ORIGINAL ARTICLE

Computation of *In Vivo* Antidiabetic Activity of *Holarrhena Antidysenterica* Seeds Extracts in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Medicinal plants have curative properties due to the presence of various complex chemical compositions, which are found as secondary plant metabolites in one or more parts of the plant. The aim of this study was to compute the antidiabetic activity of *Holarrhena antidysenterica* seeds extract in streptozotocin-induced diabetic rats. The experimental protocol designed as animals were divided into six groups (n=6) as control, diabetic control, Glibenclamide, methanol extract (MEHAD), petroleum ether extract (PEHAD) and aqueous extract (AEHAD). Except control group, other remaining groups were treated with streptozotocin (STZ) (35 mg/kg body weight) by single i.v. injection to induce diabetes. The diabetic rats were treated with the glibenclamide, MEHAD (250 mg/kg body weight), PEHAD (250 mg/kg body weight) and AEHAD (250 mg/kg body weight) for 18 days. The fasting plasma glucose level, body weight, fasting serum glucose level, serum cholesterol, serum triglyceride, total protein, blood urea, urine glucose and liver glycogen levels were determined. The diabetic rats treated with MEHAD, PEHAD and AEHAD showed significant reduction in fasting serum glucose, serum cholesterol, serum triglyceride, total protein, blood urea, urine glucose and protection from the loss of body weight and increase in liver glycogen content during the treatment period. These effects were comparable to those seen in the glibenclamide-treated group of rats. This suggests that the *Holarrhena antidysenterica* seed extracts posses antidiabetic activity and further studies are needed to elucidate the mechanism of action and to know the active principles involved in producing the effect.

Keywords: *Holarrhena antidysenterica, Antidiabetic, Glibenclamide, Streptozotocin*

Diabetes mellitus is characterized by alterations in the metabolism of carbohydrate, fat and protein, which are caused by a relative or absolute deficiency of insulin secretion and different levels of insulin resistance and also resulting from both genetic predisposition and favoring environmental factors. In the patients, late complications develop consisting of alterations and failure of various organs (especially the non-insulin sensitive ones) including the eyes (retinopathy with vision loss), kidneys (nephropathy leading to renal failure), nerves (peripheral and autonomic neuropathy), heart and blood vessels (precocious and severe cardiovascular, cerebrovascular and peripheral vascular atherosclerosis) [1-2]. People with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity. Globally the prevalence of diabetes was estimated to be 2.8 % in 2000 and 4.4 % in 2030. Worldwide the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [3].

Holarrhena antidysenterica (Family: Apocynaceae) is a plant commonly found in the forests of India, indigenous to the tropical Himalayas, Assam, Uttar Pradesh, down to Travancore [4-5]. The survey of

literature reveals that various parts of this plant are used in diarrhoea, blood dysentery, haematemesis, acute rheumatism, astringent and diabetes. The fresh bark and seeds of this plant were reportedly used as antibacterial, antidiarrhoeal agent [6-7]. The *'Bhavaprakasha'* has recommended the fresh bark and seeds of *Holarrhena antidysenterica* in the treatment of diabetes [5]. However, scientific data on antidiabetic activity of *Holarrhena antidysenterica* is not available. So, in the present study, we have made an attempt to explore the antidiabetic effect of petroleum ether, methanol and aqueous extracts of *Holarrhena antidysenterica* seed in the streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant material

The seeds of *Holarrhena antidysenterica* (HAD) been procured commercially from "Amruth Kashri", Bangalore, India. The collected material was authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai, India.

Preparation of extracts

The dried powdered seed (1000 gm) was successively extracted on a Soxhlet apparatus, employing petroleum ether, methanol and distilled water respectively. The solvents of petroleum ether extract (PEHAD), methanol extract (MEHAD) and distilled water extract (AEHAD) were removed by distillation and the last traces of solvent being removed under reduced pressure.

Animals

Healthy Male wistar albino rats (220-250 g) were used for the study. The rats were housed in polypropylene cages and maintained under standard conditions (12-h light: 12-h dark cycle; 25 ± 3°C; 35–60% humidity). The animals had free access to standard lab chow (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. Study was conducted after obtaining institutional animal ethical committee clearance.

Acute oral toxicity study

Acute oral toxicity was performed by following OECD guideline-420 fixed dose procedure for petroleum ether, methanol and aqueous extract and it was found that dose increasing up to 2000 mg/kg body weight shown no toxicity or mortality in experimental rats [8-9].

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed in overnight fasted (18 hours) normal rats. The rats were divided into six groups (*n* = 6) and were administered saline first two group, Glibenclamide (10 mg/kg), MEHAD (250 mg/kg), AEHAD (250 mg/kg) and PEHAD (250 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min prior to the administration of the extracts, except control group. Blood was withdrawn from the retro-orbital sinus after 0, 2, 4 and 6 hrs of extract administration, and the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels using a glucose oxidase–peroxidase glucose estimation kit.

Induction of non-insulin dependent diabetes mellitus (NIDDM)

Non-insulin-dependent diabetes mellitus was induced in overnight fasted adult Wistar strain albino male rats weighing 220-250 gm by a single i.v. injection of 35 mg/kg Streptozotocin, 15 minutes after i.p. administration of 120 mg/kg of nicotinamide. Streptozotocin (STZ) was dissolved in a citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hours and then on day 7, after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as > 126 mg/dL. Only those rats that were found to have permanent NIDDM were used for the study.

Experimental design

The animals were segregated into six groups of six rats each. The extract was administered for 18 days. Group I served as normal control rats, administered drinking water daily for 18 days; Group II had diabetic control rats, administered drinking water daily for 18 days; Group III diabetic rats were administered standard drug glibenclamide (10 mg/kg) p.o. for 18 days; Group IV diabetic rats were administered MEHAD (250 mg/kg) orally; Group V diabetic rats were administered PEHAD (250 mg/kg) orally; and Group VI diabetic rats were administered AEHAD (250 mg/ kg) orally for 18 days.

The fasting glucose levels were determined on days 0, 6, 12 and 18 of extract administration. During the experimental period, the rats were weighed daily and the mean change in body weight was calculated [10-11].

Estimation of biochemical parameters

The biochemical parameters were determined on day 18 after the animals were sacrificed by cervical dislocation. Total cholesterol, triglycerides, total protein, urea level and liver glycogen, were determined by using an auto-analyzer [12-13].

Statistical analysis

The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet's test. The statistical significance of difference was taken as $p < 0.05$.

RESULTS

Acute toxicity studies

The acute toxicity studies of the MEHAD, AEHAD and PEHAD was found to be safe up to a dose of 2000

Table 1. Effect of HAD extracts on changes in body weight in rats

Group	Body Weight (gm)			
	0 Day	**6 Day**	**12 Day**	**18 Day**
Normal saline	229.33 ± 2.53	251.00 ± 6.84	253.00 ± 2.58	259.00 ± 9.79
Diabetic control	190.67±3.72[a]	175.12±2.14[a]	163.50±0.99[a]	129.00±1.59[a]
Glibenclamide (10 mg/kg)	193.17 ± 2.20 *	205.00±2.29**	209.68±3.98**	212.78±2.08**
MEHAD (250 mg/kg)	186.50 ± 3.53*	193.00±2.29**	201.16±6.13**	217.17±3.23**
PEHAD (250 mg/kg)	190.83 ± 1.30 *	198.00±4.22**	201.35±4.23**	208.67±3.46**
AEHAD (250 mg/kg)	189.12 ± 3.25*	198.21±6.12**	212.32±5.20**	215.12±6.12**

Values are expressed as mean ± SEM (Number of animals, n = 6); *$p < 0.05$, **$p < 0.01$ shows level of significance difference compare to diabetic control group. [a]$p \leq 0.01$ shows significantly different compare to normal group.

mg/kg^{-1} body weight of the animals so that 1/10th (i.e. 250 mg/kg orally) was selected for anti-diabetic activity.

Body weight

The diabetic rats show a progressive loss of body weight, which was found to be significant ($p \leq 0.05$) during the 18 days of treatment period as against the gain in body weight seen in normal group of rats. But glibenclamide treatment has protected the diabetic rats from losing the body weight in a significant ($p \leq 0.05$) manner when compared with the diabetic control group of rats. The weight of diabetic rats groups treated with MEHAD (250 mg/kg), AEHAD (250 mg/kg), and PEHAD (250 mg/kg) were maintained in a significant manner ($p \leq 0.05$) throughout the study period in this group when compared with diabetic animals (Table 1).

Oral glucose tolerance test

The plasma glucose level in the normal group of rats was maintained within the normal range throughout the period of study. The fasting glucose in the diabetic control group of rats was found to be significantly ($p \leq 0.01$) higher when compared with the normal rats. These elevated fasting glucose levels were found to have been maintained throughout the 6-h treatment period indicating that the rats are rendered diabetic.

The glibenclamide (10 mg/kg) treated diabetic rats show a progressive and significant ($p \leq 0.05$) including on the 6 hr, it shows highly significant ($p \leq 0.01$) in

Table 2. Effect of extract of HAD on oral glucose tolerance test

Group	Plasma glucose concentration (mg/dL)			
	0 h	**2 h**	**4 h**	**6 h**
Normal saline	107.66 ±3.77	108.50 ± 4.34	107.33 ±3.77	108.33 ± 4.21
Diabetic control	320.50±5.37[a]	320.50±3.24[a]	321.33±4.88[a]	322.00±6.00[a]
Glibenclamide (10 mg/kg)	344.00±11.78 *	330.50±8.62*	317.00±11.08*	267.66±5.45**
MEHAD (250 mg/kg)	325.50 ±2.07*	318.35±4.93*	308.16±4.54*	273.16±8.65**
PEHAD (250 mg/kg)	330.21 ± 1.47*	320.01±7.92*	310.25±7.50*	305.49±9.03*
AEHAD (250 mg/kg)	326.25 ± 2.75*	318.29±3.41*	307.83±5.38*	284.83±6.52**

Values are expressed as mean ± SEM (Number of animals, n = 6); *$p < 0.05$, **$p < 0.01$ shows level of significance difference compare to diabetic control group. [a]$p \leq 0.01$ shows significantly different compare to normal group.
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Table 3. Effect of extract of HAD on fasting plasma glucose level in rats

Group	Fasting plasma glucose concentration (mg/dL)			
	0 Day	**6 Day**	**12 Day**	**18 Day**
Normal saline	107.66 ±3.77	107.66 ± 3.19	106.50 ±3.98	108.50 ± 4.32
Diabetic control	320.50±5.37[a]	321.33±5.51[a]	321.16±5.91[a]	320.33±4.88[a]
Glibenclamide (10 mg/kg)	344.00±11.78 *	327.16±8.86*	258.33±12.81**	136.33±3.26**
MEHAD (250 mg/kg)	325.50 ±2.07*	311.33±3.33*	269.83±7.21**	162.16±3.30**
PEHAD (250 mg/kg)	330.21±1.47 *	317.98±2.27*	295.83±2.48**	169.98±7.25**
AEHAD (250 mg/kg)	326.25 ± 2.75*	308.36±5.59*	254.33±9.16**	160.16±2.65**

Values are expressed as mean ± SEM (Number of animals, n = 6); *$p < 0.05$, **$P < 0.01$ shows level of significance difference compare to diabetic control group. [a]$p \leq 0.01$ shows significantly different compare to normal group.

reduction of fasting glucose, during the treatment period in comparison to the diabetic group of rats. This indicates that the glibenclamide treatment in diabetic rats is able to bring back the fasting glucose levels nearer to normal range throughout the study period.

The diabetic rats treated with MEHAD (250 mg/kg) and AEHAD (250 mg/kg) shows a progressive and significant ($p \leq 0.05$) and highly significant ($p \leq 0.01$) reduction of fasting glucose during the single dose of treatment period in comparison to the diabetic group of rats. The diabetic rats treated with PEHAD (250 mg/kg) show a progressive and significant ($p \leq 0.05$) reduction in fasting glucose during the treatment period in comparison to diabetic group of rats (Table 2).

Induction of non-insulin dependent diabetes mellitus

The fasting serum glucose in the normal group of rats was maintained within the normal range throughout the period of study. The fasting serum glucose in the diabetic control group of rats was found to be significantly ($p \leq 0.01$) higher when compared with the normal rats. These elevated fasting serum glucose levels were found to have been maintained throughout the 18 days of treatment period indicating that the rats are rendered diabetic.

The glibenclamide (10 mg/kg)-treated diabetic rats show a progressive and significant ($p \leq 0.05$). They showed highly significant ($p \leq 0.01$) in reduction of fasting serum glucose, during the treatment period in comparison to the diabetic group of rats. The MEHAD (250 mg/kg) treated diabetic rats show a progressive and significant ($p \leq 0.05$). They show highly significant ($p \leq 0.01$) values in reduction of fasting serum glucose during the single dose of treatment period in comparison to the diabetic group of rats.

The PEHAD (250 mg/kg) and AEHAD (250 mg/kg) treated diabetic rats shows a progressive and

significant ($p \leq 0.05$) including on the day 12 and day 18, they show highly significant ($p \leq 0.01$) values in reduction of fasting serum glucose during the treatment period in comparison to diabetic group of rats (Table 3).

Biochemical determinations

The serum cholesterol, triglyceride, total protein and blood urea level in diabetic control group of rats was found to be increasing throughout the 18 days of study period. The glibenclamide (10 mg/kg) treated diabetic rats show no significant difference when compared with the normal rats which means that the values are comparable with those of normal rats and in fact lower than the normal rats after 18 day of treatment. This shows that the cholesterol, triglyceride, total protein and blood urea levels have reduced in glibenclamide-treated diabetic rats. The diabetic rats treated with MEHAD (250 mg/kg), PEHAD (250 mg/kg) and AEHAD (250 mg/kg) show significant ($p \leq 0.01$) reduction in the serum cholesterol, triglyceride, total protein and blood urea level during the entire study period (Table 4). This indicates that the MEHAD, PEHAD and AEHAD treatment of diabetic rats is able to bring back the serum cholesterol, triglyceride, total protein and blood urea levels lower to normal range in the 18 days of treatment.

The liver glycogen in diabetic control group of rats was found to be significantly ($p \leq 0.01$) decreased when compared with the normal rats on the respective days. These liver glycogen levels were found to be decreased throughout the 18 days of study period. The glibenclamide (10 mg/kg) treated diabetic rats show significant ($p \leq 0.01$) increase as against the liver glycogen level of diabetic rats however these values also found to be having the significant difference when compared with the diabetic rats which means that the values are comparable with those of diabetic rats and in fact more close to the normal rats after 18 day of treatment. The MEHAD (250 mg/kg), PEHAD (250

Table 4. Determination of biochemical parameters after treatment with MEHAD, PEHAD, AEHAD and glibenclamide

Group	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	Total protein (g/dL)	Blood urea (mg/dL)	Liver Glycogen (mg/gm)
Normal saline	51.96 ± 1.77	139.30 ± 1.36	5.86 ± 0.19	41.83 ± 0.76	35.87 ± 1.10
Diabetic control	84.25 ± 1.44^a	187.27 ± 3.13^a	7.41 ± 0.42^a	73.70 ± 1.37^a	23.31 ± 0.85^a
Glibenclamide (10 mg/kg)	$50.80 \pm 0.22**$	$141.77 \pm 1.88**$	$5.91 \pm 0.20**$	$42.33 \pm 0.40**$	$36.11 \pm 0.98**$
MEHAD (250 mg/kg)	$55.80 \pm 3.12**$	$132.90 \pm 7.22**$	$6.18 \pm 0.13**$	$50.20 \pm 3.06**$	$44.07 \pm 1.13**$
PEHAD (250 mg/kg)	$68.92 \pm 1.54**$	$153.80 \pm 1.36**$	$6.20 \pm 0.26**$	$50.01 \pm 1.84**$	$53.63 \pm 0.85**$
AEHAD (250 mg/kg)	$63.74 \pm 1.15**$	$129.00 \pm 3.02**$	$6.09 \pm 0.25**$	$54.79 \pm 0.88**$	$45.57 \pm 2.70**$

Values are expressed as mean $\pm$ SEM (Number of animals, n = 6); *$p < 0.05$, **$p < 0.01$ shows level of significance difference compare to diabetic control group. [a]$p \leq 0.01$ shows significantly different compare to normal group.

mg/kg) and AEHAD (250 mg/kg) treated diabetic rats show significant ($p \leq 0.01$) increase as against the liver glycogen of diabetic rats during the entire study period (Table 4).

DISCUSSION

The *Holarrhena antidysenterica* fresh bark and seed extracts has been reported to have antibacterial and antidiarrhoeal activity [14], but there are no scientific data is available regarding the effect on the blood glucose levels. So we have made an attempt to use methanol, petroleum ether and aqueous extracts of *Holarrhena antidysenterica* seeds for studying anti-diabetic activity. In this study the group of diabetic rats showed progressive and significant ($p \leq 0.05$) loss in body weight throughout the study period as compared to the body weight gain of normal group of rats. This characteristic loss of body weight is due to increased muscle wasting and due to loss of tissue proteins [15]. Moreover, the fasting serum glucose of diabetic rats was significantly ($p \leq 0.05$) elevated as compared to the normal fasting serum glucose levels of diabetic rats. The serum cholesterol, serum triglyceride, total protein and blood urea of diabetic group of rats was found to be significantly ($p \leq 0.05$) increasing throughout the study period as against normal group of rats. This might have occurred in the diabetic rats as a result of lack of insulin which activates the lipase enzymes, hydrolyzing the stored triglycerides and releasing large amount of fatty acids and glycerol into the circulating blood. Consequently, the excess of fatty acids in the plasma may promote the hepatic conversation of fatty acids into phospholipids and cholesterol, the main products of lipid metabolism. At the same time glycogen, cortisol, catecholamine and growth hormones enhance lipolysis [16-17].

The liver glycogen levels of the diabetic group of rats were found to be reduced significantly ($p \leq 0.05$) as against the normal group of rats. These observations suggest that single i.v. injection of STZ (35 mg/kg) [18] produced a reproducible and consistent diabetes mellitus and appears to be a suitable model of diabetes in our laboratory conditions. The glibenclamide (10 mg/kg) treated group of rats showed significant ($p \leq 0.05$) protection from the body weight loss and progressive reduction in fasting serum glucose levels. The glibenclamide treatment also reduced the elevated serum cholesterol levels and produced significant ($p \leq 0.01$) reduction in elevated serum triglyceride, total protein, blood urea, urine glucose allowed significant ($p \leq 0.01$) recovery of reduced liver glycogen content during the period of study when compared with the diabetic group of rats. Several studies have shown protection in body weight loss, anti-diabetic activity [19-20], reduction in serum cholesterol, serum triglyceride, total protein and blood urea [21], and recovery in liver glycogen content upon glibenclamide treatment.

The all three extracts (methanol, petroleum ether and aqueous) of *Holarrhena antidysenterica* seeds have significantly ($p \leq 0.01$) protected the diabetic rats from losing the body weight at all the selected dose levels. The MEHAD-, PEHAD- and AEHAD-treated groups of rats have also shown the progressive and significant ($p \leq 0.01$; except on 6th day) reduction in fasting serum glucose of diabetic rats when compared with untreated diabetic rats at all the selected dose levels but the PEHAD-treated group at the doses of 250 mg/kg have not shown more consistent results throughout the study period. This reduction in the fasting serum glucose may be due to inhibition of intestinal absorption of glucose, possible regeneration of pancreas or stimulation of the release of endogenous insulin [22] as reported by the

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previous research workers. The entire three extracts treated group at dose of 250 mg/kg showed significant ($p \leq 0.01$) reduction in the elevated serum cholesterol and serum triglyceride levels of diabetic rats when compared with untreated diabetic rats due to increased excretion of neutral sterol and acidic steroids in feces [23-24]. These extracts were able to increase the liver glycogen content significantly ($p \leq 0.01$) as compared with the untreated diabetic rats. This improvement in liver glycogen levels may be due to the improvement of glycogenesis.

Current study gives evidence that treatment with methanol, petroleum ether and aqueous extract of *Holarrhena antidysenterica* (seed) has a favorable effect not only on blood-glucose levels, liver glycogen but also on serum lipids and body weight. This emphasized the promising effect of *Holarrhena antidysenterica* seed being a useful antidiabetic agent and furthermore in diabetic complications. Further studies are necessary to ascertain the use of *Holarrhena antidysenterica* seed in diabetic complicationsand to elucidate the proper mechanism of action.

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