Original Article

Anti-hyperlipidemic activity of *Withania coagulans* in streptozotocin-induced diabetes: A potent anti-atherosclerotic agent

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ABSTRACT: Dyslipidemia is one of the most common complications in diabetes mellitus, which increases risk of premature atherosclerosis. Drugs having antihyperlipidemic activity in addition to their hypoglycemic effect in diabetes may be suitable anti-atherosclerotic agents in diabetic patients. The present study was aimed to investigate the anti-atherosclerotic activity of an aqueous extract of *Withania coagulans* (AWC) in terms of atherogenic index (AI) in normal and streptozotocin (STZ)-induced diabetes. AWC (1,000 mg/kg body weight, BW) was orally administrated in normal and STZ (70 mg/kg)-induced diabetic rats and levels of glucose, total cholesterol (CHL), high density lipoprotein (HDL)-cholesterol and triglyceride (TG) levels in the plasma were analyzed spectrophotometrically. BW was measured and AI was calculated in each group. Results show that after sub-chronic dosing, AWC reduced plasma glucose levels both in normal and diabetic rats, while significantly decreasing plasma levels of CHL, HDL and TG only in STZ-induced diabetic rats. Repeated administration of AWC also significantly decreased AI and prevented weight loss in STZ-induced diabetic animals. Hence, AWC showed anti-hyperlipidemic activity in diabetic rats and was suggested to be a suitable candidate for the treatment of atherosclerosis associated with diabetes.

Keywords: *Withania coagulans*, diabetes, anti-hyperlipidemic, hypoglycemic, atherosclerosis

1. Introduction

Diabetes mellitus, an endocrine disorder, is a major source of morbidity in developed countries. The number of people with diabetes mellitus is rapidly increasing worldwide. There is a report by the International Diabetes and Federation in the year 2005 which shows that more than 150 million people are suffering from diabetic disease. Diabetes patients show several complications including coronary insufficiency, cerebrovascular, peripheral vascular disease, neuropathy, retinopathy, and nephropathy *etc.* (*1,2*). Diabetes and its associated complications are the major cause of disability and hospitalization which ultimately results in a significant financial burden. One of the complications of diabetes is dyslipidemia (alterations in the plasma lipid and lipoprotein profile). Dyslipidemia is one of the most common complications in diabetes mellitus, which is found in about 40% of diabetic patients. Abnormality in lipid profile increases risk of premature atherosclerosis, coronary and myocardial infarction (*3*), which is a major cause of cardiovascular (CV) morbidity and mortality in diabetic patients (*4-6*). Thus, an anti-diabetic drug having a favorable effect on lipid profile would be beneficial in the treatment of lipid abnormalities and the accompanying premature atherosclerosis of CV disease in diabetic patients.

Currently there is no oral antidiabetic drug approved for the treatment of diabetes which has a favorable effect on CV disease (*7*). Some of the available oral anti-diabetic drugs are associated with serious adverse effects (*8,9*). Injection of insulin to treat type 1 diabetes has its own limitations (*10*). Thus, there emerges the need for new, relatively non-toxic, therapeutic agents for the treatment of hyperglycemia, which also would be able to correct dyslipidemia and reduce the risk of CV complications of diabetes. With growing emphasis on therapy of dyslipidemia associated with diabetes and a need to develop drugs which do not have side effects and which avoid recurrences of cardiovascular diseases associated with diabetes on drug withdrawal, researchers during the last decade started to investigate several indigenous plants. There is a need to explore more indigenous plants through application of modern evaluation protocols, so as to evaluate the ability of these plants in the treatment of atherosclerosis associated with the diabetes. In ayurveda many indigenous plants have been mentioned and well established as anti-atherosclerotic agents. Yet there

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is a paucity of information regarding the activity of *Withania coagulans* in atherosclerosis. Thus this study was undertaken to fulfill the lacunae in this regard.

Withania coagulans Dunal (Family: Solanaceae) is commonly known as Indian cheese and is found in drier parts of India. Different parts of this plant have been reported to possess a variety of ethnopharmacological activities (*11*). Traditional healers in Varanasi and its surrounding area, use dry fruits of *Withania coagulans* for the treatment of diabetic patients. The hypoglycemic activity of an aqueous extract of the fruit of *Withania coagulans* (AWC) was previously demonstrated in streptozotocin (STZ)-induced diabetic rats (*12*). There is no report showing an anti-hyperlipidemic and anti-atherosclerotic effect of AWC in STZ-induced diabetes.

For this reason, in our present investigation we evaluated AWC for its hypolipidemic activity such as reduction in plasma total cholesterol (CHL) and triglycerides (TG) levels, body weight as well as atherogenic index after oral administration, in normal as well as STZ-diabetic rats, using the latter animals as a model for human type 1 diabetes (*13*) for elucidating its activity in atherosclerosis associated with diabetes. The sulfonylurea, glibenclamide (GLB), and the semi-essential amino acid taurine (TR) were used as reference compounds.

2. Materials and Methods

2.1. *Chemicals*

STZ, taurine and glibenclamide were procured from Sigma-Aldrich, St. Louis, MO, USA. All other chemicals used were of analytical grade.

2.2. *Plant material*

Dried fruits of *Withania coagulans* were purchased from the market and identified by chief botanist of TAMPCOL Arumbakkam, Chennai. The fruits (with persistent calyx and pedicle) were coarsely powdered and boiled with distilled water repeatedly. The aqueous extract was concentrated and dried in a vacuum dessicator. The dried extract, which was dark brown in color with coca-like smell was dissolved in distilled water and used for experimental work.

2.3. *Animals*

Male Albino rats were purchased from Central Drug Research Institute, Lucknow, Uttar Pradesh, India. The body weights of the animal ranged between 175 and 225 g. The rats were housed in polypropylene cages (one in each cage) at an ambient temperature of 25 ± 2°C and 55-60% relative humidity. The animals were acclimatized to in-house conditions and were fed a commercial pellet diet (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The experimental protocol was undertaken in accordance with "Principles of laboratory animal care" (NIH publication number 85-23, revised 1985) guidelines.

2.4. *Experimental induction of diabetes in rats*

Diabetes was induced in animals by intraperitoneal administration of a freshly prepared solution of STZ dissolved in citrate buffer (0.1 M, pH 4.5) at a dose of 70 mg/kg body weight to overnight fasted animals. Blood glucose levels were determined 72 h after STZ injection. Rats with stable (yield = 90-95%) (up to three days) blood glucose levels above 250 mg% were selected to use in further studies.

2.5. *Experimental treatment protocol*

Normal and STZ-diabetic rats were randomly assigned to groups of six each. The acute study consists of four normal and four diabetic groups while subchronic study includes four normal and four diabetic groups. Among the four groups of acute study, one received a single oral dose of distilled water, a second group received AWC at a dose of 1,000 mg/kg of body weight (BW), while the third and fourth groups received the first reference compound taurine (TR) (10 mg/kg) and the second reference drug glibenclamide (GLB) (10 mg/kg) respectively. Similarly, the four groups of STZ-diabetic rats received water, AWC, TR and GLB, at the same doses described above, respectively. Selection of the dose of AWC was based upon a previous study done by Hemalatha *et al.* (*12*) in which AWC at the stated dose showed considerable hypoglycemic activity in normal and diabetic rats. For the sub-chronic treatment, groups of normal and STZ-induced diabetic rats received oral doses (by gavage) of water or the three test materials (AWC, TR and GLB) daily for 28 days. For the acute study, blood was collected before administered dose (0 h) and then at 6 h after the single dose. For the sub-chronic study, blood samples were collected before treatment *i.e.*, for baseline estimation (D0), after 14 days of treatment (D14) and after 28 days of treatment (D28). Plasma, obtained by centrifugation, was stored at −20°C until analyzed for glucose, plasma high density lipoprotein (HDL)-cholesterol and TG levels.

2.6. *Analytical methods*

Blood glucose (collected at 0 and 6 h post dose in the single dose study and 6 h post dose on D0, D14 and D28 in the sub-chronic study), total cholesterol, HDL-cholesterol and triglyceride levels in serum (6 h post dose on D0, D14 and D28 in the sub-chronic study) were measured spectrophotometrically with the Span Diagnostic kit (Span Diagnostics Ltd., Surat, India). Atherogenic index was calculated by using the

following formula (*14*):

Atherogenic index = (CHL – HDL-cholesterol)/ HDL-cholesterol

2.7. *Statistical analysis*

All grouped data were statistically evaluated with SigmaStat 3.5 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Dunnett test for more than two groups while using student's *t*-test for two groups. $p < 0.05$ was considered to indicate statistical significance. All the results were expressed as mean ± S.E.M. for six animals in each group.

3. Results

3.1. *Effect of a single (acute) and sub-chronic oral doses of test substances (AWC, TR, and GLB) on body weight*

Effect of a single (acute) and sub-chronic oral doses of test substances (AWC, TR, and GLB) on body weight are shown in Table 1. In normal rats, body weight significantly ($p < 0.05$) increased after 28 days treatment with distilled water, AWC, TR as well as GLB compared to the baseline (D0) values. There was no significant difference in weights between the treatment groups. However, in the STZ-diabetic rats, a very significant ($p < 0.05$) weight loss occurred in rats that were given water. The TR group also showed a slight but significant ($p < 0.05$) loss in body weight. On the other hand, there was no change in the weight of rats given daily doses of AWC. GLB administration caused a small but significant ($p < 0.05$) increase in body weight.

3.2. *Effect of a single (acute) and sub-chronic oral doses of test substances (AWC, TR and GLB) on plasma glucose levels*

The effect of single (acute) oral doses of water (10 mL/kg) and the test materials (AWC, TR, and GLB) on blood glucose levels in normal and STZ-diabetic rats is presented in Table 2; the values are compared with the baseline values (before treatment). In normal rats, with a single dose of water, AWC caused little decrease (not significant) in plasma glucose levels but a significant ($p < 0.05$) decrease occurred in glucose levels with TR. GLB had no effect. In the diabetic rats, two standard drugs TR and GLB showed significant ($p < 0.05$) hypoglycemia. TR produced normal glycemia post dose. With GLB glucose levels remained slightly higher than the normal values.

The effect of daily oral dosing for 28 days with the test materials (AWC, TR, and GLB) and water at the doses indicated above on blood glucose levels in normal and STZ-induced diabetic rats is presented in Table 3. The values are compared with the baseline values (D0). In normal rats, daily dosing with the AWC reduced plasma glucose significantly ($p < 0.05$) after 14 days as well as 28 days. The hypoglycemic response to TR was significant ($p < 0.05$) after 28 days. Administration of GLB, like water, had no effect on plasma glucose levels. In the diabetic rats, daily dosing with AWC produced significant hypoglycemia ($p < 0.05$) on D14 as well as on D28. Rats became normoglycemic after 2 weeks of daily treatment with levels going down further at the end of the 28 days of treatment. GLB treatment reduced glucose levels on D14, but the decrease was far less than that produced by AWC. Continuous administration of GLB resulted in a further decrease ($p < 0.05$) in glucose levels. After 28 days of treatment, once again the reduction was not as great as with AWC, and the rats did not achieve normoglycemia even after 4 weeks of continuous treatment. TR significantly ($p < 0.05$) reduced glucose levels after 2 weeks. However, normoglycemia was achieved after 28 days of treatment

Table 1. Effect of repeated daily oral treatment with the aqueous extract of *Withania coagulance* (AWC), taurine (TR) and glibenclamide (GLB) on body weight in normal and STZ-induced diabetic rats

Treatment	Dose (mg/kg)	Body weight (g)	
		D0	D28
Normal			
Water	a	201.2 ± 2.8	251.5 ± 5.4*
AWC	1,000	198.4 ± 3.8	236.7 ± 9.6*
TR	10	228.6 ± 1.5	273.1 ± 4.8*
GLB	10	187.4 ± 2.7	218.3 ± 6.3*
STZ-induced diabetic rats			
Water	a	223.3 ± 1.7	191.9 ± 2.4*
AWC	1,000	249.1 ± 10.2	237.0 ± 13.8
TR	10	210.2 ± 1.6	195.5 ± 1.4*
GLB	10	197.6 ± 9.2	224.7 ± 4.3*

The values are expressed as mean ± S.E.M. ($n = 6$). a: 10 mL/kg. * $p < 0.05$, when compared to baseline values. Data of D0 and D28 were analyzed within the treatment groups by *t*-test.

Table 2. The effect of a single oral dose of aqueous extract of *Withania coagulance* (AWC), taurine (TR), and glibenclamide (GLB) on plasma glucose levels after acute oral administration in normal and STZ-diabetic rats

Treatment	Dose (mg/kg)	Plasma glucose level (mg%)	
		Before treatment	After treatment
Normal			
Water	a	94.6 ± 2.9	93.5 ± 3.1
AWC	1,000	93.5 ± 4.5	81.9 ± 2.1
TR	10	95.5 ± 1.6	77.4 ± 0.8*
GLB	10	96.5 ± 1.3	98.3 ± 2.4
STZ-induced diabetic rats			
Water	a	303.4 ± 6.6	288.0 ± 2.5
AWC	1,000	330.3 ± 5.2	307.8 ± 4.7
TR	10	365.2 ± 5.0	106.6 ± 3.6*
GLB	10	370.8 ± 2.5	191.9 ± 9.5*

The values are expressed as mean ± S.E.M. ($n = 6$). a: 10 mL/kg. * $p < 0.05$, when compared to baseline values. Data of treatment groups before treatment and after treatment were analyzed by *t*-test.

with TR. Water had no significant effect on glucose levels. Since acute administration of AWC was not effective, further study was conducted in the chronic administered group.

3.3. *Effect of repeated oral doses (sub-chronic) of test substances (AWC, TR, and GLB) on plasma lipids (CHL, HDL, and TG)*

The effect of oral sub-chronic treatment with AWC, TR, and GLB on plasma CHL levels in normal and STZ-induced diabetic rats are represented in Table 4. In normal rats, repeated daily administration of AWC induced a significant ($p < 0.05$) fall in plasma total CHL levels on the 28th day of continuous treatment. The effect of daily treatment with TR was similar to that of AWC. GLB and water had no significant effect on total CHL levels. In the STZ-diabetic rats all the materials tested (except water) at the daily doses indicated above, showed a significant ($p < 0.05$) hypocholesterolemic effect. AWC induced a significant fall in plasma CHL levels on D14, with an additional decrease occurring with continued treatment on D28. TR was more effective than AWC in decreasing CHL levels on D14, while less so on D28. GLB was less effective than AWC on D28 ($p < 0.05$). Water had no effect.

The effect of oral sub-chronic treatment with AWC, TR, and GLB on plasma CHL levels and HDL levels in normal and STZ-induced diabetic rats are represented in Table 5. Oral sub-chronic treatment with AWC, TR, and GLB are ineffective in plasma HDL level in normal animals while AWC and TR at doses indicated above show a significant ($p < 0.05$) increase in plasma HDL-cholesterol levels on D14 as well as D28 in normal and

Table 3. The effect of sub-chronic oral administration of the aqueous extract of *Withania coagulance* (AWC), taurine (TR), and glibenclamide (GLB) for up to 28 days on plasma glucose levels in normal and STZ-diabetic rats

Days of chronic treatment	Plasma glucose level (mg%)			
	Water (10 mL/kg)	AWC (1 g/kg)	TR (10 mg/kg)	GLB (10 mg/kg)
Normal				
D0	98.2 ± 2.7	103.7 ± 3.1	106.2 ± 3.5	99.6 ± 3.7
D14	100.4 ± 2.0	87.6 ± 1.0*	95.1 ± 2.6	98.2 ± 2.2
D28	91.7 ± 4.7	73.6 ± 2.7*	89.0 ± 3.1*	96.2 ± 3.7
STZ-induced diabetes				
D0	333.6 ± 14.5	363.4 ± 9.9	391.0 ± 16.5	31.0 ± 19.8
D14	372.8 ± 13.8	107.1 ± 10.2*	178.8 ± 9.4*	177.0 ± 8.8*
D28	376.3 ± 10.1	83.1 ± 6.3*	2.9 ± 9.1*	27.6 ± 10.8*

The values are expressed as mean ± S.E.M. ($n = 6$). * $p < 0.05$, when compared to baseline values. Data within the treatment groups was analyzed by one way ANOVA followed by Dunnett test.

Table 4. The effect of sub-chronic oral administration of the aqueous extract of *Withania coagulance* (AWC), taurine (TR), and glibenclamide (GLB) for up to 28 days on plasma total cholesterol levels (mg/dL) in normal and STZ-diabetic rats

Days of chronic treatment	Plasma total cholesterol level (mg/dL)			
	Water (10 mL/kg)	AWC (1 g/kg)	TR (10 mg/kg)	GLB (10 mg/kg)
Normal				
D0	66.2 ± 1.2	62.3 ± 1.1	66.6 ± 2.5	62.4 ± 3.7
D14	65.1 ± 2.1	58.8 ± 1.6	64.8 ± 1.1	60.1 ± 2.3
D28	64.6 ± 2.0	55.2 ± 0.9*	60.0 ± 1.2*	59.6 ± 2.1
STZ-induced diabetes				
D0	131.3 ± 4.3	122.5 ± 3.4	106.3 ± 2.7	116.3 ± 2.2
D14	123.8 ± 3.9	94.6 ± 1.1*	91.2 ± 2.0*	107.3 ± 3.4
D28	115.0 ± 5.1	60.2 ± 1.4*	70.2 ± 1.0*	105.8 ± 2.0*

The values are expressed as mean ± S.E.M. ($n = 6$). * $p < 0.05$, when compared to baseline values. Data within the treatment groups was analyzed by one way ANOVA followed by Dunnett test.

Table 5. The effect of sub-chronic oral administration of the aqueous extract of *Withania coagulance* (AWC), taurine (TR), and glibenclamide (GLB) for up to 28 days on plasma HDL-cholesterol levels (mg/kg) in normal and STZ-diabetic rats

Days of chronic treatment	Plasma HDL-cholesterol level (mg/dL)			
	Water (10 mL/kg)	AWC (1 g/kg)	TR (10 mg/kg)	GLB (10 mg/kg)
Normal				
D0	29.0 ± 0.4	28.8 ± 0.6	28.7 ± 0.9	26.5 ± 0.9
D14	29.7 ± 0.5	29.0 ± 0.3	27.5 ± 0.9	25.9 ± 0.5
D28	28.2 ± 0.4	27.4 ± 0.6	27.0 ± 0.7	25.2 ± 0.5
STZ-induced diabetes				
D0	23.3 ± 0.7	20.3 ± 0.4	20.0 ± 0.1	21.7 ± 0.4
D14	21.1 ± 0.4	26.6 ± 0.4*	22.5 ± 0.3*	22.9 ± 0.9
D28	23.5 ± 0.6	29.0 ± 0.3*	22.5 ± 0.7*	23.8 ± 0.3*

The values are expressed as mean ± S.E.M. ($n = 6$). * $p < 0.05$, when compared to baseline values. Data within the treatment groups was analyzed by one way ANOVA followed by Dunnett test.

STZ-diabetic rats.

The effect of oral sub-chronic treatment with AWC, TR, and GLB, at doses indicated above, on plasma TG levels in normal and STZ-diabetic rats is shown in Figure 1. In normal rats, daily dosing with either AWC or TR reduced TG levels significantly ($p < 0.05$) only after 28 days of treatment (Figure 1A). GLB showed only an insignificant reduction in TG levels, while water had no effect (Figure 1A). In the STZ-diabetic rats, daily administration of either AWC or TR caused a significant ($p < 0.05$) decrease in plasma TG levels on D14 (Figure 1B). The decrease with GLB was insignificant. With continued administration, serum TG values fell further significantly ($p < 0.05$) with AWC and TR on D28 with respect to baseline (D0), while GLB and water had no significant effect.

3.4. *Effect of repeated oral doses (sub-chronic) of test substances (AWC, TR and GLB) on AI (atherogenic index)*

The effect of oral sub-chronic treatment with AWC, TR, and GLB, at doses indicated above, on AI in normal and STZ-diabetic rats is shown in Figure 2. In normal rats, daily dosing with neither AWC, TR nor GLB reduced AI significantly on D14 as well as D28 of continuous treatment. In the STZ-diabetic rats, daily administration of either AWC ($p < 0.05$) or TR ($p < 0.05$) caused a significant decrease in plasma TG levels on D14 (Figure 2B). The decrease with GLB was insignificant. With continued administration, AI values fell further significantly ($p < 0.05$) with AWC and TR on D28, while GLB and water had no significant effect.

4. Discussion

Until now, no previous study has been performed with *Withania coagulans* on dyslipidemia associated with diabetes, which results in premature atherosclerosis and is one of the important causes of CV disease in diabetic patients. In the present study, we investigated, whether AWC has any effect on levels of lipids (plasma CHL and TG), in addition to its hypoglycemic action, in normal and STZ-diabetic rats as well as on the atherogenic index.

One report showed that AWC has a hypolipidemic effect in high fat diets as well as triton induced hypercholesterolemia (*15*). However, the mechanism underlying diabetes induced dyslipidemia is different from hyperlipidemia, resulting from administration of triton and high fat diets in normal

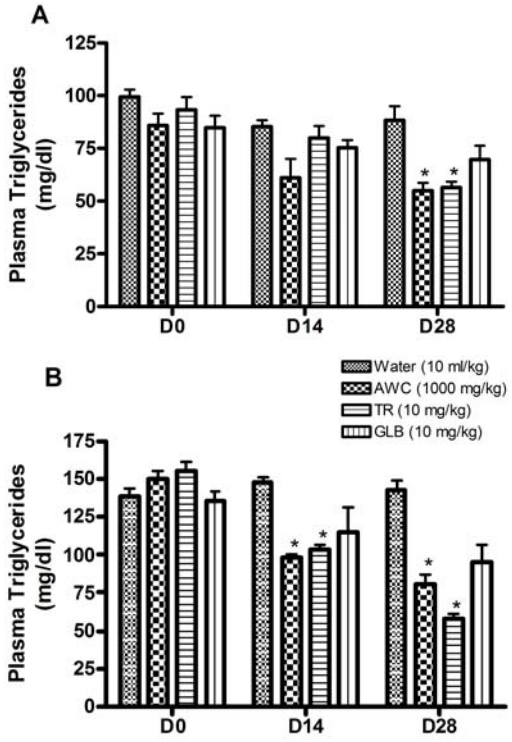

Figure 1. Plasma triglyceride levels (mg/dL) at baseline (D0), on 14th day (D14) and on 28th day (D28) after repeated daily oral administration (sub-chronic dosing) of water (10 mL/kg/day), aqueous extract of *Withania coagulace* (AWC) (1,000 mg/kg/day), taurine (TR) (10 mg/kg/day) and glibenclamide GLB (10 mg/kg/day) in normal (A) and STZ-diabetic (B) rats. Values are expressed as mean ± S.E.M. ($n = 6$). * $p < 0.05$ compared with respective baseline values (D0). Data within the treatment groups was analyzed by one way ANOVA followed by Dunnett test.

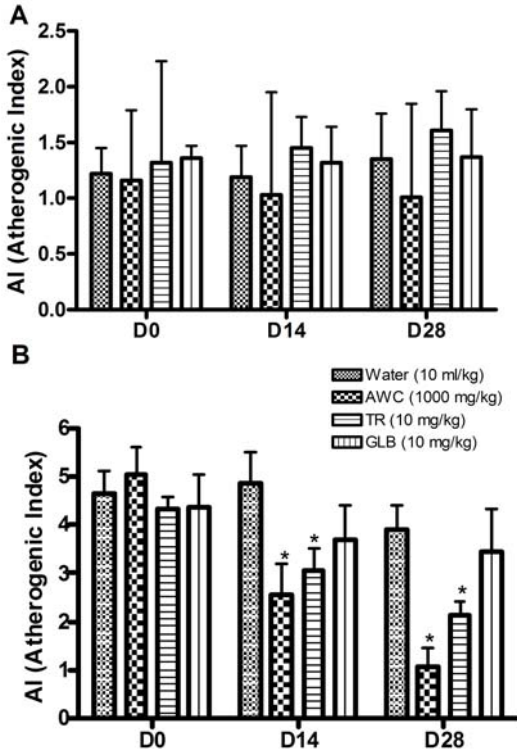

Figure 2. Atherogenic index (AI) at baseline (D0), on 14th day (D14) and on 28th day (D28) after repeated daily oral administration (sub-chronic dosing) of water (10 mL/kg/day), aqueous extract of *Withania coagulace* (AWC) (1,000 mg/kg/day), taurine (TR) (10 mg/kg/day) and glibenclamide (GLB) (10 mg/kg/day) in normal (A) and STZ-diabetic (B) rats. Values are expressed as mean ± S.E.M. ($n = 6$). * $p < 0.05$ compared with respective baseline values (D0). Data within the treatment groups was analyzed by one way ANOVA followed by Dunnett test.

animals. Triton first causes a sharp increase in serum cholesterol levels (phase I) followed by a decrease in hypercholesterolemia nearly to control levels (phase II). The mechanism of triton induced hypercholesterolemia in phase I is thought to be due to increased hepatic synthesis of cholesterol through the ability of triton to interfere with the uptake of plasma lipids by the tissues. Drugs interfering with cholesterol biosynthesis were shown to be active in phase I, while those interfering with cholesterol excretion and metabolism were active in phase II triton induced hyperlipidemia (*16-19*).

In high fat diets, higher levels of glucose and lipid are maintained in blood for a longer duration of time and result in insulin resistance. STZ-induced diabetes mimics type 1 diabetes where insulin secretion decreases greatly. Therefore, drugs active in STZ induced diabetes and high fat diets are effective in decreased insulin secretion as well as in insulin resistance (*20-23*). Thus different models are used for screening different antihyperlipidemic drugs. Moreover, different hypercholesterolemia affecting drugs show different activities using different tests (*17*). Therefore, the study of the effect of drugs on lipid profiles in different models reveals a clearer picture about their mechanism of action.

Induction of diabetes in rats by STZ, also led to the development of dyslipidemia (hypercholesterolemia and hypertriglyceridemia) (Tables 4 and 5) and weight loss as has been reported previously (*24,25*). Administration of daily doses of AWC protected the STZ-diabetic rats from weight loss, which was not the case with rats given water, TR or GLB (Table 1). A single oral dose of AWC and water had no effect on glucose levels in normal as well as in STZ-induced diabetes. TR caused lowering of glucose levels in normal as well as STZ-induced diabetes. GLB had no effect on normal animals and caused a lowering of glucose levels in STZ-induced diabetes (Table 2). Daily dosing with AWC (sub-chronic study) caused a gradual decrease in plasma glucose in normal rats, which became significant on D28 (Table 3). In the diabetic rats, daily dosing with AWC produced a significant euglycemic effect on D14, with glucose levels going down further by D28. GLB was less potent and not able to decrease glucose levels up to normal. TR had a slower onset of action and caused a euglycemic effect on D28 (Table 3). The lower hypoglycemic response in normoglycemic animals compared to diabetic rats may be due to homeostasis mechanisms of glucose/carbohydrate metabolism (*26*). AWC and TR reduced TG levels in normal rats, while TR performed at a slower rate (Figure 1A); GLB and water had no effect. In the diabetic rats, daily dosing with either AWC or TR reduced TG levels significantly, and the effect increased with repeated dosing (Figure 1B). GLB had no significant effect. Thus, our study shows that repeated oral administration of AWC caused significant hypolipidemia, especially in the diabetic rats.

The mechanism(s) of hypolipidemic and hypoglycemic actions of AWC are not known. However, in view of the similar reduction in plasma glucose as well as the hypolipidemic effect of AWC and TR, the mechanism of hypoglycemic action of AWC may be like that of TR, which involves insulin and in turn causes increased glucose utilization *via* insulin sensitization in peripheral tissues (*27,28*). This also normalizes plasma lipids in STZ-diabetic rats (*25*). Plants like *Momordica charantia* fruit (*29*) and *Scoparia dulcis* leaves (*30*) also show similar effects. The mechanism of action of AWC is not like that of the sulfonylurea GLB, since it has only a weak hypolipidemic action. Additionally its hypolipidemic activity in triton induced as well as high fat diet induced hyperlipidemia also reveals its interference in synthesis, metabolism and excretion of lipids. Insulin receptor sensitization would also explain its mechanism of action.

The chronic elevation of glucose levels in diabetes causes oxidative stress (*31*). It leads to protein oxidation, glycation (*32*), and dyslipidemia. Together they play a significant role in the manifestation and development of premature atherosclerosis. Thus drugs effective in lipid profiles in diabetic animals are assumed to be effective in protection from atherosclerosis. It is further supported by the result of the atherogenic index (Figure 2) which shows AWC decreases the AI significantly in STZ-induced diabetes.

A preliminary phytochemical analysis of AWC revealed that it contains several withanolides and lactones (*33-35*). There is report which shows that withanolides are effective in cardiovascular complications (*36*). Thus hypolipidemic and hypoglycemic activities of AWC may be attributed to one or more of the identified or as-yet-unidentified compounds. Studies are still in progress to isolate and identify the active principle(s) of *Withania coagulans*, which may be valuable in the treatment of dyslipidemia and atherosclerosis in diabetic patients.

It can be concluded that AWC exhibited strong hypolipidemic activity in addition to its hypoglycemic action in diabetic animals. This has clinical implications in that the relatively nontoxic AWC, if used as a hypoglycemic agent, may also reverse dyslipidemia associated with diabetes (types 1 and 2), and prevent the CV complications which are very prevalent in diabetic patients. Our results suggest that AWC has the potential to be a suitable candidate for further investigations as an anti-atherogenic agent in humans with diabetes. Further studies are warranted to confirm our results and fractionate AWC to isolate and identify the active principle(s), and to determine the exact mechanism(s) of action.

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