

Effect of *Holostemma annularis* on the progression of diabetes induced by a high fructose diet in rats and in diabetic C57BL/6J *ob/ob* mice

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Abstract: The roots of *Holostemma annularis* K. Schum (Asclepiadaceae) are used in traditional medicine to treat diabetes. This medicinal plant, widely used in more than 34 ayurvedic preparations, was evaluated in a high fructose diet in induced insulin resistance and in C57BL/6J *ob/ob* diabetic mice for its antidiabetic activity. Graded doses of both chloroform and methanolic extracts of the roots of *H. annularis* were administered to normal and experimental diabetic rats for 21 days. Serum glucose, triglycerides, cholesterol levels and total protein in urine were analyzed. Significant results were observed in the estimated parameters. These data justify the use of the plant in the treatment of diabetes mellitus and is a potential source for the isolation of new active agents for diabetes mellitus.

Keywords: *Holostemma annularis*, high fructose diet, antihyperglycemic, C57BL/6J *ob/ob* mice

Introduction

Diabetes is associated with sustained high glucose concentrations in the blood beyond a certain level that leads to long term damage, dysfunction and failure of various organs including eyes, kidney, nerves, heart and blood vessels. Insulin plays a central role in the regulation of glucose homeostasis and acts in a coordinated fashion on cellular events that include the regulation of ion and amino acid uptake, protein synthesis and degradation, gene transcription and mRNA turnover, and cellular growth and differentiation.^{1,2} An impairment of insulin action (insulin resistance) is involved in many diseases, including noninsulin dependent diabetes, obesity, hypertension and cardiovascular disease.³ Development of safe antidiabetic agents is still a challenge for scientists working in this area. The development of new treatment modalities requires animal models that mimic the range of pathophysiological changes seen in diabetic humans.⁴ The most common is the rat model because of the complications of diabetes induced by streptozotocin. However, streptozotocin induces type 1 diabetes and experimental results from this model may be relevant only to a small proportion of diabetic patients. Type 2 diabetes is associated with complications such as hypertension, endothelial damage, cardiac hypertrophy, inflammation, atherosclerosis, ventricular contractile dysfunction, fibrosis, retinopathy, neuropathy and nephropathy. Diet-induced models of type 2 diabetes, rather than the streptozotocin-induced model of type 1, may serve as a better vehicle to investigate possible interventions for these complications.⁵ Both human and animal studies have shown that fructose is a highly lipogenic nutrient that contributes to insulin resistance, metabolic defects and development of a pre-diabetic or diabetic state.⁶ High fructose diet in rats ($\geq 60\%$ of the

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diet) has been used to induce cardiovascular symptoms such as hypertension, hypertriglyceridemia, increased collagen deposition in the heart and kidneys associated with increased oxidant concentration and decreased antioxidant defences.⁷⁻⁹ These features are almost identical to clinical type 2 diabetes. Hence high fructose diet-induced diabetes in rats is used to evaluate antidiabetic drugs. This study was designed to examine the possibility of the antidiabetic effect of *Holostemma annularis* K. Schum (Asclepiadaceae) (HA) in diabetic C57/BL6J *ob/ob* mice; which was considered a good model for type 2 diabetes as it displays many of the characteristics of the human diseases including hyperglycemia, insulin resistance and progressive obesity.¹⁰ In humans, the occurrence of type 2 (non-insulin dependent) diabetes mellitus has been related to a strong genetic influence. In mice, the autosomal recessive diabetes (*db*) mutation results in metabolic changes similar to those observed in type 2 diabetes in humans. The relative diabetes susceptibility observed among certain inbred strains carrying either the *db* mutation on chromosome 4, or the obesity (*ob*) mutation on chromosome 6, provides evidence of genetic differences. While the nature of this genetic influence is unknown, both *db/db* and *ob/ob* mice exhibit profound resistance to insulin.¹¹ There is increasing evidence that indicates that oxidative stress produced under hyperglycemia can cause, or lead to, insulin resistance and diabetic complications.¹²

Moreover, several studies have shown that antioxidants ameliorate a number of altered physiological and metabolic parameters that occur as a result of type 2 diabetes.^{13,14} The roots of HA, which is reported to have a potent antioxidant property¹⁵ and used traditionally in Indian system of medicine to treat diabetes, was selected to screen for possible antidiabetic activity in high fructose diet-induced and C57BL/6J *ob/ob* diabetic mice. Also the roots are reported to contain β -sitosterol, lupeol, and alpha-amyrin as the main constituents which are reported to have both antioxidant and antidiabetic properties.^{16,17} The roots of this plant are also reported to have an antidiabetic effect in streptozotocin-induced diabetic rats (Type-1).¹⁸ However, there is no available evidence of such an effect of HA root in type 2 diabetes or an insulin resistant animal model. Based on these profiles of HA, work was undertaken to screen the roots of the plant for its antidiabetic activity in type 2 diabetes.

Material and methods

Preparation of plant extract

Roots of HA were collected from the Herbal Folk Research Centre (Reg. No. 37 of 1998) Tirupathi, Andhra Pradesh

(India). It was authenticated by taxonomist Prof. Vedhavathy, and a voucher specimen was deposited in SV University. The roots were dried at room temperature, until moisture free. Finally, the dried roots were powdered coarsely and then passed through a sieve (no. 44) to obtain a uniform powder. The coarse powder obtained was extracted with petroleum ether to remove any fatty substances; the marc was further extracted exhaustively with chloroform and methanol separately in a Soxhlet apparatus and filtered. Excess solvent was completely removed by using a rotary flash evaporator to obtain a chocolate colored semisolid extract. The obtained semisolid mass was completely dried in mini lyophilizer.

Chemicals and instruments

Streptozotocin was purchased from Cisco Research Laboratories Ltd, (Mumbai, India). Glibenclamide was obtained as gift sample from Ranbaxy Laboratories Ltd. Fructose, beef extract, sodium chloride, and potassium chloride, were purchased from SD Fine Chemicals Ltd, (Mumbai). All chemicals used in this study were of analytical grade. Auto-Analyzer (Star 21 plus) was used to estimate various biochemical parameters using standard procedures. A Mini Lyophilizer (LTE Scientific Ltd, UK) and rotary flash evaporator were used for preparing extracts.

Animals

Healthy adult male 5- to 6-old Wistar albino rats (180–200 g) were used for the high fructose diet model. C57BL/6J mice 10–14 weeks old and (weighing 50–60 g) were procured from the National Institute of Nutrition, Hyderabad and used as the diabetic *ob/ob* mice model. For toxicity evaluation mice were procured from HSK College of Pharmacy, Department of Pharmacology (Bagalkot, Karnataka, India). The rats and mice were housed in polypropylene cages and maintained under suitable nutritional and environmental (12-hour light–12-hour dark cycle: $25 \pm 3^\circ\text{C}$ and 35%–60% humidity) conditions throughout the experiment. All the experimental protocols were approved by the institutional animals' ethics committee (HSKCP/IAEC. Clear 2004–05. Dated: 27/12/2004), HSK College of Pharmacy, (Bagalkot, Karnataka, India).

Toxicity evaluation in mice

The alcohol and chloroform extracts were tested for their acute and short term toxicity in mice. To determine acute toxicity of a single oral administration of the HA extract, different doses (0.5, 1.0, 1.5, 2 g/kg) were administered to

different groups of mice (2 mice were used for each group, control mice received Tween 80). Mortality and general behavior of the animals was observed periodically for 48 hours. The animals were observed continuously for the initial 4 hours and intermittently for the next 6 hours and then again at 24 hours and 48 hours following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion. To study short term toxicity, 3 groups of mice each containing 6 male mice (20–25 g, body weight [bw]) were used. Group I was kept as control and group II and group III received 200 and 400 mg/kg of HA extracts in Tween 80. The drug was administered daily for 14 days per os (po). The control group received 5% Tween 80 in an identical manner. The behavior of the animals was observed daily for 1 hour in the forenoon for 14 days. Initial and final bw, water and food intake, state of stool and body temperature were observed. The animals were killed on the 15th day. Hematological and serum biochemical parameters such as hemoglobin content, white blood cells (WBC), glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase (ALP) were determined. Liver, spleen, kidneys and brain were dissected and weights of these organs were determined. Both extracts did not produce any behavioral changes, biochemical changes and mortality up to the dose of 3000 mg/kg bw. Hence 1/10th of this dose (high dose) was used for the study.¹⁹

High fructose diet

The high fructose diet contained fructose (624 g/kg diet), fat as vegetable oil (50 g/kg diet), protein (223 g/kg diet) and a 1:3.6 sodium/potassium ratio. The standard diet contained 1:3 sodium, potassium rodent chow diet containing vegetable starch (527 g/kg diet), fat as vegetable oil (35 g/kg diet), animal protein (220 g/kg diet) and a 1:3.3 (3 g Na⁺/kg diet and 10 g K⁺/kg diet).²⁰

High fructose diet-induced diabetic rat model

Male Wistar rats (180–200 g) were randomly divided into 7 groups. Group I served as solvent control and received the vehicle (2% Tween 80) at 0.5 mL/kg bw and received the standard diet. Group II served as positive control and received glibenclamide (5 mg/kg bw po), along with the high fructose diet. Group III (diabetic control) received the high fructose diet alone. Group IV and V received the freshly prepared chloroform extract of HA (CEHA) suspension in 2% Tween 80 at two different dose levels, 150 mg/kg and

300 mg/kg bw po along with high the fructose diet. Group VI and VII received the freshly prepared methanol extract of HA (MEHA) suspension in 2% Tween 80 at two different dose levels, 150 mg/kg and 300 mg/kg bw po along with the high fructose diet. The animals were treated for 21 days. The animals were kept in metabolic cages and urine was collected for estimation of total urine protein. On days 1, 7, 14 and 21 animals were anesthetized and blood was collected by retro-orbital puncture for the assessment of the activity.

C57 BL/6J *ob/ob* diabetic mice model

Thirty-six diabetic C57BL/6J *ob/ob* mice 10–14 weeks old were randomly divided into six groups. Group I received a standard diet for 21 days. Group II served as positive control and received glibenclamide (5 mg/kg po), Group III and IV received CEHA at two different dose levels, 150 mg/kg and 300 mg/kg bw po, Group V and VI received MEHA at two different dose levels 150 mg/kg and 300 mg/kg bw po. All the animals received standard diet along with drug treatment for 21 days. On days 1, 10, and 21 animals were anesthetized and blood was collected by retro-orbital puncture for biochemical estimation and assessment of activity.

Glucose tolerance test

As described by Babu and colleagues.²¹

Biochemical analysis

Blood was collected in heparin-coated tubes and centrifuged at 3000 rpm for 10 minutes. Serum glucose level was estimated by GOD-POD method²¹ using a commercial kit (Span Diagnostics, India). Serum triglycerides and total cholesterol levels were measured according to Allian et al²² and Friedewald et al.²³ Total protein in urine was estimated by protein CSF kit using auto-analyzer.

Statistical analysis

The statistical analysis was carried out by one-way analysis of variance (ANOVA). The values are means ± standard error of mean (SEM). Comparison of mean values of different groups treated with different dose levels of extracts and positive control with normal were estimated by Tukey's multiple comparison test. *P* values less than 0.05 were considered to be statistically significant.

Results

Body weight changes

There was no significant difference in mean bw values between the fructose-fed control and HA-treated groups

(260 ± 10 g, 258 ± 7 g and 256 ± 8 g, respectively) during the 21 days of the experimental period. Similar results were observed in the diabetic *ob/ob* mice model.

Effect of HA extracts in high fructose diet-induced change in serum and urine biochemical parameters

Fructose feeding significantly increased serum glucose when compared to normal rats (188 ± 7.2 mg/dL, $P < 0.001$ [Table 1]). Administration of chloroform and methanolic extract of HA (150 and 300 mg/kg) along with fructose feeding significantly ($P < 0.001$) reduced the serum glucose values in a dose dependent manner during 21 days of the experimental study when compared to the group fed fructose alone (Table 1). Hypertriglyceridemia and hypercholesterolemia are common features in animal models of insulin resistance induced by a high fructose diet.^{24,25} Increased levels of triglycerides and cholesterol are the main predictors and/or causative agents for inducing the insulin resistance in type 2 diabetes. The triglyceride and cholesterol levels were significantly higher ($P < 0.001$, 153.83 ± 1.7 g/dL, $P < 0.05$, 89.01 ± 7.39 g/dL, respectively) in the diabetic control group than in the normal animals. In the HA administered group of animals the triglyceride (83.33 ± 6.3 g/dL, $P < 0.001$) and cholesterol levels (72.60 ± 6.57 g/dL, $P < 0.05$) were significantly lower

than in the diabetic control group of animals (Table 1). On day 21 the urine analysis was performed. The total protein in the urine of the HA (300 mg/kg)-treated group of animals reduced significantly (1.2 ± 0.1 g/dL, $P < 0.05$) (Table 1), suggesting partly the nephroprotective activity of the drug. Low dose of HA (150 mg/kg) was not significant in showing nephroprotective activity. However the nephroprotective effect of HA needs further study. The results of our study indicate that the development of hyperglycemia and progression of diabetes by feeding a high fructose diet and HA might be prevented and/or delayed.

Oral glucose tolerance test (OGTT)

The fasting plasma glucose level was elevated in the high fructose diet test group when compared to the normal group at the end of the 21-day study. After 21 days of dietary treatment the OGTT test showed a significant increase of plasma glucose levels in the high fructose diet test group after 2 hours of glucose challenge compared with the normal diet group (Table 3).

Effect of HA extracts in serum bio-chemical changes in C57BL/6J mice

Serum glucose levels in diabetic C57BL/6J *ob/ob* mice after 4 hours fasting were measured on days 0, 10, and 21 after daily

Table 1 Effect of chloroform and methanol extracts of *Holostemma annularis* on high fructose diet induced change in serum glucose, triglycerides, and cholesterol and urine total proteins

Group	Serum glucose (mg/dL)				Serum triglycerides (mg/dL)	Total cholesterol (mg/dL)	Total protein in urine (g/dL)
	Day				Day	Day	Day
	1	7	14	21	21	21	21
Normal control	57 ± 6.0	62 ± 4.8	64 ± 3.0	71 ± 1.4	86.34 ± 6.6	70.57 ± 6.40	Nil
Diabetic control (5% Tween 80, p.o)	65 ± 2.2	87 ± 3.8	137 ± 6.0	188 ± 7.2***	153.83 ± 1.7***	89.01 ± 7.39*	1.9 ± 0.14***
Glibenclamide (5 mg/kg, po)	63 ± 3.1	60 ± 2.8	71 ± 5.1	82 ± 2.9***	99.53 ± 6.1***	75.45 ± 5.72**	1.1 ± 0.11***
CEHA (150 mg/kg)	59 ± 3.1	63 ± 2.8	78 ± 2.6	126 ± 2.9**	114.69 ± 11.1*	80.37 ± 5.90*	1.8 ± 0.13
CEHA (300 mg/kg)	49 ± 3.0	75 ± 5.2	103 ± 6.6	96 ± 6.9***	89.35 ± 6.8***	74.87 ± 5.55*	1.3 ± 0.09*
MEHA (150 mg/kg)	64 ± 4.6	66 ± 6.5	98 ± 6.5	117 ± 7.6**	126.64 ± 6.8*	82.53 ± 5.4*	1.7 ± 0.18
MEHA (300 mg/kg)	54 ± 5.4	71 ± 6.5	87 ± 3.8	94 ± 3.4***	83.33 ± 6.3***	72.60 ± 6.57*	1.2 ± 0.12*

Notes: Effect of normal (saline), Tween 80 (2%), glibenclamide (5 mg/kg), chloroform extract of *Holostemma annularis* (CEHA) 150 and 300 mg/kg and methanol extract of *H. annularis* (MEHA) 150 and 300 mg/kg on serum glucose, triglycerides, cholesterol and total protein level in urine in high-fructose diet induced diabetic rats. The results were analyzed by Student's t test and by comparing mean of treatment group, control group with normal. Conclusions are presented as mean ± SEM. P value < 0.05 was considered as significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Abbreviation: SEM, standard error of mean.

Table 2 Effect of chloroform and methanol extracts of *Holostemma annularis* on serum glucose, triglyceride and cholesterol in C57BL/6j mice

Group	Serum Glucose (mg/dl)			Serum Triglyceride (mg/dl)	Serum Cholesterol (mg/dl)
	Day			Day	Day
	0	10	21	21	21
C57BL/6j diabetic mice	183.3 ± 10.6	191.5 ± 9.8	201.6 ± 11.8	68.2 ± 42.2	169 ± 12.4
Glibenclamide (5 mg/kg)	188.5 ± 17.6	169.5 ± 5.8	149.4 ± 10.3**	49.3 ± 38.4**	117 ± 16.4**
CEHA (150 mg/kg)	187.6 ± 12.3	171.4 ± 11.2	166.5 ± 10.2*	60.2 ± 10.4*	144 ± 15.5*
CEHA (300 mg/kg)	190.0 ± 18.1	178.7 ± 15.1*	163.5 ± 12.9**	56.1 ± 9.7*	121 ± 18.3*
MEHA (150 mg/kg)	189.4 ± 13.3	175.4 ± 12.3	164.3 ± 11*	59.4 ± 12.3*	148 ± 17.3*
MEHA (300 mg/kg)	195.8 ± 15.9	172.2 ± 12.0*	156.1 ± 13.9**	53.3 ± 11.2*	127 ± 14.7*

Notes: Effect of glibenclamide (5 mg/kg), chloroform extract of *Holostemma annularis* (CEHA) 150 and 300 mg/kg and methanol extract of *H. annularis* (MEHA) 150 and 300 mg/kg on serum glucose, triglycerides and cholesterol level in C57BL/6j *ob/ob* diabetic mice. The results were analyzed by Student's t test and by comparing mean of treatment group, control group with glibenclamide. Conclusions are presented as mean ± SEM. *P* value <0.05 was considered as significant. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Abbreviation: SEM, standard error of mean.

oral administration of HA or vehicle. As shown in Table 2, diabetic *ob/ob* mice had remarkably high fasting serum glucose levels (>180 mg/dL), which decreased significantly (*P* < 0.01) after administration of high-dose (300 mg/kg) CEHA and MEHA (163.5 ± 12.9 and 156.1 ± 13.9 mg/dL, respectively). On day 10 serum glucose level were 172.2 ± 12.0 mg/dL (*P* < 0.05) compared to the vehicle group, 191.5 ± 9.8 mg/dL. On day 21, the high-dose group serum glucose level was significantly reduced (156.1 ± 13.9 mg/dL) compared to the vehicle group (201.6 ± 11.8 mg/dL, *P* < 0.01). At the low dose (150 mg/kg), however, the serum glucose decreased from

191.5 ± 9.8 mg/dL to 171.4 ± 11.2 mg/dL on day 10 and 164.3 ± 11 mg/dL on day 21 (*P* < 0.05). HA inhibited serum glucose level in a dose-dependent manner. The glucose-lowering effect of CEHA and MEHA were compared to glibenclamide which reduced the serum glucose significantly (*P* < 0.01). We also evaluated the effects of HA (150 and 300 mg/kg) on serum triglyceride and cholesterol in *ob/ob* mice. As shown in Table 2, after administration of HA high-dose treatment (300 mg/kg), serum triglyceride levels significantly decreased from 68.2 ± 42.2 mg/dL (Day 21) to 53.3 ± 11.2 mg/dL (*P* < 0.05) and cholesterol level from 169 ± 12.4 mg/dL to 121 ± 18.3 mg/dL (*P* < 0.05). In the low-dose group (150 mg/kg), serum triglyceride and cholesterol level were lowered significantly on day 21 (59.4 ± 12.3 mg/dL) and 144 ± 15.5 mg/dL, *P* < 0.05 versus vehicle group, respectively. The results showed that HA (150 and 300 mg/kg) caused antihypertriglyceridemia and antihypercholesterolemia effects after oral administration.

These results suggest that HA suppressed the elevation of serum glucose, triglycerides and cholesterol in both models. Antihyperlipidemic effect needs further study. The foregoing observations lead us to conclude that HA inhibited the development of early hyperglycemia and hyperglyceridemia. The drug needs a further thorough study to identify the anti-diabetic mechanism.

Table 3 Effect of chloroform and methanol extract of *Holostemma annularis* on glucose tolerance

Treatment	Initial	Serum glucose (mg/100 mL)	
		Time (minutes) after glucose administration	
		30	90
Control (Tween 80)	52 ± 5.0	138 ± 2.8	129 ± 4.0
CEHA (150 mg/kg)	55 ± 3.8	114 ± 4.5**	119 ± 5.6**
MEHA (150 mg/kg)	59 ± 4.5	126 ± 5.3**	118 ± 5.2**

Notes: The rats of all groups were loaded with glucose (3 g/kg po) 30 minutes after the drug treatment. Values are mean ± SD. n = 6 in each group. ****P* < 0.001 (compared to control value). **P* < 0.01 (compared to control value).

Abbreviations: SEM, standard error of mean; CEHA, chloroform extract of *Holostemma annularis*; MEHA, methanol extract of *H. annularis*.

Discussion

A high fructose diet induces insulin resistance, alterations in lipid metabolism, and oxidative stress in rat tissues.²⁶ Fructose is readily absorbed and rapidly metabolized by human liver. Westernization of diets has resulted in significant increases in added fructose, leading to typical daily consumptions amounting to 85–100 g fructose per day. The exposure of the liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and triglycerides accumulation; which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance. These negative effects of fructose are the reason that fructose metabolism has gained recent research attention.^{27,28} The long-term negative effects can include changes in digestion, absorption, plasma hormone levels, appetite, and hepatic metabolism, leading to the development of insulin resistance, diabetes, obesity, and inevitably cardiovascular disease.²⁹ Because of its lipogenic properties, excess fructose in the diet can cause glucose and fructose malabsorption, together with greater elevations in triglycerides and cholesterol compared to other carbohydrates.³⁰ In the liver, fructose is metabolized into glyceraldehyde and dihydroxyacetone phosphate. These particular fructose end products can be readily taken up in the glycolytic pathway. Of key importance is the ability of fructose to bypass the main regulatory step of glycolysis; the conversion of glucose-6-phosphate to fructose 1, 6-bisphosphate, controlled by phosphofructokinase. Thus, while glucose metabolism is negatively regulated by phosphofructokinase, fructose can continuously enter the glycolytic pathway. Therefore, fructose can uncontrollably produce glucose, glycogen, lactate, and pyruvate, providing both the glycerol and acyl portions of acyl-glycerol molecules. These particular substrates, and the resultant excess energy flux due to unregulated fructose metabolism, will promote the overproduction of triglycerides.^{31,32} A high fructose diet can have hypertriglyceridemic and pro-oxidant effects, and fructose-fed rats have shown less protection from lipid peroxidation.³³ Oxidative stress has often been implicated in the pathology of insulin resistance induced by fructose feeding, and lipid peroxides, and reactive substances are undeniably elevated in fructose-fed animals, especially accompanying a deficient antioxidant system.³⁴ Administration of HA has been shown to prevent these changes, and improve insulin sensitivity.¹⁸ In our study, treatment with HA also prevented several deleterious effects of fructose feeding; such as the increases in serum glucose, cholesterol and triglyceride levels. In the other model the antihyperglycemic effects of HA root were evaluated in diabetic C57BL/6J *ob/*

ob mice. Diabetes and obesity are complex genetic diseases caused by a combination of genetic predisposition and environmental exposure.^{35,36} The genetic contribution can be either monogenic or polygenic, with polygenic inheritance being the predominant mode of inheritance in human type 2 diabetes and obesity. The leptin mutations arose spontaneously in the C57BL/6J mice, and resulted in the severe early-onset of obesity, hyperphagia, hyperinsulinemia, and insulin resistance with modest hyperglycemia.³⁷ Leptin deficiency is associated with dyslipidemia (abnormal levels of cholesterol and triglycerides in the blood) and insulin resistance, a precursor to diabetes in animals and humans with lipoatrophy (fat loss).³⁸

HA has been documented as a traditional treatment for diabetes. In the present study, CEHA and MEHA (150 mg/kg and 300 mg/kg) significantly decreased the serum glucose, triglycerides and cholesterol in the high fructose-induced diabetic rats and in the diabetic C57BL/6J *ob/ob* mice. It also rendered nephroprotection by decreasing the total protein levels in urine. The alcoholic root extract of HA has been shown to have a potential antioxidant activity in both *in vitro* and *in vivo* models.¹⁵ However there is no evidence of any scientific studies into its antidiabetic activity. The chemical investigation of the root extract of HA revealed the presence of β -sitosterol, lupeol, α -amyrin and amino acids.³⁹ The presence of β -sitosterol, lupeol supports its antioxidant potential.^{40,41} The HA root has been reported to have chemoprotective activity in cyclophosphamide-induced toxicity.⁴² Many studies have validated the role of β -sitosterol and lupeol in the management of diabetes and hypercholesterolemia.^{17,41} β -sitosterol has been shown to normalize blood sugar and insulin levels in type 2 diabetics. The mechanism for this effect is that β -sitosterol stimulates the release of insulin in the presence of non-stimulatory glucose concentrations,⁴³ and inhibits glucose-6-phosphatase.⁴⁴ β -sitosterol reduces elevated blood glucose levels by the downregulation of glucose-6-phosphatase. Human liver microsome studies show that β -sitosterol inhibits cholesterol absorption.⁴⁵ In a clinical study β -sitosterol was shown to lower cholesterol and triglyceride levels.⁴⁶ These results indicate a possible antihyperglycemic use for β -sitosterol in the prevention and treatment of diabetes. In addition to this the other antioxidant constituent of HA, lupeol, also supports antidiabetic activity. Lupeol present in the roots of HA has the ability to protect cells and tissues from oxidative stress, which induces the formation of cytoprotective enzymes like catalase and superoxide dismutase.⁴⁷ Furthermore, the treatment of chloroform and methanolic extracts of HA root showed an improved

lipid profile by reducing the level of total cholesterol and triglycerides. Plant sterols (β sitosterol) found in the root may have contributed to the improvement in lipid profile. Plant sterols along with lupeol were well known for their antihypercholesterolemia by lowering the cholesterol level.^{41,48–51} This antihyperlipidemic effect could represent a protective mechanism against the development of hypercholesterolemia complications associated with diabetes. CEHA and MEHA decreased the serum glucose, cholesterol and triglyceride levels of the diabetic C57BL/6J mice, suggesting an antihyperglycemic effect. The principal antioxidant compounds (sitosterols and lupeol) of HA root may also be responsible for hypoglycemic effects shown in this study.

Disclosures

The authors disclose no conflicts of interest.

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