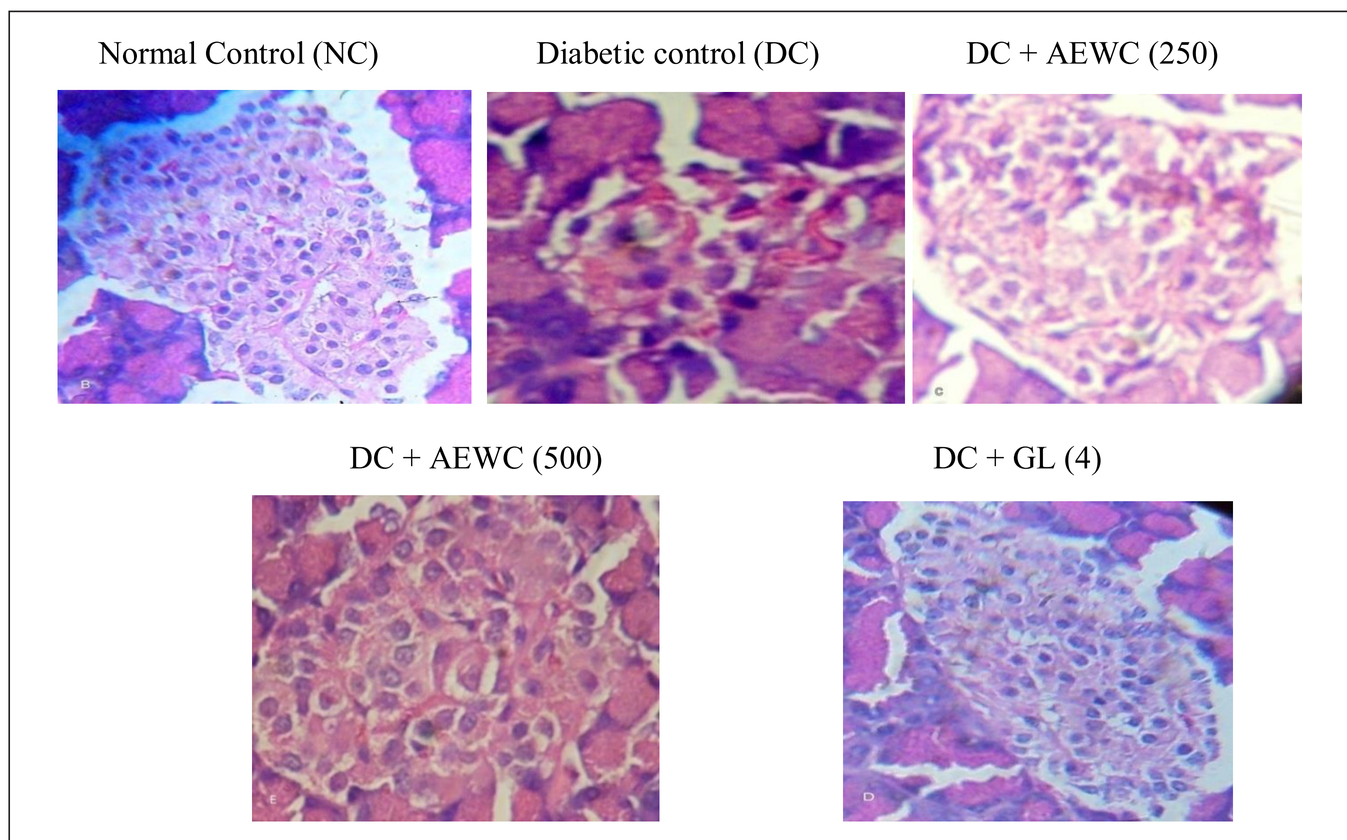

Fig. 1: Effect of AEWC on histopathology of pancreas in STZ-diabetic rats

Figure shows photomicrographs of histopathological sections of rat pancreas of different experimental groups; NC, Normal Control; DC, Diabetic Control; AEWC, Aqueous extract of W. coagulans fruits; GL, Glibenclamide; Photomicrographs are taken using electronic bilobed microscope (Labomed, Mumbai) under 40X magnification

Table I: Effect of AEWC on fasting blood glucose in STZ-induced diabetes in rats

Experimental Group	Fasting blood glucose				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
NC	75.33±3.36	74.5±2.72	71.33 ±3.21	75.33±2.7	76.5±2.79
DC	293.83±7.38[a]	298.3±7.38[a]	305.16 ±8.6[a]	295.8±8.09[a]	311.5±9.25[a]
DC + AEWC (250 mg kg^{-1})	295.16±7.28	254.8±5.91**	224.7±8.5**	199.33±5.1**	187.5 ±9.0**
DC+ AEWC (500 mg kg^{-1})	291.16±6.86	239.33±7.9**	210.8±6.6**	161.66±7.5**	149.6±6.94**
DC+ GL (4 mg kg^{-1})	294.33±6.91	215.16±8.7**	193.2±5.1**	146.5±6.97**	130.7±6.0**

*Values are expressed as mean ± S.E.M of six rats per group; NC, Normal control; DC, Diabetic control; AEWC, Aqueous extract of W. coagulans fruits; GL, Glibenclamide; Data analyzed by one way analysis of variance (ANOVA) followed by Dunnett's multiple test of comparison; [a]p<0.01 Significant difference of diabetic control from normal control; **p<0.01, *p<0.05 Significant difference of treated groups from diabetic control*

Table II: Effect of AEWC on lipid profile in STZ- induced diabetes in rats

Experimental Group	Lipid Profile				
	T-CH	TG	HDL-CH	LDL-CH	VLDL-CH
NC	79.5±2.24	63.66±1.87	29.83±1.19	36.93±2.61	12.73±0.37
DC	195.33±2.77[a]	168.5±3.34[a]	20.16±1.13[a]	141.46±4.2[a]	33.7±0.66[a]
DC + AEWC (250 mg kg^{-1})	130.83±2.7**	120.33±2.6**	23.66±1.02	83.1±3.45**	24.06±0.51**
DC+ AEWC (500 mg kg^{-1})	104.16±3.1**	81.66±1.98**	25.16±0.48*	62.7±3.56**	16.33±0.4**
DC+ GL (4 mg kg^{-1})	90.66±2.21**	75.16±1.77**	26.16±1.1**	49.46±2.86**	15.03±0.4**

*Values are expressed as mean ± S.E.M of six rats per group; NC, Normal control; DC, Diabetic control; AEWC, Aqueous extract of W. coagulans fruits; GL, Glibenclamide; T-CH, Total cholesterol; TG: Triglycerides; HDL-CH, High density lipoprotein cholesterol; LDL, Low density lipoprotein cholesterol; VLDL, Very low density lipoprotein cholesterol;. Data analyzed by one way analysis of variance (ANOVA) followed by Dunnett's multiple test of comparison; **p<0.01, *p<0.05 Significant difference of treated groups from diabetic control*

Table III: Effect of AEWC on biochemical parameters in STZ-induced diabetes in rats

Experimental Group	Liver glycogen (g $100g^{-1}$)	Glycosylated Hb (%)	Serum insulin (μIU mL^{-1})	Glucose uptake by diaphragm (mg 100 g^{-1})
Normal Control (NC)	3.73±0.17	5.7±0.2	3.5±0.03	13.66±0.62
Diabetic control (DC)	0.78±0.03[a]	9.5±0.4[a]	1.73±0.04[a]	5.31±0.14[a]
DC + AEWC (250 mg kg^{-1})	2.26±0.1**	8.23±0.3**	2.4±0.04**	5.95±0.19*
DC+ AEWC (500 mg kg^{-1})	2.93±0.07**	7.13±0.36**	2.9±0.08**	7.8±0.17**
DC+ GL (4 mg kg^{-1})	3.15±0.14**	6.25±0.27**	3.1±0.05**	12.1±0.68**

*Values are expressed as mean ± S.E.M of six rats per group; NC, Normal control; DC, Diabetic control; AEWC, Aqueous extract of W. coagulans fruits; GL, Glibenclamide; Data analyzed by one way analysis of variance (ANOVA) followed by Dunnett's multiple test of comparison; [a]p<0.01 Significant difference of diabetic control from normal control; **p<0.01, *p<0.05 Significant difference of treated groups from diabetic control*

Table IV: Effect of AEWC on liver SOD and CAT in streptozotocin induced diabetes in rats

Experimental Group	SOD (Units $min^{-1}mg^{-1}$ protein)	CAT (moles of H_2O_2 consumed $min^{-1}mg^{-1}$ protein)
Normal control (NC)	5.83±0.15	40.16±1.78
Diabetic control (DC)	2.0±0.09[a]	26.33±1.28[a]
DC + AEWC (250 mg kg^{-1})	4.45±0.12**	29.66±1.54
DC+ AEWC (500 mg kg^{-1})	5.21±0.08**	33.66±1.78**
DC + GL (4 mg kg^{-1})	5.5±0.16**	35.83±1.25**

*Values are expressed as mean ± S.E.M of six rats per group; NC, Normal control; DC, Diabetic control; AEWC, Aqueous extract of W. coagulans fruits; GL, Glibenclamide; SOD, Superoxide dismutase; CAT, Catalase; Data analyzed by one way analysis of variance (ANOVA) followed by Dunnett's multiple test of comparison; [a]p<0.01 Significant difference of diabetic control from normal control;**p<0.01, Significant difference of treated groups from diabetic control*

Table V: Effect of AEWC on AST, ALT, ALP and total proteins in STZ-induced diabetes in rats

Experimental Group	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})
Normal Control (NC)	73.5±2.59	43.66±3.56	140.33 ±4.53
Diabetic control (DC)	130.16±3.55[a]	105.66±4.1[a]	250.83±5.52[a]
DC + AEWC (250 mg kg^{-1})	128.83±3.0	95.33±4.82	215.66±4.11[**]
DC+ AEWC (500 mg kg^{-1})	100.3±5.19[**]	71.16±2.6[**]	193.3±6.28[**]
DC+ GL (4 mg kg^{-1})	98.33±2.69[**]	50.83±3.02[**]	165.5±4.97[**]

Values are expressed as mean ± S.E.M of six rats per group; NC, Normal control; DC, Diabetic control; AEWC, Aqueous extract of W. coagulans fruits; GL, Glibenclamide; AST, Aspartate transaminase; ALT, Alanine transaminase; ALP, Alkaline phosphatase; Data analyzed by ANOVA followed by Dunnett's multiple test for comparison; [a]p<0.01:Significant difference of diabetic control from normal control; [**]p<0.01,[*]p<0.05 Significant difference of treated groups from diabetic control

Table VI: Effect of AEWC on body weight, food intake, water intake and urine output in streptozotocin induced diabetes in rats

Experimental Group	Body weight (g)	Food (g day^{-1})	Water (mL day^{-1})	Urine (mL day^{-1})
Normal control (NC)	256±3.0	20±1.73	38±2.06	15±1.82
Diabetic control (DC)	163.83±2.89[a]	35±2.12[a]	62±1.46[a]	25±1.52[a]
DC + AEWC (250 mg kg^{-1})	194.83±4.67[**]	28.3±3.2	56±0.9	21±1.15
DC+ AEWC (500 mg kg^{-1})	204.33±3.19[**]	23±2.1[**]	51±3.38[**]	18±1.69[**]
DC + GL (4 mg kg^{-1})	215.66±1.8[**]	21±1.52[**]	44.6±2.2[**]	15±1.21[**]

*Values are expressed as mean ± S.E.M of six rats per group; NC, Normal control; DC, Diabetic control; AEWC, Aqueous extract of W. coagulans fruits; GL, Glibenclamide; Data analyzed by one way analysis of variance (ANOVA) followed by Dunnett's multiple test of comparison; [a]p<0.01:Significant difference of diabetic control from normal control; **p<0.01, *p<0.05 Significant difference of treated groups from diabetic control*

to normal control. Treatment of diabetic rats with AEWC (250 and 500 mg kg^{-1}) significantly improved the body weight. Diabetic control group showed significant increase in food and water intake with simultaneous increase in urine output as compared to normal control, as shown in Table VI. Diabetic rats treated with AEWC (250 and 500 mg kg^{-1}) showed considerable reduction in food and water intake with simultaneous decrease in urine output.

As shown in Fig. 1, STZ-treated pancreatic sections showed clear decrease in the area occupied by the beta cells, probably due to reduction in the number of beta cells. Sections of pancreas treated with AEWC have clearly shown the protective effect on histology of pancreatic cells.

DISCUSSION

Sedentary life style of the modern era, lack of exercise, high stress, irregular routine of life, poor food habits and obesity have been identified as the major causes of diabetes to become epidemic. Diabetes mellitus is very often associated with a marked increase in well known parameters of cardiovascular risk including hypertension, hypertriglyceridemia and low levels of high density lipoprotein cholesterol[21]. The goals of managing diabetes mellitus are therefore to optimize the control of blood glucose and to normalize disturbances in lipid metabolism that could predispose patients to cardiovascular complications. For any preclinical evaluation, it is an important prerequisite that an experimental model of the human disease should be available. An experimental model can be produced by means of chemical agents that are having specific beta cytotoxic effects in islet of Langerhans. For the present study, we have used streptozotocin induced diabetes model in male Wistar rats. It is now well established that STZ selectively destroys the pancreatic beta cells

irreversibly, causing degranulation or reduction of insulin secretion and produces hyperglycemia[22-23]. It has been reported that in this model of non-insulin dependent diabetes mellitus (NIDDM), insulin is markedly depleted, but not absent[24-25]. The presence of trace levels of insulin in the plasma of STZ-induced diabetic rats suggests the presence of residual beta cell activity to incomplete destruction of all the islet cells by STZ treatment. Hence, the animals survived without insulin treatment and showed improvement by glibenclamide, which acts by stimulating residual beta cells of the pancreas[26]. Prolonged exposure of pancreatic β-cells to high glucose levels is known to cause β-cell dysfunction, called glucose toxicity. Such damaged β-cells often display extensive degranulation and are clinically associated with the development of non-insulin dependent diabetes mellitus[27].

Administration of AEWC produced a decrease in blood glucose that was observed after one week of drug treatment and continued up to four weeks. To find out the possible mechanism by which AEWC showed antidiabetic effect, we studied various biochemical parameters amongst which serum insulin is an important one. Ability of AEWC to improve serum insulin levels in diabetic rats indicates ability of AEWC in stimulation of beta cells and releasing insulin. Protection of beta cells could be, at least in part, a result of the reduction in blood glucose, thereby eliminating glucotoxicity of beta cells. The involvement of pancreatic mechanism is further supported by the pancreatic histology which showed protection of pancreatic beta cells of rats treated with AEWC.

The risk of coronary heart disease is increased in diabetes mellitus due to profound alterations in plasma lipids and lipoprotein profile. It is well known that the level of glycemic control is the major determinant of serum level of very low density lipoproteins and triglycerides[28]. In the present study, streptozotocin-induced diabetic rats exhibited clear cut abnormalities in lipid metabolism as shown by significant disturbances in lipid profile. The rise in the plasma lipids, especially triglycerides, in diabetic rats indicates increased mobilization of free fatty acids from peripheral depots through a mechanism called the glucose-fatty acid cycle[29]. Total cholesterol reducing potential of AEWC indicates that the extract may help to increase transport of peripheral tissue cholesterol to liver and thereby decrease blood cholesterol level. The effect of plant extract on diabetic hypercholesterolemia and hypertriglyceridemia could be through its control on hyperglycemia. This is in agreement with the fact that the level of glycemic control is the major determinant of total and very low density lipoprotein triglyceride concentrations[30]. Reduction of triglycerides following treatment with AEWC would also facilitate the glucose oxidation, its utilization and subsequently the reduction of hyperglycemia. AEWC by its ability to decrease the levels of LDL-cholesterol and triglycerides in diabetic rats may play a role in alleviating cardiovascular complications of diabetes such as atherosclerosis and coronary heart disease. Thus glucose lowering action of AEWC could be due to its consequence of an improved lipid metabolism apart from the direct effect on glucose homeostasis.

Under diabetic conditions, reactive oxygen species are produced mainly through glycation reactions, which occur in various tissues and may play an important role in the development of diabetic complications[31]. To establish a scientific basis for the utility of AEWC in the treatment of diabetes, it was decided to evaluate *in vivo* antioxidant potential of the extracts by estimating liver superoxide dismutase (SOD) and liver catalase (CAT) enzymes. Superoxide dismutase is an important defense enzyme that catalyses the dismutation of superoxide radicals. Catalase is the hemoprotein that reduces the hydrogen peroxide produced by dismutation reaction and prevents generation of hydroxyl radicals thereby protecting the cellular constituents from oxidative damage in peroxisomes[32]. In the present study, a significant decrease in SOD and CAT activities is observed in the liver of diabetic rats when compared with normal control. Improvement in liver SOD and CAT enzymes after treatment with AEWC indicates its beneficial action in scavenging reactive oxygen species and thereby inhibiting lipid peroxidation. Thus, the therapeutic utility of AEWC may also be partly due to its ability to decrease the generation of reactive oxygen species, a process that results in oxidative stress and aggravates the diabetic state. In addition to the antihyperglycemic and antihyperlipidemic effects, AEWC also possesses antioxidant potential that may be beneficial for correcting the hyperglycemia and preventing the diabetic complications that may occur due to lipid peroxidation and free radicals.

Glycosylated hemoglobin is a good measure to indicate the average blood glucose concentration over the preceding weeks while a single glucose determination gives a value which is true only at the time the blood sample is drawn[33]. The present study showed increased HbA_{1c} levels in the diabetic control group. The AEWC treatment showed improvement in glycosylated hemoglobin as compared to diabetic control. Therefore, prolonged intake of AEWC may further reduce HbA_{1c} levels and probably help in achieving better glycemic control.

The estimation of glucose content in rat hemi-diaphragm is a commonly employed and reliable method for *in vitro* study of peripheral uptake of glucose. Oral administration of AEWC enhances the uptake of glucose by isolated rat-hemi diaphragm in diabetic rats by increasing secretion of insulin. Thus antihyperglycemic action of AEWC could be due to its consequence of improved peripheral glucose consumption.

The liver plays an important role in buffering the postprandial hyperglycemia and is involved in the synthesis of glycogen. The activation of key enzymes involved in glycogen synthesis appears to be defective in NIDDM animals[34]. Increase in liver glycogen content after administration of the AEWC and glibenclamide in diabetic rats indicates its possible effect through increase in insulin secretion and thereby enhancing the activity of key enzymes of glycogen synthesis and by sensitizing the tissues like liver for uptake of glucose.

Inadequate insulin levels in NIDDM decrease the peripheral uptake of glucose and synthesis of glycogen thereby affecting the body weight of animals. Loss of body weight after administration of STZ may be due to the loss of degradation of structural proteins[35]. Besides reduction in body weight, diabetic rats showed weakness, polyuria and polyphagia. Improvement in body weight after administration of AEWC in diabetic rats indicates improved glycemic control.

Disturbances in carbohydrate, lipid and protein metabolism together with oxidative stress are likely to affect hepatic functions in diabetic condition. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels are used as indicators of hepatocyte damage[36]. ALT and AST enzymes are directly associated with the conversion of amino acids to keto acids[37]. Moreover liver dysfunction causes leakage of these enzymes together with ALP into blood stream in diabetes. Diabetic rats have increased activities of these enzymes in serum that may be due to hepatic damage and insulin deficiency which causes increased gluconeogenesis and ketogenesis[38]. Insulin suppressed the genes that encoded gluconeogenic enzymes. Treatment with AEWC decreased the activities of these enzymes, probably through its insulin secretary activity, its insulin like effect and its antioxidant property. It may be due to presence of flavonoids in the extract which are reported to be hepatoprotective agents[39].

CONCLUSION

From the results of the study, it can be concluded that besides the stimulation of regeneration of islets of Langerhans in pancreas of STZ-diabetic rats, AEWC may reduce glucose absorption from the intestine and it may stimulate the peripheral utilization of glucose by promoting the conversion of glucose into glycogen. The antidiabetic, antihyperlipidemic and antioxidant potential of AEWC may be attributed to the presence of phytochemicals like alkaloids, saponines, tannins and flavonoids present in the extract.

In view of the increasing incidence of diabetes mellitus in rural populations throughout the world, inability of current therapies to control all the metabolic defects of the disease and the great expense of modern therapy, study of *W. coagulans* fruits may prove an alternative strategy for diabetic therapy.

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