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Antidiabetic activity of *Hiptage Benghalensis* in chemical-induced diabetic rats

A V S Ravi Sai Nadh
a2zravisai@gmail.com
Jawaharlal Nehru Technological University, Kakinada, Andhra Pradesh

P Rajeswara Rao
a2zravisai@gmail.com
Lydia College of Pharmacy, Ravulapalem, Andhra Pradesh

A Prameela Rani
a2zravisai@gmail.com
University College of Pharmaceutical Sciences, Warangal, Telangana

ABSTRACT

The present study was an attempt to investigate the effect of extracts of Hiptage benghalensis on glycemia, lipid profile, lipoprotein level and antioxidant profile in STZ induced diabetic rats for 21 days. Diabetes was induced using streptozotocin (50 mg/kg i.p) and after the induction of diabetes the animals were given with HBEE (100 mg/kg, 200 mg/kg) orally for 21 days. Blood glucose levels were determined by using GOD-POD method with diagnostic kits. The lipid and lipoprotein level was estimated by using the respective kits. The administration of the extracts orally for 21 days showed that there was an amelioration of the lipid and lipoprotein levels significantly. After 21 days the parameters like HDL, LDL, VLDL, TC, TG, Albumin, Creatinine, total protein and glucose were estimated. The treatment with the extracts and fractions of Hiptage benghalensis improved the lipid level and lipoprotein level to a normal condition which may be attributed to its potent antidiabetic activity. The levels of urea and creatinine were significantly decreased after the treatment of STZ diabetic rats with HBEE. The treatment of diabetic rat with Hiptage benghalensis caused noticeable elevation in serum total protein and albumin levels as compared with normal levels. The treatment with HBEE ameliorated the changes induced by STZ.

Keywords: *Hiptage Benghalensis, HBEE, STZ, GOD-POD.*

1. INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia along with alterations of carbohydrate, fat, lipid metabolism resulting from defective insulin secretion or action leading to micro vascular and macro vascular complications. Diabetes mellitus affects multiple organ systems, and the allied vascular complications are considered as the major cause of death around the world. Patients suffering from diabetes are at high risk of accelerated progression of atherosclerosis due to dyslipidemia and oxidative stress. Although, sulfonylureas fail to control hyperglycemia on long term therapy, they are still widely used to treat patients with type 2 diabetes mellitus.

There were about 285 million adults with diabetes in the world in 2010 and approximately 90-95% of them had type 2 diabetes mellitus. [36] The prevalence of diabetes patients increased from 194 million in 2003, and it is expected to be approximately 439 million by 2030. Type 2 diabetes mellitus is very under-diagnosed and even half of the patients have not been diagnosed. Type 2 diabetes mellitus is associated with severe and disabling comorbidities, such as nephropathy and retinopathy. The treatment of the comorbidities is expensive and often leads to hospitalizations.

When patients are diagnosed with diabetes, a large number of medications become appropriate therapy. These include medications for dyslipidemia, hypertension, anti platelet therapy, and glycaemia control. So many medications can be overwhelming, and it is imperative that patients are thoroughly educated about their drug regimen.

The use of herbal medicine is widespread, which are used by the people for the treatment of disparate diseases even at this modern era. There are diverse medicinal plants in the world, which are the impeding sources of the drugs. These drugs are invariably single plant extracts or fractions or mixtures of extracts/fractions from different plants, which have been carefully standardized for their safety and efficacy. Now a days, scientists and researchers are very much tiring on research of natural plant products all over the world and a large number of substantiation have shown the immense potential of medicinal plants used traditionally. In addition to the known plants, there are unexplored group of plants used by tribal and folk medical practitioners which are a promising source of effective antidiabetic agents. [28] Despite its ancestral use in the treatment of diabetes mellitus there are

insufficient scientific data to support folkloric medicine. This formed the basis of present study which was aimed at investigating the effects of oxidative stress, nephropathy and dyslipidemia in streptozotocin-induced diabetic rats by oral administration of extract.

In the Indian system of medicine, *H. benghalensis* is widely cultivated in the tropics for its attractive and fragrant flowers; it can be trimmed to form a small tree or shrub or can be trained as a vine. It is also occasionally cultivated for medicinal purposes in the alternative medicine practice ayurveda: the leaves and bark are hot, acrid, bitter, insecticidal, vulnerary and useful in the treatment of biliousness, cough, burning sensation, thirst and inflammation; it also has the ability to treat skin diseases and leprosy.[36] But the pharmacological and scientific evidence for its antidiabetic effect is yet to be proved. So based on above fact it can be evaluated for antidiabetic and antioxidant property in streptozotocin (STZ) induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Chemicals used: STZ, diagnostic kits, HBEE (100 mg/kg and 200 mg/kg). The doses were selected based on the acute toxicity studies under OECD guidelines 423. The acute toxicity study was carried out for 14 days and there were no signs of toxicity.

2.2 Extraction and fractionation of the plant:

The plant was collected and authenticated by the botanist. The leaves of the plant were dried and made into a coarse powder and then extracted using soxhlet apparatus with ethanol as solvent and the menstruum was dried by evaporating ethanol and the solid was used for experimental studies.

2.3 Animals used:

Healthy, adult Wistar rats of both sexes (180-220g) were obtained and the animals were kept in a well-ventilated room and the animals were exposed to 12 hrs day and night cycle with a temperature between 20±3 ^{0} C. The animals were housed in large spacious, hygeinic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed *ad libitum,* supplied by this institution. All the experiments were performed after obtaining prior approval from IAEC.

2.4 Induction of diabetes:

Non-Insulin dependent diabetes mellitus (NIDDM) was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of 50mg/kg streptozotocin. The elevated glucose levels in plasma, determined at 72hr, confirmed hyperglycemia**.** The rats with permanent NIDDM (250-350 mg/dL) were used for the study.

3. EXPERIMENTAL MODELS

3.1 Oral glucose tolerance test (OGTT): [47]

The oral glucose tolerance test was performed in overnight fasted (18 h) normal animals. Rats divided in to six group (n-6) were administered with 10 mg/kg Glibenclamide, 100 mg/kg and 200 mg/kg ethanolic extract respectively. Glucose (2 g/kg) was fed 30 min. after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation (to minimize the distress) at 0, 30, 60, 90, and 120 min. of extract administration. The fasting blood glucose levels were estimated by glucose oxidase-peroxidase method.

3.2 STZ induced diabetic model:

The Wistar rats weighing 180-220 gm of either sex were used for the experimental study. The animals were divided into five groups of 6 animals each.

GROUPING OF THE ANIMALS

GROUP I	-	Untreated Control
GROUPII	-	Diabetic control
GROUPIII	-	Positive control (Glibenclamide 10 mg/kg b.w i.p)
GROUPIV	-	HBEE 100 mg/kg, p.o
GROUPV	-	HBEE 200 mg/kg, p.o

The test drug was administered for 21 days at different dose levels of 100, 200 mg/kg for ethanolic extract made in aqueous and given by orally. The blood was collected by sinous orbital under light diethyl ether anesthesia. The blood was centrifuged at 3000 rpm for 10 minutes. Body weight, urine sugar, glucose was analyzed every week and fluid intake was analyzed every day and lipid and lipoprotein profile from serum and tissue homogenate (TC, TG,) were analyzed after 21 days. [29, 30]

Total protein, albumin, creatinine, urea were also analyzed by serum. On the day of termination of the study, the animals were sacrificed; liver and kidney were excised and stored in 10% buffered neutral formalin for histopathological studies.

4. RESULTS AND DISCUSSION

The present study was an attempt to investigate the effect of extracts and fractions of *Hiptage benghalensis*on glycemia, lipid profile, lipoprotein level and antioxidant profile in STZ induced diabetic rats. The phytochemical screening showed the presence of alkaloids, tannins, terpenes, phenols, flavonoids that are responsible for the antidiabetic activity and also for free radical scavenging activity.

The goal of blood glucose tests is to find out whether there is the availability of large amounts of glucose in the blood. The combination of increased hepatic glucose production and reduced metabolism in peripheral tissues leads to elevated plasma

glucose levels. [41] The treatment with HBEE diabetic rats significantly decreased the elevated serum glucose levels from first week onwards.

Serum lipid profile is usually raised during diabetes and presents a risk for the coronary heart disease. Serum total lipids, TG and TC were increased in STZ diabetic rats. The treatment with the extracts of *Hiptage benghalensis* decreased the raise of lipids in serum. The treatment with the extracts of *Hiptage benghalensis* improved the lipid level and lipoprotein level to a normal condition, which may be attributed to its potent antidiabetic activity. The diabetic hyperglycemia induces elevation of the serum levels of urea, creatinine which are considered as significant marker of renal dysfunction. The result showed a significant increase in the level of serum urea, creatinine in the diabetic rats when compared with respective control rats. The levels of urea and creatinine were significantly decreased after thetreatment of STZ diabetic rats with HBEE. Reduction in serum total protein and albumin level was observed in diabetic rats. [49]

Effect of administration of HBEE on body weight and fluid intake in normal and diabetic rats for 21 days.

S. No	TREATMENT	Body weight (g)		Fluid intake mL/animal/day
		Before treatment	After treatment	
1.	Untreated control	194.00±1.88	220.5±1.84	22.05±0.25
2.	Diabetic control	202.66±2.33	168.5±2.51 ###	75.28±0.22 ###
3.	Diabetic+Glibenclamide(10 mg/kg)	206.66±1.75	222.3±1.96 ***	53.61±0.37 ***
4.	Diabetic+HBEE (100 mg/kg)	185.50±6.56	196.0±2.89 ***	33.31±1.60 ***
5.	Diabetic+HBEE (200 mg/kg)	186.33±11.32	196.8±13.10 ***	33.07±2.69 ***

All values are expressed as mean ± S.E.M (n=6).
** P<0.01, *** P<0.001 as compared to diabetic control
P<0.001 as compared to untreated control

The decrease in total protein and albumin may be due to the microproteinuria and albuminuria, which is important clinical marker of diabetic nephropathy, may be due to protein catabolism. The result of present study demonstrated that the treatment of diabetic rat with *Hiptage benghalensis* caused noticeable elevation in serum total protein and albumin levels as compared with normal levels. It has been established that insulin stimulated the incorporation of amino acids into proteins.[39]

During diabetes liver shows decrease in weight due to enhanced catabolic processes such as glycogenolysis, lipolysis and proteolysis, whereas increase in kidney weight is attributed to the overutilization and subsequent enhancement in glycogen synthesis, lipogenesis and protein synthesis. But the treatment with HBEE ameliorated the changes induced by STZ. [41]

Increase in kidney lipid profiles during diabetes appears to be due to increased glucose flux and reducing equivalents leading to an overall enhanced biosynthetic pathway and an increase in hepatic lipid level may be due to increased uptake from portal system mainly due to decreased lipogenesis and increased lipolysis. [49]

Glucose tolerance signifies the ability of the body to dispose off additional glucose entered into the body. It is useful in distinguishing a person with normal glucose tolerance and a person with impaired glucose tolerance namely diabetic. The increase in blood sugar level after glucose loading is sharp and the magnitude of increase is generally greater than normal ones. In the present study, the treatment with HBEE tolerated by the animals compared with that of normal untreated group of animals.

Histopathology reports of liver and kidney gave additional support to the study. Liver sections of normal animals showed the normal architecture with well brought out central vein, well preserved cytoplasm and prominent nucleolus whereas the diabetic group section showed the presence of feathery degeneration, micro and macro cellular fatty changes and inflammatory cells around portal tract. The other groups showed good protection from STZ induced changes in the liver. The sections of normal rat kidney showed the normal nephro-morphology whereas the diabetic section showed fatty degeneration. The other groups showed the less pathological changes of the kidney. [31]

Effect of administration of HBEE on serum glucose

S.No	TREATMENT	Serum glucose (mg/dL)			
		0 day	**7th day**	**14th day**	**21th day**
1.	Untreated control	84.83±5.41	85.33±5.87	84.66±5.77	84.83±5.09
2.	Diabetic control	298.16±17.20	367.33±4.70 ###	413.83±16.61 ###	410.00±2.05 ###
3.	Diabetic+Glibenclamide (10mg/kg)	280.33±2.44	205.33±1.14 **	165.00±1.29 ***	114.83±1.302 **
4.	Diabetic+HBEE (100 mg/kg)	319.33±6.76	217.83±6.46 **	147.17±5.36 ***	121.00±2.67 ***
5.	Diabetic+HBEE (200 mg/kg)	322.17±10.20	217.17±9.10 **	142.17±7.10 ***	115.00±4.50 ***

All value are expressed as mean ± SEM (n=6).
***P<0.001, **P<0.01 as compared to diabetic control.
##P<0.01, ###P<0.001 as compared to untreated control.

Effect of HBEE on serum lipid and lipoprotein profile

S.No	TREATMENT	TC(mg/dL)	TG(mg/dL)	HDL(mg/dL)	LDL(mg/dL)	VLDL(mg/dL)
1.	Untreated control	86.50±0.76	54.83±1.14	54.80±0.69	18.38±0.40	8.79±0.40
2.	Diabetic control	156.68±0.72 ###	183.5±11.59 ###	21.33±0.44 ###	37.73±6.75 ###	37.10±2.85 ###
3.	Diabetic + Glibenclamide (10mg/kg)	69.33±2.81***	116.5±5.21***	32.63±2.30 ***	16.27±1.39 ***	23.96±1.13 **
4.	Diabetic+HBEE (100 mg/kg)	73.18±4.59 *	123.3±6.32 **	41.13±0.52 **	18.48±1.51 ***	23.90±0.87 ***
5.	Diabetic+HBEE (200 mg/kg)	66.15±7.50 **	120.33±5.37 ***	31.62±1.74 ***	15.27±0.79 ***	20.00±1.49 ***

All value are expressed as mean ± SEM (n=6).
* P<0.05,** P<0.01, *** P<0.001, as compared to diabetic control
P<0.01, ### P<0.001,as compared to untreated control

Effect of HBEE on kidney marker enzymes

S.No	TREATMENT	Urea (mg/dL)	Creatinine (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
1.	Untreated control	23.70±0.15	0.9±0.025	6.195±0.26	3.48±0.15
2.	Diabetic control	37.59±0.73 ###	2.41±0.123 ###	3.99±0.12 ###	1.63±0.10 ###
3.	Diabetic+Glibenclamide (10mg/kg)	21.50±0.39 ***	1.35±0.114 ***	6.09±0.174 ***	3.26±0.08 ***
4.	Diabetic+HBEE (100 mg/kg)	19.43±2.24 ***	1.86±0.098 ***	10.33±1.28 **	3.72±1.43 **
5.	Diabetic+HBEE (200 mg/kg)	18.14±1.67 ***	1.42±0.098 ***	7.83±1.21 ***	3.24±1.77 ***

All value are expressed as mean ± SEM (n=6).
*** P<0.001, ** P<0.01 as compared to diabetic control
P<0.001 as compared to untreated control

Effect of HBEE on liver and kidney weight

S.No	TREATMENT	LIVER		KIDNEY	
		Liver wt.(g)	Liver wt./100g b.w	Kidney wt.(g)	Kidney wt/100g b.w
1.	Untreated control	6.42±0.10	3.36±0.12	1.05±0.01	0.65±0.01
2.	Diabetic control	4.55±0.13 ###	2.71±0.08 ###	1.54±0.56 ##	0.77±0.01 ##
3.	Diabetic+ Glibenclamide (10mg/kg)	6.38±0.10 ***	3.83±0.18 ***	1.12±0.01 **	0.65±0.04 **
4.	Diabetic+HBEE (100 mg/kg)	4.74±0.08 ***	3.29±0.14 ***	1.18±0.06 **	0.59±0.01 **
5.	Diabetic+HBEE (200 mg/kg)	4.71±0.12 ***	2.73±0.07 ***	1.25±0.01 **	0.54±0.01 **

All values are expressed as mean ± SEM (n=6).

***P<0.001, **P<0.01 as compared to diabetic control

P<0.001, ##P<0.01 as compared to untreated control

Effect of HBEE on tissue TC and TG

S. No	TREATMENT	Total cholesterol (mg/g tissue)		Triglycerides (mg/g tissue)	
		Liver	Kidney	Liver	Kidney
1.	Untreated control	4.37±0.12	1.60±0.01	9.78±0.41	6.16±0.11
2.	Diabetic control	2.56±0.11 ###	1.26±0.03 ###	13.75±0.19 ###	8.95±0.24 ###
3.	Diabetic+Glibenclamide(10mg/kg)	3.90±0.09 ***	1.63±0.02 ***	9.63±0.40 ***	6.98±0.13 ***
4.	Diabetic+HBEE (100 mg/kg)	3.41±0.08 ***	1.66±0.02 ***	10.70±0.42 ***	7.21±0.06 ***
5.	Diabetic+HBEE (200 mg/kg)	3.69±0.04***	1.58±0.02 ***	10.08±0.45 ***	7.21±0.15 ***

Value are expressed in mean ±SEM (n=6) *** P<0.001 as compared to diabetic control
P<0.001 as compared to control

Effect of HBEE on OGTT

S.No	TREATMENT	Blood glucose concentration(mg/dL)				
		0 min	30min	60min	90min	120min
1.	Untreated Control	88.16±1.25	110.5±1.33	103.1±0.73	102.5±0.76	96.33±0.98
2.	Glibenclamide(10mg/kg)	82.50±0.76	94.2±4.01 ###	80.5±2.18 ###	78.1±2.12 ###	66.5±0.76 ###
3.	HBEE(100mg/kg)	80.10±1.40	110.3±2.51 **	101.8±3.81 **	89.5±2.86 ***	85.6±2.21 **
4.	HBEE(200mg/kg)	77.83±2.60	94.2±2.61 ***	89.2±1.90 ***	85.8±2.77 ***	79.2±2.61 ***

All value are expressed as mean ± SEM

***P<0.001, **P<0.01, as compared to Glibenclamide

P<0.001, as compared to untreated control

5. CONCLUSION

The antidiabetic activity of the plant has been further confirmed by biochemical parameters and histopathological studies. The phytochemical studies revealed the presence of phenols, tannins, flavonoids, steroids & triterpenoids. These components may be responsible for the antidiabetic activity. Further studies are needed to characterize the active principle of *Hiptage benghalensis* which can offer antidiabetic properties and to establish that the whole plant is of utmost importance in serving the mankind's

urgent health needs.

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