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Attenuation of diabetic disorders in experimentally induced diabetic rat by methanol extract of seed of *Holarrhena antidysenterica*

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Abstract: The study was undertaken to evaluate the therapeutic efficacy of methanol extract of seed of *Holarrhena antidysenterica* in streptozotocin (STZ) induced diabetic rats. Extract was administered orally at a dose of 400 mg / kg body weight / day for 21 days, to STZ-induced diabetic rats. The fasting blood glucose level was decreased significantly after the treatment of methanolic extract to diabetic rats. Activities of hexokinase and glucose-6-phosphate dehydrogenase in liver as well as glycogen levels in liver and skeletal muscle all were increased significantly in extract treated diabetic group in compare with untreated diabetic control group. Activities of hepatic glucose-6-phosphatase were recovered significantly in extract treated diabetic group in respect to untreated diabetic group. To assess the antihyperlipidemic activities of this extract, we have also measured the serum levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and high density lipoprotein cholesterol (HDLc). The said extract also resulted a significant recovery in above mentioned biomarkers of lipid profile when treated to STZ-induced diabetic rat. Resettlement of all these parameters after the treatment of methanol extract of seed of *H. antidysenterica* is promising which has been reflected here from the comparison with the antidiabetic drug i.e. glibenclamide. After the monitoring of GOT and GPT activities in serum, it has been noted that the extract significantly correct the activities of these enzymes in STZ-induced diabetic rat. The result of this experiment demonstrated that the extracts of the said plant part possess a promising antidiabetic efficacy.
Key words: *Holarrhena antidysenterica,* Antidiabetic, Antihyperlipidemic, Carbohydrate metabolic enzymes, Glycogen

Introduction

Diabetes mellitus is a syndrome, initially characterized by loss of glucose homeostasis resulting from defects in insulin secretion or insulin action that resulting impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins[1]. Despite the great strides that have been made in the understanding and management of diabetes, the disease and disease related complications are increasing at an alarming rate[2]. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease[3]. More than 400 species have been reported to display the hypoglycemic effects, but only few of them have been investigated[4] and the world health organization has recommended that this area warrants attention[5].

In this study our attention has been focused on *Holarrhena antidysenterica* under the family of Apocynaceae is one such plant, popularly known as "Indrajav" is a shrub, distributed through out India upto an altitude of 4,000 ft. In Indian traditional medicine, the plant has been considered a popular remedy for the treatment of dysentery, diarrhea, intestinal worms[6-7] and the seeds of this plant are also used as an antidiabetic remedy in Asian countries[8]. The scientific study about this plant for the management of diabetes is scanty[9]. From the pilot experiments using the different organic

solvents for extraction from seed of *H. antidysenterica,* the result of methanol extract about antidiabetic activity is reported and highly encouraging.

Materials and Methods

Preparation of extract:

The seeds of *H. antidysenterica* were collected from local market in the Midnapur town and it was authenticated by taxonomists at the department of Botany & Forestry, Vidyasagar University, Midnapore where voucher specimen was preserved having the No Bio-Med / V.U / H.A / 16 / 08. Fresh seeds were dried at 37^0C in an incubator for 48 hrs. Then the seeds were ground into powder in an electric grinder. The methanol extract was prepared by mixing 200 g of powder with 1 litre of methanol in a container for 48 hrs for two times at room temperature and filtering through No. 3 Whatman filter paper. The extract was evaporated under reduced pressure using a rotary evaporator and the residue was collected and was stored in a refrigerator at 2-8^0C for use in the experiments[10].

Animals:

Adult male albino rats, 3 months of age weighing 150-170 g were taken for this study. Animals were acclimated for 15 days in our disease free animal house prior to start the experiment. The animals were kept in clean and dry plastic cages, with 12 h light: 12 h dark cycle at 25±2^0C temperature and 45-60% relative humidity. Animals were free access to standard feed and water. For experimental purpose the animals were kept overnight fasting but allowed free access to water. The Institutional Animal Ethics Committee approved the study and all the instructions given by our Animal Ethics Committee were followed throughout the experimentation.

Induction of diabetes:

Diabetes was induced by a single intramuscular injection of STZ (Sigma Aldrich, USA) at the dose of 40 mg/kg of body weight. Streptozotocin was dissolved in 0.1M citrate buffer (pH 4.5). Diabetes was confirmed 7 days after STZ injection by determining the fasting blood glucose (FBG) concentration. Only animals with FBG levels more than 300 mg/dl were considered for the experiment[11].

Experimental design:

The rats were divided into five groups (six in each group). Group A, control rats + vehicle (solution of 3.0% tween 80); Group B, untreated diabetic rats + vehicle; Group C, diabetic rats + methanol extract (400 mg / kg body weight/day); Group D, diabetic rats + glibenclamide (0.6 mg / kg body weight/day)[12].

The above mentioned dose of methanol extract has been selected here from dose dependent pilot study where the applied dose exhibits most promising results. The extracts were administered continuously for 21 days orally using a gavage after confirmation of diabetes that is after 7 days or from 8^{th} day of STZ-injection. The results were compared with the standard drug glibenclamide which was also given continuously for 21 days orally with gavage. Starting from the 1^{st} day (8^{th} day of STZ-injection) of extract administration to diabetic rats, FBG level was measured in every 8^{th} day using glucometer[13]. On the 22^{nd} day of extract administration (29^{th} day after STZ-injection) all the animals were sacrificed by decapitation, blood was collected from dorsal aorta by a syringe and the serum was separated at 3000g for 10 min for the assessment of serum lipid profile. The different tissues were dissected out and stored at -20^0C for biochemical analysis of the activities of enzymes and of glycogen content in respective tissue sample.

Biochemical assays:

Activities of carbohydrate metabolic marker enzymes like hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase were measured biochemically[14-16]. Glycogen level was measured according to standard method[17]. Serum lipid profile like serum levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and high density lipoprotein cholesterol (HDLc) were measured biochemically[18-21]. Serum activities of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were assessed by the standard method[22].

Statistical analysis:

Analysis of Variance (ANOVA) followed by multiple comparison two-tail‘t’ test was used for statistical analysis of collected data[23]. All the values have been indicated by Mean ± SEM. Differences were considered significant at $p<0.05$.

Results

Levels of FBG:

Diabetes induced by STZ resulted in a significant elevation in the levels of FBG in comparison to the control group. After the treatment of methanol extract of seed of *H. antidysenterica* or glibenclamide to the diabetic animals for 21 days, a significant reduction ($p<0.05$) in FBG level was noted in respect to untreated diabetic group. At the end of the experiment, the methanol extract showed 77.1% reduction and glibenclamide showed 76.4% reduction in the levels of FBG in respect to untreated diabetic group. When the level of FBG was compared between methanol extract treated diabetic group and glibenclamide treated diabetic group, there was no significant difference was noted (Table 1).

Table 1. Fasting blood glucose levels in every 8th day (7 days interval) in control and different experimental groups of rats

	Fasting blood glucose level (mg/dl)				
Groups	1st day	8th day	15th day	22nd day	29th day
Group A	74±4.2[a]	67±3.8[a]	72±4.8[a]	68±4.0[a]	69±5.2[a]
Group B	76±5.3[a]	351±4.2[b]	361±2.9[b]	339±3.5[b]	346±5.4[b]
Group C	73±4.2[a]	358±4.1[b]	238±3.3[c]	122±5.2[c]	82±6.2[a]
Group D	69±3.8[a]	364±5.2[b]	232±4.1[c]	124±4.6[c]	86±4.3[a]

Data were given as Mean ± SEM (n=6). Values not sharing same superscripts in each column differ significantly at $p<0.05$

Carbohydrate metabolic enzyme activities:

STZ-induced diabetic animal resulted in a significant diminution ($p<0.05$) in hexokinase and glucose 6-phosphate dehydrogenase activities along with elevation in glucose 6-phosphatase activity in liver with respect to the control group. After the treatment of methanol extract or glibenclamide to STZ-induced diabetic rat, a significant recovery ($p<0.05$) was noted in respect to diabetic group. Percentage of recovery in the activities of hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase were 18.9%, 37.6%, 65.1% by methanol extract and 18.5%, 34.4%, 63.1% by glibenclamide, when compared to untreated diabetic group. There was no significant variation in the activities of hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase when comparison was made between methanol extract treated diabetic group and glibenclamide treated diabetic group (Fig. 1).

Glycogen content:

In diabetic control the glycogen levels in liver and skeletal muscle were diminished significantly ($p<0.05$) from the control group but after treatment of methanol extract of seed of *H. antidysenterica* or glibenclamide to diabetic rat, a significant recovery ($p<0.05$) was noted in the level of glycogen in skeletal muscle in respect to diabetic group but in liver only methanol extract show the significant correction in the levels of glycogen. Percentage of recovery in this parameter was 38.9%, 37.3% in methanol extract treated diabetic group in liver and skeletal muscle respectively and 11.1%, 38.9% in glibenclamide treated diabetic group in liver and skeletal muscle respectively. When comparison was made between methanol extract treated diabetic group and glibenclamide treated diabetic group, no significant variation was noted in the levels of glycogen in skeletal muscle. But in case of liver a significant variation in the level of glycogen was observed after comparison was made between methanol extract and glibenclamide treated diabetic group (Fig. 2).

Serum lipid profile:

Serum levels of TG and TC were increased significantly ($p<0.05$) in STZ-induced diabetic control group when compared with control. After the treatment of methanol extract of said plant part or glibenclamide, a significant ($p<0.05$) recovery was noted in respect to diabetic group. In the levels of TG and TC in serum, methanol extract treated diabetic group showed 53.9%, 61.6% recoveries whereas glibenclamide treated diabetic group showed 21.1%, 65% recoveries in respect to diabetic control respectively. When comparison was made between the methanol extract treated diabetic group and glibenclamide treated diabetic group, a significant difference was noted in the levels of serum TG and TC. (Table 2)

Serum levels of LDLc and VLDLc were increased significantly ($p<0.05$) in untreated diabetic group in respect to control. But after treatment of methanol extract of seed of *H. antidysenterica* or glibenclamide to diabetic rats the level of these biomarkers were corrected significantly ($p<0.05$) in respect to diabetic group. Percentage of recoveries in the serum levels of LDLc and VLDLc were 85.4%, 26.9% by methanol extract where 91.1%, 25.9% by glibenclamide in respect to diabetic control respectively. After comparison of the data between methanol extract treated diabetic group and glibenclamide treated diabetic group, no significant variation was observed in the levels VLDLc though in the levels LDLc a significant variation was noted when the comparison was made between of methanol extract treated diabetic group with glibenclamide treated diabetic group (Table 2).

HDLc level was decreased significantly ($p<0.05$) in diabetic control group in respect to the control. But after treatment of the said plant extract or glibenclamide, a significant recovery ($p<0.05$) was noted in respect to

diabetic control. The recovery in serum level of HDLc was 38.2% in methanol extract treated diabetic group where 43.5% recovery was noted in glibenclamide treated diabetic group. After comparative analysis of the data related to this parameter there was no significant variation was observed between methanol extract and glibenclamide treated diabetic groups (Table 2).

Table 2. Levels of serum lipid profile in control and different experimental groups of rats

Groups	Serum lipid profile				
	TG (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)	TC (mg/dl)
Group A	58.7±0.76[a]	35.6±0.66[a]	15.6±0.63[a]	11.5±0.69[a]	62.7±0.56[a]
Group B	94.4±0.61[b]	20.7±0.55[b]	114±0.99[b]	18.2±0.59[b]	153±0.67[b]
Group C	43.5±0.65[c]	28.6±0.63[c]	16.7±0.81[a]	13.3±0.57[a]	58.6±0.56[c]
Group D	74.5±0.57[d]	29.7±0.58[c]	10.2±1.1[c]	13.5±0.83[a]	53.4±0.58[d]

Data were given as Mean ± SEM (n=6). Values not sharing same superscript in each column differ significantly at $p<0.05$

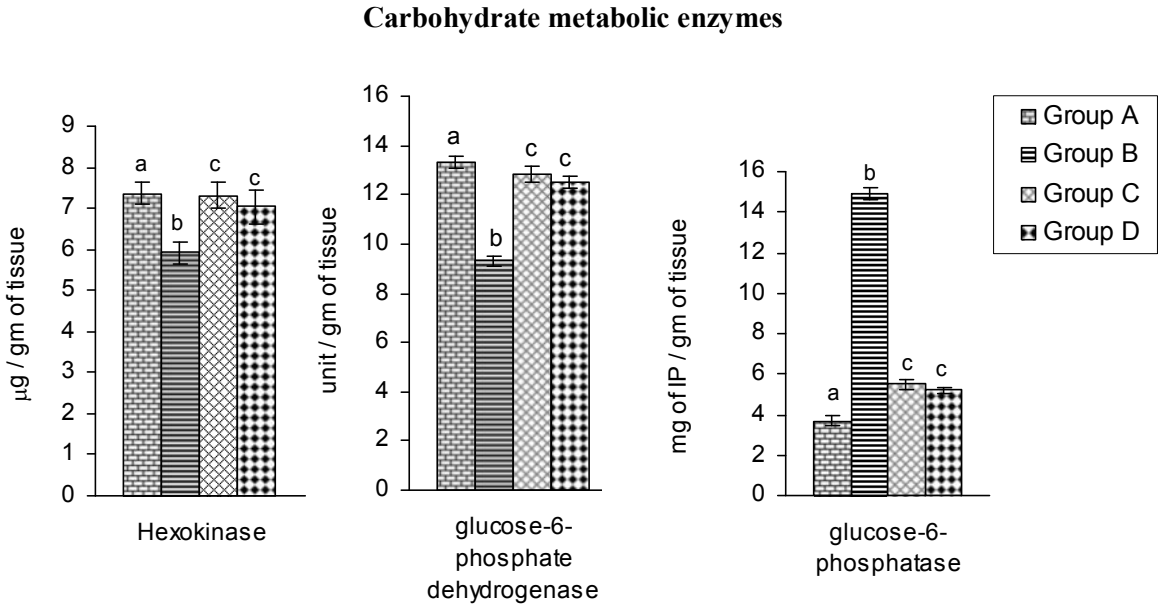

Figure 1. Activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase and glucose 6-phosphatase in control and different experimental groups of rats. Data were expressed as Mean ± SEM (n=6). Bar with different superscript differ significantly at $p<0.05$

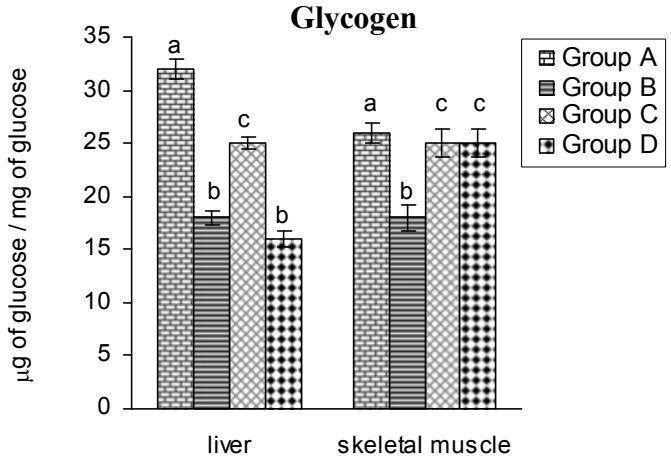

Figure 2. Levels of glycogen in liver and skeletal muscle in control and different experimental groups of rats. Data were expressed as Mean ± SEM (n=6). Bar with different superscript differ significantly at p<0.05

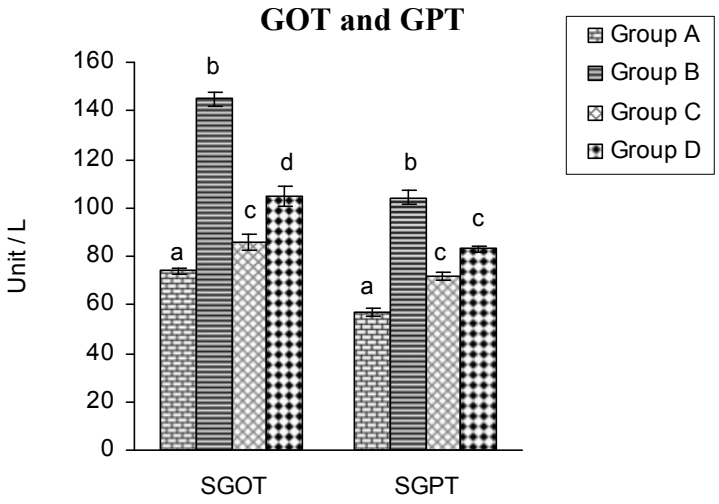

Figure 3. Activities of serum GOT and GPT in control and different experimental groups of rats. Data were given as Mean ± SEM (n=6). Bar with different superscript differ significantly at p<0.05.

Activities of GOT and GPT in serum:

Activities of GOT and GPT in serum were increased significantly ($p<0.05$) in diabetic group compared to the control group. After the treatment of methanol extract or glibenclamide to diabetic rats, activities of GOT and GPT in serum showed a significant reduction in respect to diabetic control. Recovery of GOT, GPT activities in serum were noted 40.7%, 30.8% in methanol extract treated diabetic group respectively where 27.6%, 20.2% recoveries were noted in glibenclamide treated diabetic group respectively. When comparison was made between methanol extract treated diabetic group and glibenclamide treated group, there was no significant variation was observed in the activities of serum GPT. In the activities of serum GOT, a significant variation was noted in between methanol extract treated and glibenclamide treated diabetic groups (Fig. 3).

Discussion

The STZ is a broad spectrum antibiotic extracted from *Streptomyces acromogenes*. The STZ-induced diabetes causes the partial destruction of β-cells of the islets, which leads to a reduction in insulin release[24]. An insufficient release of insulin, leads high blood glucose namely hyperglycemia. In continuation to other workers, STZ-induced diabetic model which is one of the best models in this field of study has been accepted [10]. In the present study, administration of the methanol extract of *H. antidysenterica* effectively reduced the blood glucose level in STZ-induced diabetic rats. Since the methanol extract of *H. antidysenterica* reduced the FBG level, it may be assumed that the extract may directly results stimulations on the remaining β-cells of pancreas for insulin secretion or it helps the regeneration of pancreatic β-cells which has been proposed by other using other plant products[25].

Another view for the regenerative activities of pancreatic β-cell of this extracts against STZ-induced diabetes is in the recovery of activities of hexokinase, glucose-6-phosphate dehydrogenase, glucose 6-phosphatase in liver and the levels of glycogen in liver and skeletal muscle. As these bio-markers are under positive control of insulin level[26] so, it may predict that the extract may recover the pancreatic insulin synthesis and secretion through β-cell regeneration. The same line of observation has been noted in our previous work using other plants in this purpose[27].

Lipid profile, which is altered in the serum of STZ-induced diabetic rats, appears to be a vital factor in the development of atherosclerosis which is noted in diabetes [28]. Elevated levels in serum TG and TC in diabetes is in consistent with our previous observation[29] and by others[30]. In this study, methanol extract significantly recovered the levels of serum lipid profile in treated diabetic rats when compared to untreated diabetic rats. From this result, it may be stated that the methanol extract leads to regeneration of the β-cells of the pancreas and potentiation of insulin secretion from surviving β-cells. The increase in insulin secretion and consequent decrease in blood glucose level may lead to inhibition of lipidperoxidation and control of lipolytic hormones. In this context, a number of other plants have also been reported to have insulin stimulatory along with antihyperlipidemic effects[28, 31].

The methanol extract of seed of *H. antidysenterica* has no metabolic toxicity induction which has been studied here by GOT and GPT activities in serum as these are the sensitive biomarkers in this concern[32]. So, on the basis of the result in this experiment it may be stated that the seed of *H. antidysenterica* has a beneficial effect in preventing diabetes and it complications as well as improving lipid metabolism in diabetics. Further studies will be conducted to purify the bioactive compound (s), and use the purified compound (s) for bioassay guided experiments in near future.

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