Anti-diabetic Potential of Ethanolic Extract of *Holarrhena antidysenterica* Linn Leaves

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ABSTRACT

The anti-diabetic activity of ethanolic extract of *Holarrhena antidysenterica* leaves (EEHA) was evaluated against alloxan (100 mg/kg, i.p.) and streptozotocin (STZ) (50 mg/kg, i.p.) induced diabetes in Wistar rats. Oral glucose tolerance test was also performed. The effect was compared with antidiabetic activity of glibenclamide (5 mg/kg, p.o.). In alloxan and STZ induced hyperglycemic rats, a marked rise in blood glucose level was observed in diabetic control compared to normal control rats. The EEHA exhibited a significant dose dependent antihyperglycemic activity when compared with diabetic control. The effect of test extract on blood glucose level was seems to be less potent than reference standard glibenclamide. The results of the present study indicated that *Holarrhena antidysenterica* leaves extract possesses significant dose dependent anti-diabetic activity. Thus the traditional use of this plant in the treatment of diabetes mellitus might be validated by this investigation.

Keywords: Alloxan, Anti-diabetic activity, Glibenclamide, Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) consists of a group of syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, and proteins; resulting from defects in insulin secretion, its action, or both. It is a chronic metabolic disorder which affects a significant population world wide. It is a major cause of morbidity and mortality. Present drugs which are used for the treatment of this disease are mainly insulin, sulphonylureas and biguanides. All these drugs are associated with adverse effect and not able to control metabolism adequately. Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. There is growing interest in herbal remedies due to these reasons [1].

Diabetes mellitus is a disease in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated by hormone insulin resulting in elevation of fasting and postprandial blood glucose levels. The major chronic complications associated with diabetes include retinopathy, neuropathy, nephropathy, atherosclerotic coronary artery disease and peripheral atherosclerotic vascular diseases. Besides hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis. Despite the great strides that have been made in the understanding and management of this disease, the graph of diabetes-related mortality is raising unabated [2]. Although a number of synthetic drugs are available in the market, diabetes and its related complications still remain uncontrolled.

From ancient time plant species are being used in the treatment of various diseases. But yet only about five percent of the total plant species has been thoroughly tested for its safety and efficacy. *Holarrhena antidysenterica* commonly known as Kurchi or Kutaj is one of the important medicinal herb of the family *Apocynaceae*. Recent study reflects the use of these traditional medicinal plants against malaria, multiple antibiotic resistant enteropathogenic *E. coli* and in gut motility disorder. The leaves are used in chronic bronchitis, boils, ulcers and dysentery [3].

The survey of literature reveals that various parts such as bark, seeds and leaves of *H. antidysenterica* Linn have been traditionally used for anti diabetic activity. This plant is also known to contain various polyphenolic compounds like flavonoids, tannins, etc related to antidiabetic potentials. But there is no published scientific data for anti diabetic activity of leaves of *Holarrhena antidysenterica*, hence to ascertain the claim, the ethanolic extract of *Holarrhena antidysenterica* leaves (EEHA) has been selected to evaluate antidiabetic activity in the present study.

MATERIALS AND METHODS

Collection and authentication of plant material

The fresh leaves of *Holarrhena antidysenterica* used for the present study were collected from Kottakkal, Kerala, in May 2013. It was authenticated by Mr. Prabhu Kumar, Scientist Kottakkal Aryavaidya Sala, Kerala. The leaves were dried under shade. The dried leavers were pulverized separately into coarse powder by a mechanical grinder and were used for extraction.

Preparation of Ethanolic Extract

The powdered material (150 g) was packed in Soxhlet extractor and extracted using ethanol as solvent. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was concentrated to syrupy consistency by using rotary flash evaporator. The concentrated extract was then air dried at room temperature and stored in air tight container in 2–8°C until used.

Preliminary phytochemical screening

Freshly prepared ethanol extract of the leaves of *Holarrhena antidysenterica* was subjected to phytochemical screening tests for the detection of various constituents [4].

Experimental animals

Healthy Wistar albino rats (150–200 g) of either sex were used for the experiment. They were maintained under standard conditions (temperature 27 ±2°C, relative humidity 60 ± 5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing paddy husk as bedding. They had free access to standard chow and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol (Approval No. SCP/CPCSEA/P19/F150/2012). All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health". The animals were acclimatized for at least one week before use.

Acute toxicity study

Acute toxicity study of ethanolic extract of the leaves of *Holarrhena antidysenterica* was determined in Wistar albino rats according to OECD guidelines No. 425 [5]. The animals were fasted overnight and the ethanolic extract 2000 mg/kg was administered orally. Animals were observed continuously for first 3 h and monitored for 14 days for mortality and general behavior of animals, signs of discomfort and nervous manifestations.

Anti-diabetic activity

Alloxan induced anti-diabetic activity

Hyperglycemia was induced by single i.p. injection of 100 mg/kg of alloxan monohydrate in normal saline. After 3 days of alloxan injection, the hyperglycemic rats (glucose level > 200 mg/dl) were separated and used for the anti-diabetic study [6]. Animals were randomly divided into 5 groups of 6 each and assigned as follows,

Group I: Vehicle control (normal saline)

Group II: Diabetic control (Alloxan, 100 mg/kg, i.p.)

Group III: Diabetic + Glibenclamide (5 mg/kg, p.o.)

Group IV: Diabetic + *H. antidysentrica* (200 mg/kg, p.o.)

Group V: Diabetic + *H. antidysentrica* (400 mg/kg, p.o.)

The treatment (p.o.) was started from 3 days after alloxan injection except diabetic control groups. The animals had free access to feed and water *ad libitum*. Fasting blood glucose level was determined after depriving food for 16 h with free access to drinking water.

Streptozotocin (STZ) induced anti-diabetic activity

Hyperglycemia was induced by single i.p. injection of 50 mg/kg of STZ in citrate buffer, freshly prepared and injected within 5 minute of preparation to prevent degradation. After administration of STZ the animals had free access to feed and water *ad libitum*. The development of hyperglycemia in rats were confirmed by fasting blood glucose estimation 72 h post STZ injection, wherein animals were fasted overnight again for blood collection. The rats with fasting blood glucose level of above 200 mg/dl at 72 h after STZ injection were considered diabetic and included in the study [7]. Fasting blood glucose was determined after depriving food for 16 h with free access to drinking water. Animals were randomly divided into 5 groups of 6 each and assigned as below.

Group I: Vehicle control (Citrate buffer)

Group II: Diabetic control (Streptozotocin, 50 mg/kg, i.p.)

Group III: Diabetic + Glibenclamide (5 mg/kg, p.o.)

Group IV: Diabetic + *H. antidysentrica* (200 mg/kg, p.o.)

Group V: Diabetic + *H. antidysentrica* (400 mg/kg, p.o.)

Collection of blood and serum samples

The above treatment was carried out in each group of animals for both the models for 21 days. Fasting blood glucose level was measured using single touch glucometer. Blood samples were withdrawn under mild anesthesia from retro-orbital of the overnight fasted animals on 1st, 7th, 14th, and 21st day. Body weight of the animals was also measured during these days. On 21st day the blood was collected for biochemical estimations by retro orbital puncture. The serum was obtained by centrifugation at 3000 rpm for 10 min and they were used for estimation of SGPT, SGOT [8] by using a corresponding kit from Agappe Diagnostics Pvt. Ltd and the intensity of the coloured complex formed after treating with these reagents were estimated in semi-auto analyzer.

Oral glucose tolerance test

The oral glucose tolerance test was performed in overnight fasted normal rats. Rats were divided into four groups (each group containing six animals). After 30 minutes glucose (2 g/kg) was fed to all groups. Blood was withdrawn from the retro- orbital sinus just prior to the glucose administration and at 30, 60, 120, 180 and 240 min after glucose loading and glucose levels were measured [9]. The groups were assigned as below.

Group I: Vehicle control (Citrate buffer)

Group II: Diabetic + Glibenclamide (5 mg/kg, p.o.)

Group III: Diabetic + Extract (200 mg/kg, p.o.)

Group IV: Diabetic + Extract (400 mg/kg, p.o.)

Statistical analysis

Results of biochemical estimation were reported as mean ± S.E.M. The total variation present in a data was analyzed by one way analysis of variance (ANOVA). P value less than 0.05 was considered as statistically significant.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical investigation of the ethanolic extract of *Holarrhena antidysentrica* leaves revealed the presence of flavonoids, alkaloids, tannins and steroids.

Acute toxicity study

There was no mortality and any signs of discomfort amongst the dosed groups of animals suggest that the EEHA is relatively safe in or non-toxic to rats and hence 200 and 400 mg/kg, p.o. doses were chosen for the study.

Alloxan induced anti-diabetic activity7

Fasting blood glucose (FBG) level was within the range of 80-95 mg/dl in all the groups prior to diabetic induction. Treatment with alloxan in normal saline (100 mg/kg, i.p) had increased the FBG level above 200 mg/dl after 72 h. However treatment with glibenclamide and ethanolic extract of *Holarrhena antidysenterica* leaves significantly normalized the elevated blood glucose level as shown in (Table 1).

Body weight of animals in all groups was recorded at 0, 7th, 15th and 21st day. Highest change (decrease) in body weight during study period was found to be in diabetic control group. Treatment with ethanolic extract of *Holarrhena antidysentrica* leaves showed increase in body weight as compared to diabetic control group (Table 2).

Streptozotocin induced anti-diabetic activity

Fasting blood glucose (FBG) level was within the range of 80-90 mg/dl in all the groups prior to STZ administration. Treatment with STZ in normal saline (50 mg/kg, i.p) had increased the FBG level above 300 mg/dl after 72hrs. Hitherto treatment with ethanolic extract of *Holarrhena antidysenterica* leaves significantly normalized the elevated blood glucose level as shown in (Table 3).

Body weight of animals in all groups was recorded at 0, 7th, 15th and 21st day. Highest change (decrease) in body weight during study period was found to be in diabetic control group. Treatment with ethanolic extract of *Holarrhena antidysenterica* leaves showed increase in body weight as compared to diabetic control group (Table 4).

Serum biomarkers

After 21 days of experiment, serum biomarkers such as SGPT and SGOT level were significantly elevated in diabetic control group. However, in animals treated with ethanolic extract of *Holarrhena*

antidysenterica leaves, SGPT and SGOT levels were decreased significantly ($p < 0.01$) when compared with diabetic control (Table 5).

Oral glucose tolerance test

Treatment with ethanolic extract of *Holarrhena antidysentrica* leaves showed dose dependent significant reduction in the blood glucose level after glucose loading as compared to normal control (Table 6).

DISCUSSION

In the present study, diabetes was induced by using alloxan and streptozotocin. Alloxan is a cyclic urea derivative, is reported as a potent diabetogenic agent widely has been used for the induction of experimental diabetes in animal species by damaging the insulin secreting pancreatic β-cells, resulting in a decrease in endogenous insulin release [10]. Alloxan produces oxygen free radicals which causes pancreatic injury and could be responsible for increased blood glucose in animals. Streptozotocin is a broad spectrum antibiotic, induces diabetes in a wide variety of animal species by damaging the insulin-secreting cells of the pancreas [11].

In glucose tolerance test a significant reduction in plasma glucose levels was found in extract treated rats when compared with normal control rats. It is probably due to its antihyperglycemic effect by retarding the carbohydrate absorption from intestine through the inhibition in α- glucosidase activity [12]. This study showed the efficacy of EEHA in alloxan and streptozotocin induced diabetic rats. Alloxan and STZ causes the destruction of pancreatic β cells in albino rats in turn developed marked hyperglycemia. Over production of glucose and decreased utilization by the tissues is the fundamental basis of hyperglycemia in diabetes mellitus.

The possible hypoglycemic activity by EEHA might be through potentiating the action of β cells of islets or stimulation of blood glucose uptake by peripheral tissue or/and inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles [13]. Decrease in body weight in alloxan and streptozotocin induced diabetic rats and weight gain in EEHA treated group is due to loss of tissue protein and muscle wasting in the former and having beneficial effect in preventing loss of body weight and in catabolic process in EEHA treated groups. Significant increase in the weight of the animas treated with EEHA in comparison to vehicle treated diabetic rats indicating that ethanolic extract had beneficial effect in preventing loss of body weight of diabetic rats. Insulin deficiency leads to various metabolic abnormalities in animals. Restoration of the level of biochemical enzymes such as SGPT and SGOT in EEHA and glibenclamide treated albino rats are indication of better control of blood sugar level in this group [14].

Phytochemical studies of the extract revealed the presence of various polyphenolic compounds viz. flavonoids, alkaloids, tannins and steroids. However, number of investigators reported that the flavonoids, saponins, tannins and other polyphenolic compounds are known to possess antidiabetic activity in animals [15]. Thus, the presence of flavonoids, tannins and other polyphenolic compounds in the ethanolic extract of *Holarrhena antidysenterica* might responsible for its antidiabetic activity.

CONCLUSION

From the above observation it can be concluded that ethanolic extract of leaves *Holarrhena antidysenterica* posses a potent anti-diabetic property as it significantly reduced the fasting blood glucose level in alloxan and STZ induced diabetic rats as compared to diabetic control group. Thus justifies the traditional use of this plant in the treatment of diabetes mellitus. However, further studies are required to determine the exact mechanism of action and to isolate and characterize the bioactive principles responsible for the claimed activity.

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Table 1: Serum glucose in alloxan induced diabetic rats

Groups	Blood glucose level (mg/dl)				
	Before diabetic induction	**Day 0**	**Day 7**	**Day 14**	**Day 21**
Normal control	86.97± 2.91	87.8± 1.086	89.52± 1.31	92.81± 0.61	94.95± 0.87
Diabetic control	87.6± 4.458	208.60±3.62	217.81± 3.13	244.17± 2.74	260.98± 3.32
Glibenclamide (5mg/kg)	83.13± 1.53	197.32± 3.94	160.08± 2.14***	134.27± 2.98***	101.65± 3.54***
EEHAD (200 mg/kg)	87.06± 4.367	214.43±7.65	195.021±5.23**	173.74± 6.29**	141.60± 5.76**
EEHAD (400 mg/kg)	92.24± 2.03	221.21±4.25	180.425±5.12***	158.23± 4.214***	128.14± 4.25***

Values are expressed as mean ± SEM; n=6. One way ANOVA followed by Dunnett's 't' test. **P<0.01 and ***P<0.001 compared with diabetic control.

Table 2: Body weight in alloxan induced diabetic rats

Groups	Body Weight (Grams)				Change in body weight (%)
	Day 0	**Day 7**	**Day 14**	**Day 21**	
Normal control	180.45±5.38	184.5± 4.86	189.55± 6.92	192.45± 4.66	-6.65
Diabetic control	185.5± 6.32	164.65± 5.67	160.85± 6.25	154.25± 5.25	1.67
Glibenclamide (5mg/kg)	186.45±2.58	185.30± 2.26***	183.22± 1.67***	190.55± 1.46***	-2.15
EEHAD (200 mg/kg)	188.31±3.12	170.61± 2.61**	176.56± 5.48**	179.05± 4.65**	4.78
EEHAD (400 mg/kg)	185.31±4.12	176.31± 4.12***	179.31± 4.12***	183.31± 4.12***	1.08

Values are expressed as mean ± SEM; n=6. One way ANOVA followed by Dunnett's 't' test. **P<0.01 and ***P<0.001 compared with diabetic control.

Table 3: Serum glucose in STZ induced diabetic rats

Groups	Blood glucose level (mg/dl)				
	Before diabetic induction	**Day 0**	**Day 7**	**Day 14**	**Day 21**
Normal control	85.62± 1.38	86.10± 1.86	86.34± 1.92	87.37± 2.56	85.25± 3.16
Diabetic control	85.08± 4.58	329.54± 13.46	334.33± 5.62	341.87± 7.56	353.42± 5.23
Glibenclamide (5mg/kg)	84.93± 1.13	331.16± 8.15	234.21± 6.54***	163.83± 16.15***	111.36± 3.45***
EEHAD (200 mg/kg)	85.406± 4.67	323.48± 7.56	297.56± 4.32**	217.45± 5.92**	142.46± 4.67**
EEHAD (400 mg/kg)	83.25± 3.12	329.52± 8.46	261.46± 7.21**	195.74± 6.21***	124.15± 2.154***

Values are expressed as mean ± SEM; n=6. One way ANOVA followed by Dunnett's 't' test. **P<0.01 and ***P<0.001 compared with diabetic control.

Table 4: Body weight in STZ induced diabetic rats

Groups	Body Weight (Grams)				Change in body weight (%)
	Day 0	**Day 7**	**Day 14**	**Day 21**	
Normal control	190.5± 5.38	192.45± 5.86	198.05± 5.62	197.45± 4.66	3.64
Diabetic control	185.5± 6.32	162.65± 5.67	152.85± 6.25	147.15± 5.25	2.06
Glibenclamide (5mg/kg)	190.51± 4.12	186.61± 2.61***	184.56± 5.48***	182.55± 4.65***	4.21
EEHAD (200 mg/kg)	185.64± 2.12	180.51± 1.13**	172.81± 4.12**	160.11± 1.36**	1.31
EEHAD (400 mg/kg)	188.51± 4.12	183.61± 1.6***	178.56± 2.48***	179.55± 4.65***	4.78

Values are expressed as mean ± SEM; n=6. One way ANOVA followed by Dunnett's 't' test. **P<0.01 and ***P<0.001 compared with diabetic control.

Table 5: SGPT and SGOT levels in diabetic rats

Groups	Alloxan induced group		STZ induced group	
	SGPT	**SGOT**	**SGPT**	**SGOT**
Normal control	56.43± 1.28	64.52± 1.45	58.61± 2.65	62.63± 3.75
Diabetic control	142.51± 1.95	150.38± 1.65	139.48± 2.38	148.72± 1.05
Glibenclamide (5mg/kg)	89.38± 1.65***	87.45± 2.25**	79.52± 3.54***	76.57± 1.95***
EEHAD (200 mg/kg)	105.87± 3.21**	101.43± 1.67**	95.14± 2.75**	91.14± 1.24**
EEHAD (400 mg/kg)	102.81± 2.65***	98.91± 2.85***	92.80± 2.11***	84.64± 1.69***

Values are expressed as mean ± SEM; n=6. One way ANOVA followed by Dunnett's 't' test. **P<0.01 and ***P<0.001 compared with diabetic control.

Table 6: Blood glucose levels on OGTT in normal rats

Groups	Blood glucose level (mg/dl)				
	0 min	30 min	60 min	120 min	240 min
Normal control	82.12± 1.38	136.22± 1.86	120.49± 2.92	114.77± 2.66	85.44± 4.12
Glibenclamide (5mg/kg)	84.61± 2.58	95.30± 2.26**	86.22± 4.67**	77.16± 5.46**	64.14± 4.32**
EEHAD (200 mg/kg)	81.31± 1.12	126.61± 2.61*	111.06± 5.48*	97.95± 4.65*	84.62± 3.55*
EEHAD (400 mg/kg)	84.25± 2.15	111.61± 2.61**	91.06± 5.48**	82.95± 4.65**	71.62± 3.55**

Values are expressed as mean ± SEM; n=6. One way ANOVA followed by Dunnett's 't' test. *P<0.05 and **P<0.01 compared with normal control.