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Antihyperglycemic effect of *Hypericum perforatum* ethyl acetate extract on streptozotocin-induced diabetic rats

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ABSTRACT

Objective: To evaluate the antihyperglycemic activity of ethyl acetate extract of *Hypericum perforatum* (*H. perforatum*) in streptozotocin (STZ)-induced diabetic rats. **Methods:** Acute toxicity and oral glucose tolerance test were performed in normal rats. Male albino rats were rendered diabetic by STZ (40 mg/kg, intraperitoneally). *H. perforatum* ethyl acetate extract was orally administered to diabetic rats at 50, 100 and 200 mg/kg doses for 15 days to determine the antihyperglycemic activity. Biochemical parameters were determined at the end of the treatment. **Results:** *H. perforatum* ethyl acetate extract showed dose dependant fall in fasting blood glucose (FBG). After 30 min of extract administration, FBG was reduced significantly when compared with normal rats. *H. perforatum* ethyl acetate extract produced significant reduction in plasma glucose level, serum total cholesterol, triglycerides, glucose-6-phosphatase levels. Tissue glycogen content, HDL-cholesterol, glucose-6-phosphate dehydrogenase were significantly increased compared with diabetic control. No death or lethal effect was observed in the toxic study. **Conclusions:** The results demonstrate that *H. perforatum* ethyl acetate extract possesses potent antihyperglycemic activity in STZ induced diabetic rats.

1. Introduction

Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, and proteins, and an increased risk of complications from vascular disease[1]. Apart from currently available therapeutic options for diabetes like oral hypoglycemic agents and insulin, which have limitations of their own, many herbal medicines have been recommended for the treatment of diabetes[2]. A variety of ingredients present in medicinal plants are thought to act on a variety of targets by various modes and mechanisms. They have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications[3]. Hence the present study was carried out to evaluate the antidiabetic activity of *Hypericum perforatum* (*H. perforatum*) ethyl acetate extract.

H. perforatum is widely used in the treatment of

depression in many countries and represents as an accepted alternative to synthetic antidepressants or behavioral therapy, particularly for mild to moderate depression[4]. Recently, antidepressants have been reported to have neuroprotective and antioxidant effect against immobilization stress[5]. However, its exact status in stressful conditions is still not clear so far. *H. perforatum* has also been reported for its traditional use in treatment of DM[6]. Based on the above knowledge *H. perforatum* is selected to evaluate its antidiabetic property in streptozotocin induced diabetic rats.

2. Materials and methods

2.1. Plant material

H. perforatum plant was collected from Western Ghats of Nilgiris, Tamil Nadu and was botanically authenticated by Dr. Somusundaram S, National Institute of Siddha, Chennai. The leaves were air dried at room temperature, finely powdered with auto-mix blender and stored in a

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deep freezer until the time of use. The ethyl acetate extract was prepared using Soxhlet and concentrated by rotary evaporator at 40 °C and stored in a cool place.

2.2. Chemicals

Streptozotocin was obtained from Sigma Chemicals, Bangalore, India. Kits to estimate total cholesterol, triglycerides and HDL-cholesterol were purchased from Merck, Mumbai, India. All other chemicals were of analytical grade.

2.3. Animals

Healthy adult Wistar male albino rats with body weight around (170 ± 5) g at 60–70 days from birth were procured from Madavaram Veterinary Medical College, Chennai, Tamil Nadu. They were housed at poly propylene cages and maintained under standard conditions [12 h light and 12 h dark cycle, (25 ± 3) °C]. The rats were fed with standard rat pellet diet (Pranav Agro Industry Ltd, Maharashtra) and given water *ad libitum*.

2.4. Acute toxicity study

Healthy adult Wistar albino rats starved overnight were divided into five groups ($n = 6$) and were orally fed with the *H. perforatum* ethyl acetate extract at 100 mg/kg bw, 500 mg/kg bw, 1 g/kg bw, 3 g/kg bw and 5 g/kg bw, respectively^[7]. The following profiles of animals were observed continuously for 2 h^[8].

Behavioral profile: Alertness, restlessness, irritability, and fearfulness; Neurological profile: Spontaneous activity, reactivity, touch response, pain response and gait; Autonomic profile: Defecation and urination.

After a period of 24 h and 72 h lethality or death was observed.

2.5. Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed on overnight fasted (18 h) normal rats^[9]. Rats were divided into four groups ($n = 6$), and were administered drinking water, *H. perforatum* ethyl acetate extract at 50, 100 and 200 mg/kg bw^[10], respectively. Glucose (2 g/kg bw) was given 30 min after the administration of extract. Blood was withdrawn from the retro orbital sinus under ether inhalation at 30, 60 and 120 min of glucose administration and glucose levels were estimated using a GOD-POD method^[11].

2.6. Induction of non-insulin dependent diabetes mellitus (NIDDM).

NIDDM was induced in overnight fasted adult Wistar strain albino rats weighing (170 ± 5) g by single intraperitoneal

injection of freshly prepared streptozotocin (STZ), (Sigma–Aldrich, Bangalore) (40 mg/ kg bw) in 0.1 M citrate buffer (pH = 4.5)^[12]. After seven days of STZ administration, blood glucose level was determined. Rats with blood glucose level above 200 mg/dL were considered diabetic and included in the study.

2.7. Experimental design

In the experiment totally 36 rats (6 normal and 30 STZ diabetic surviving rats) were used. These rats were divided into six groups of 6 rats each. The extract was dissolved in 2% tween 80 solutions and administered orally for 2 weeks.

Normal control rats served as Group I; diabetic control rats served as Group II; diabetic rats treated with 50 mg/kg bw *H. perforatum* ethyl acetate extract served as Group III; diabetic rats treated with 100 mg/kg bw *H. perforatum* ethyl acetate extract served as Group IV; diabetic rats treated with 200 mg/kg bw *H. perforatum* ethyl acetate extract served as Group V; diabetic rats treated with 600 µg/kg bw glibenclamide served as Group VI^[13].

At the end of the two week study, the animals were euthanized between 9:00–11:00 am to minimize diurnal variation. Fasting glucose level was estimated by glucose oxidase–peroxidase method^[11]. Insulin level was estimated in plasma of normal and STZ induced diabetic rats by ELISA method. The glycogen level of liver and skeletal muscles was measured by anthrone method^[14]. Lipid profile [total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride] levels in serum were determined according to the instructions of the manufacturer (Merck, Mumbai, India). Glucose–6–phosphatase was determined by the method of Koide and Oda^[15]. Glucose–6–phosphate dehydrogenase was estimated by the method of Bergmeyer^[16].

2.8. Statistical analysis

One-way ANOVA and Student's *t*-test (SPSS program; version 11.5) were carried out to compare the data with the level of significance set at $P < 0.05$.

3. Result

Acute toxicity studies revealed the non-toxic nature of the *H. perforatum* ethyl acetate extract. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period. In OGTT, *H. perforatum* ethyl acetate extract, from 30 min onwards showed significant reduction in plasma glucose levels (Table 1).

Induction of diabetes in the experimental rats was confirmed by the presence of a high fasting plasma glucose level. The effect of *H. perforatum* ethyl acetate extract on fasting plasma glucose levels was presented in Table 2. *H. perforatum* ethyl acetate extract treated rats significantly

Table 1Effect of the *H. perforatum* ethyl acetate extract on oral glucose tolerance test (Mean $\pm$ SEM) (mg/dL).

Groups	Blood glucose level			
	0 min	30 min	60 min	120 min
Normal control	69.03 $\pm$ 2.41	146.29 $\pm$ 1.15	134.05 $\pm$ 1.35	123.68 $\pm$ 1.88
<i>H. perforatum</i> (50 mg/kg bw)	65.07 $\pm$ 1.15	135.25 $\pm$ 0.60*	119.47 $\pm$ 0.71**	103.16 $\pm$ 1.88**
<i>H. perforatum</i> (100 mg/kg bw)	65.44 $\pm$ 0.92	128.71 $\pm$ 1.39**	110.28 $\pm$ 1.12**	96.34 $\pm$ 0.86**
<i>H. perforatum</i> (200 mg/kg bw)	65.89 $\pm$ 1.20	123.60 $\pm$ 1.34	100.38 $\pm$ 1.15**	78.15 $\pm$ 1.12**
Glibenclamide (600 μ g/kg bw)	65.93 $\pm$ 0.92	123.41 $\pm$ 0.67**	114.64 $\pm$ 0.76**	74.83 $\pm$ 1.76**

*: $P<0.05$; **: $P<0.01$.**Table 2**Effect of the *H. perforatum* ethyl acetate extract on fasting blood glucose level and plasma insulin level (Mean $\pm$ SEM).

Groups	Fasting blood glucose level (mg/dL)			Plasma insulin (U/mL)
	0 day	7th day	15th day	
Normal control	74.24 $\pm$ 1.04	75.47 $\pm$ 1.46	76.29 $\pm$ 0.58	130.08 $\pm$ 1.66
Diabetic control	220.14 $\pm$ 3.39	235.78 $\pm$ 2.33	247.36 $\pm$ 2.46	54.48 $\pm$ 1.54
Diabetic control + <i>H. perforatum</i> (50 mg/kg bw)	212.89 $\pm$ 3.66	185.84 $\pm$ 1.63**	167.45 $\pm$ 2.37**	65.48 $\pm$ 3.20*
Diabetic control + <i>H. perforatum</i> (100 mg/kg bw)	215.16 $\pm$ 3.51	167.08 $\pm$ 3.12**	134.07 $\pm$ 2.30**	86.71 $\pm$ 1.54**
Diabetic control + <i>H. perforatum</i> (200 mg/kg bw)	216.11 $\pm$ 1.85	158.02 $\pm$ 1.03**	113.29 $\pm$ 1.79**	114.44 $\pm$ 1.33**
Diabetic + glibenclamide (600 μ g/kg bw)	218.83 $\pm$ 1.59	153.68 $\pm$ 0.33**	98.00 $\pm$ 2.17**	116.21 $\pm$ 0.56**

*: $P<0.05$; **: $P<0.01$; comparing with diabetic control group.**Table 3**Effect of the *H. perforatum* ethyl acetate extract on liver and muscle glycogen content in STZ induced diabetic rats after 15 day treatment (Mean $\pm$ SEM) (mg/100 mg wet weight).

Groups	Glycogen content	
	Liver	Muscle
Normal control	46.07 $\pm$ 1.60	10.79 $\pm$ 1.26
Diabetic control	13.83 $\pm$ 0.08	1.44 $\pm$ 0.03
Diabetic + <i>H. perforatum</i> (50 mg/kg bw)	21.50 $\pm$ 1.29*	3.21 $\pm$ 0.19*
Diabetic + <i>H. perforatum</i> (100 mg/kg bw)	29.00 $\pm$ 1.53**	5.54 $\pm$ 0.36*
Diabetic + <i>H. perforatum</i> (200 mg/kg bw)	42.55 $\pm$ 1.12**	7.44 $\pm$ 0.20**
Diabetic + glibenclamide (600 μ g/kg bw)	46.50 $\pm$ 0.62**	8.70 $\pm$ 0.29**

*: $P<0.05$; **: $P<0.01$; comparing with diabetic control group.**Table 4**Effect of the *H. perforatum* ethyl acetate extract on glucose metabolism enzymes in STZ induced diabetic rats after 15 day treatment (Mean $\pm$ SEM).

Groups	Glucose metabolism enzymes	
	Glucose-6-phosphate (Ua/min/mg protein)	Glucose-6-phosphate dehydrogenase (Ub/mg protein)
Normal control	0.16 $\pm$ 0.002	4.41 $\pm$ 0.10
Diabetic control	0.39 $\pm$ 0.006	2.18 $\pm$ 0.13
Diabetic + <i>H. perforatum</i> (50 mg/kg bw)	0.33 $\pm$ 0.009*	2.54 $\pm$ 0.03
Diabetic + <i>H. perforatum</i> (100 mg/kg bw)	0.30 $\pm$ 0.003**	2.79 $\pm$ 0.04*
Diabetic + <i>H. perforatum</i> (200 mg/kg bw)	0.23 $\pm$ 0.009**	3.19 $\pm$ 0.05**
Diabetic + glibenclamide (600 μ g/kg bw)	0.22 $\pm$ 0.002**	3.42 $\pm$ 0.15*

*: $P<0.05$; **: $P<0.01$; comparing with diabetic control group. a: μ mol of Pi liberated per hour; b: nmol of NADPH formed per minute.**Table 5**Effect of the *H. perforatum* ethyl acetate extract on total cholesterol, triglycerides and HDL-cholesterol in STZ induced diabetic rats after 15 day treatment (Mean $\pm$ SEM) (mg/dL).

Groups	Serum lipid profile		
	Total cholesterol	HDL-cholesterol	Triglycerides
Normal control	50.59 $\pm$ 1.40	72.51 $\pm$ 1.29	52.72 $\pm$ 1.70
Diabetic control	203.06 $\pm$ 10.99	24.51 $\pm$ 1.41	204.45 $\pm$ 1.80
Diabetic + <i>H. perforatum</i> (50 mg/kg bw)	111.38 $\pm$ 2.38*	42.06 $\pm$ 1.24**	166.48 $\pm$ 2.76**
Diabetic + <i>H. perforatum</i> (100 mg/kg bw)	102.35 $\pm$ 1.45*	66.54 $\pm$ 1.72**	107.18 $\pm$ 2.26**
Diabetic + <i>H. perforatum</i> (200 mg/kg bw)	95.18 $\pm$ 1.42*	74.84 $\pm$ 1.23**	74.07 $\pm$ 1.18**
Diabetic + glibenclamide (600 μ g/kg bw)	97.82 $\pm$ 3.60*	72.86 $\pm$ 1.12**	74.04 $\pm$ 1.52**

*: $P<0.05$; **: $P<0.01$; comparing with diabetic control group.

reduced its fasting blood glucose in dose dependent manner compared with diabetic control.

Plasma insulin level was decreased in diabetic control when compared with normal control rats. After 15 days treatment with *H. perforatum* ethyl acetate extract the level of plasma insulin was significantly increased when compared with diabetic control (Table 2).

Table 3 presents the level of muscle and liver glycogen content in normal and diabetic control rats. Glycogen content of *H. perforatum* ethyl acetate extract treated diabetic rats were increased significantly when compared with diabetic control.

Table 4 shows the activities of carbohydrate metabolism enzymes in the liver of normal and STZ induced diabetic rats. The activity of glucose-6-phosphatase enzyme increased in diabetic rats whereas the activity of glucose-6-phosphate dehydrogenase decreased when compared with normal control rats. Oral administration of *H. perforatum* ethyl acetate extract decreased the activity of glucose-6-phosphatase and increased glucose-6-phosphate dehydrogenase enzymes significantly in dose dependent manner when compared with diabetic control rats.

Table 5 represents the serum lipid profile. Over five fold increases was observed in total cholesterol, triglycerides and two fold decreases in HDL cholesterol level in diabetic control compared with normal control. *H. perforatum* ethyl acetate extract treated diabetic rats significantly reduced total cholesterol, triglycerides and increased HDL cholesterol compared with diabetic control.

4. Discussion

The present paper discussed the antidiabetic effect of the *H. perforatum* ethyl acetate extract on STZ induced diabetic rats. In our study STZ was selected for induction of diabetes in rats rather than alloxan. STZ is well known for its selective pancreatic β -cell cytotoxicity and has been extensively used to induce DM in animals[17] and it is less toxic than alloxan and allows a consistent maintenance of DM. The experimental diabetic model in this study is type 2 diabetic since low dose of STZ (40 mg/kg bw) destroys half of the population of pancreatic beta cells and there are residual beta cells which secrete insufficient insulin causing type 2 diabetes[18]. Over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues are the fundamental basis of hyperglycemia in DM[19–27]. When *H. perforatum* ethyl acetate extract was administered to fasted normal rats, hypoglycemia was observed after 30 min. The decline in blood sugar level reached its maximum at 2 h. In our study the difference between the initial and final fasting plasma glucose levels of different groups revealed a significant elevation in blood glucose in diabetic control group as compared with normal animals at the end of the

15th day experimental period. Our investigations indicate the efficiency of the *H. perforatum* ethyl acetate extract maintained the blood glucose levels in normal and STZ induced diabetic rats. Rats treated by administration of *H. perforatum* ethyl acetate extract showed a significant decrease in the level of blood glucose and an increase in the level of serum insulin. The possible mechanism by which *H. perforatum* brings about its hypoglycemic action in diabetic rats may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

H. perforatum is reported to contain several phytochemical constituents such as rutin, flavonoids including quercetin, isoquercetin[6]. For example, rutin has been reported to enhance insulin release and decrease blood glucose level in diabetic animals[28].

DM impairs the normal capacity of the liver to synthesize glycogen. Synthase phosphatase activates glycogen synthase resulting in glycogenesis and this activation appears to be defective in diabetes. Skeletal muscle is also a major site of insulin-stimulated glucose uptake[3]. In our study decreased levels of muscle and hepatic glycogen were observed in diabetic control rats. Treatment with *H. perforatum* ethyl acetate (50, 100 and 200 mg/kg bw) for 15 days significantly increased muscle and liver glycogen content, demonstrating the defective glycogen storage of the diabetic state was partially corrected by the extract.

Glucose-6-phosphatase is an important enzyme for the final step of gluconeogenesis or glucogenolysis which catalyzes the hydrolysis of glucose-6-phosphatase to glucose and phosphate. Glucose is transported out of liver to increase blood glucose concentration. Normally insulin inhibits the hepatic glucose production by suppressing glucose-6-phosphatase and fructose 1, 6-bisphosphatase activity[29]. Administration of *H. perforatum* ethyl acetate extract decreased the activity of glucose-6-phosphatase when compared with diabetic control rats and thereby decreased gluconeogenesis.

Since lipid abnormalities accompanying with premature atherosclerosis are the major causes of cardiovascular diseases in diabetic patients, therefore ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile. Cardiovascular diseases are listed as the cause of death in 65% people suffering from diabetes[30]. *H. perforatum* ethyl acetate extract showed significant reduction in serum triglycerides and total cholesterol in STZ-diabetic rats. Thus, it is reasonable to conclude that *H. perforatum* ethyl acetate extract could modulate blood lipid abnormalities.

Thus, the significant antidiabetic effect of *H. perforatum* ethyl acetate extract could be attributed to the presence of various phytoconstituents detected in the phytochemical screening which alone or in synergism can impart therapeutic effect.

In conclusion, ethyl acetate extract of *H. perforatum*

possesses potent antihyperglycemic activity in STZ induced diabetic rats and further study is needed to identify the compounds responsible for its promising *in vivo* anti-diabetic activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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