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RESEARCH ARTICLE

In vivo antioxidant and hypoglycaemic potentials of *Rivina humilis* extract against streptozotocin induced diabetes and its complications in wistar rats

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Abstract

Purpose This current research study was designed to investigate beneficial effects of *R. humilis* (*Rivina humilis)* against streptozotocin-induced diabetic rats.

Methods The *R. humilis* ethanol extract was prepared using soxhlet and its phenol content was determined. The type-2 diabetes was induced in rats by giving fructose mixed drinking water and single dose of streptozotocin. Oral glucose tolerance test (OGTT) was performed after 72 h of streptozotocin to check ability of extract to utilize oral glucose load with 2 h. The extract was also tested for its potentials to reduce blood glucose (BGL) and diabetic complications by administering to diabetic rats for 21 days. Blood glucose was determined on day 1, 7, 14 and 21. At 21st day, blood samples were collected from experimental rats were euthanized to collect pancreas and liver. Liver and kidney function tests, HbAc1 and lipid profile was established from blood samples. Pancreas was subjected to histopathological examination and liver was used to determine antioxidant enzymes. In vitro study was done to investigate the effect of extract on glucose utilization by rat hemidiaphragm.

Results In OGTT, administration of extract could stimulate glucose utilization which was witnessed by significant BGL reduction at 90 and 120 min in therapeutic groups compare to diabetics. In chronic study, we observed significant reduction in BGL on 21st day and all tests performed to determine liver and kidney function, HbAc1, vitamin E were normal in extract treated groups. There was significant increase in liver antioxidant enzymes in therapeutic groups which revealed regeneration of β-cells in therapeutic groups.

Conclusion The results of research demonstrated significant antidiabetic potentials in *R. humilis*.

Keywords Hypoglycaemic · Anti-oxidant · Serum glucose · Lipid profile · ALT · AST · Urea · *Rivina humilis* and streptozotocin

Abbreviations

ALT	Alanine transferase
AST	aspartate transferase
CAP	Catalase peroxidase
DB	Diabetes mellitus
EERH	Ethanol extract of *Rivina humilis*
GSH	Glutathione reductase
GST	Glutathione S transferase
SOD	Superoxide dismutase
TC	Total cholesterol
TG	Triglycerides

Introduction

Diabetes mellitus arises as a result of disruptions in the body's metabolism brought on by an absolute or relative lack of insulin or by insulin resistance, which ultimately causes changes in the metabolism of the nutrients lipids, carbs, and amino acids [1]. According to a World Health Organization (WHO) survey, India will have the highest prevalence of

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diabetes in the world by 2020, with an estimated 220 million people worldwide having the disease. Due to the great incidence of the condition and its long-term effects, there is therefore always room for the development of anti-diabetic medications [2]. The insulin a peptide hormone produced from recombinant DNA technology is used in insulin dependent diabetes mellitus (Type 1 or IDDM) and oral hypoglycemic drugs are used in non-insulin dependent diabetes mellitus (Type 2 or NIDDM) to bring hyperglycemic to euglycemic condition in individuals [3, 4]. Despite being a very helpful tool in the control of diabetes and hyperglycemia, insulin is known to be associated with a number of risk factors, including local lipodystrophy at the injection site and weight gain following extended administration. The search for innovative anti-diabetic drugs with less adverse effects on patient health has resulted from this [5]. Although there is availability of several pharmacological agents for the management of diabetes mellitus, still there is no truly satisfactory drug for its effective management with less side effects. Hence identification and development of newer therapeutic agents remains highly desirable. In view of the toxic effects and adverse reactions associated with the therapy using presently available oral hypoglycemic drugs and insulin, searching for more potent and less toxic hypoglycemic drug from plant origin is under pipeline throughout the world since herbal medicine play essential role in this segment due to their minimum side effects [6]. Since ancient period Ayurveda physicians Charka and Sushruta had mentioned the usefulness of several medicinal plants for the effective management diabetes with fewer side effects in Ayurveda, the traditional medicinal system of India. Herbal remedies for diabetes mellitus constituting of plant substances, either a single agent or in combination with other drugs, which are considerably safe and free from adverse reactions compared to synthetic agents [7, 8].

Ayurveda uses a variety of botanicals that have been demonstrated to have anti-diabetic properties. Numerous investigations on herbals by scientists have revealed that natural remedies may be superior to synthetic medications in treating hypoglycemia. Utilizing herbal treatments that have historically been employed in the pharmaceutical business is essential for the efficient management of diabetes and the secondary issues that are associated to it [9]. The *R. humilis* is a species of flowering plant in the family *Petiveriaceae*. It is commonly known as blood berry and used folklore and other traditional medicine for the treatment of various diseases (Fig. 1). *R. humilis* is one among those plants used traditionally for the treatment of diabetes mellitus but there is no scientific data available for the same [10, 11]. In order to prove the scientific validity of *R. humilis* for antidiabetic potentials against streptozotocin-induced diabetes and its consequences in wistar rats.

Materials and methods

Preparation of ethanol extract

The *R. humilis* was collected in chittore forest and authenticated by Dr. Madhavachetty, Asst.Prof., Department of

Fig. 1 Rivina humilis plant

Botany, Sri Venkateshwara university, Tirupati and specimen herbarium sample was kept for future reference at institute herbarium library (Herbarium No.0701). The whole plant was dried and grounded into powder which then passed through sieve No. 22 mesh. The coarse powder about 300g was defatted followed by extraction using ethanol in soxhlet apparatus. The percentage yield of ethanol extract was found to be 8.12 and it was light green in colour [12].

Preliminary phytochemical investigation

The ethanol extract of *R. humilis* (EERH) was tested for the preliminary phytoconstituents by procedure given in Khandelwal [13].

Assessment of total phenolic content in EERH

Using the Folin Ciocalteu reagent, the total phenolic content of the ethanol extract of *R. humilis* was determined (1mL). The following protocol has been reached in a brief: 1 mL of a solution containing 1 mg of ethanol extract was added to a 100-mL Erlenmeyer flask, and the final volume was subsequently changed to 46 mL by adding distilled water. With the use of the Foline Ciocalteu reagent and 3 mL of 2% sodium carbonate, the aforementioned reaction mixture was brought to a reaction after 3 min. The mixture's absorbance at 760 nm was measured after two hours of room temperature mixing. The following equation, which was taken from a standard reference, was used to express the amount of the total phenolic content in EERH as micrograms of pyrocatechol [14].

$$\text{Absorbance} = (1 \times 10^3) \times \text{Pyrocatechol } (\mu g) + 0.0033.$$

Experimental animals

Vaarunya Biolabs Pvt Ltd. in Bengaluru provided male wistar rats weighing between 120 and 140 g. All test animals were housed in ideal laboratory conditions with constant temperature of 22 °C and relative humidity of 55 °C, and they were fed a standard pril diet from Amrut, Pranav Agro Industries Ltd., Sangli, India, along with unlimited access to water. The animals were handled in accordance with the CCSEA regulations and were given unlimited access to food and water. The protocols used in the study were approved by Institutional Animal Ethics Committee, East West College of Pharmacy, Bengaluru – 560,091. (Ref.NO.EWCP/CPCSEA/IAEC/V/2021/05).

Induction of diabetes

All experimental animals, with the exception of the control group in both sets, were given full access to fructose (10%) in drinking water for two weeks to induce insulin resistance after seven days of acclimatisation. A single dosage of streptozotocin (60 mg/kg, b.w.) was then administered intraperitoneally to induce type-2 diabetes mellitus (DM). Blood glucose testing was done 48 h after the injection of streptozotocin to identify the development of DM [15].

Evaluation of hypoglycaemic potentials of EERH

Acute anti-diabetic property of EERH was determined by Oral glucose tolerance test and chronic anti-diabetic study for 21 days was performed to check long term effect of ethanol extract using above diabetic rats. The details of treatment protocol are given following table.

In both the sets, all 30 animals were divided into five groups consisting of six in each group and details of treatment protocol are given in Table 1.

Oral glucose tolerance test [OGTT

On the third day after the creation of diabetes in experimental rats, oral feeding tubes were utilised to administer the glibenclamide and EERH solutions to overnight-starved animals. An hour after receiving treatment with EERH and glibenclamide, all experimental animals had blood drawn by retro-orbital vein puncture, and the basal blood glucose level was determined. Blood samples were obtained from each animal at predetermined intervals of 30 min, 60 min, 90 min, and 120 min after giving them an oral glucose solution (2 g/kg). Then, a Glucometer (Acucheck) was used to measure each sample's plasma glucose [15–17].

Table 1 Study design for hypoglycaemic properties of EERH

Sl. No.	Group	Treatment
I.	**Normal**	Wistar rats administered with once a day for 21 normal Saline 2ml/kg
II.	**Diabetic control**	Diabetic rats administered with single dose of 2% of tween 20 in OGTT and once a day for 21 days in chronic study.
III.	**Standard**	Diabetic rats administered with single dose of glibenclamide (5 mg/kg., p.o) in OGTT and once a day for 21 days in chronic study.
IV.	**EERH-200 mg**	Diabetic rats administered with single dose of ethanol extract of *R. humilis* (200 mg/kg., p.o) in OGTT and once a day for 21 days in chronic study.
V.	**EERH-400 mg**	Diabetic rats administered with single dose of ethanol extract of *R. humilis* (400 mg/kg., p.o) in OGTT and once a day for 21 days in chronic study.

Chronic study model

According to the groups listed in the above table, diabetic animals were treated 2% tween 20, glibenclamide and EERH once in a day for 21 days in the chronic anti-diabetic study model. On days 1, 7, 14, and 21, blood samples (about 1ml) by retro-orbital vein puncture from each experimental animal (diabetic and normal) were taken in order to measure the plasma glucose levels. Blood samples were also tested on the 21st day of the investigation for the presence of insulin, lipid profile (total cholesterol, triglycerides, HDL and VLDL), Kidney function tests (Creatinine, Urea, Blood urea nitrogen and Uric acid), liver test function tests such as Alanine transferase (ALT), Aspartate transferase (AST), Alkaline phosphatase (ALP) and Acid phosphatase (ACP and estimation of free haemoglobin and glycosylated haemoglobin (HbAc1) were performed [15–17].

Analysis of oxidant, antioxidant enzymes and non-enzymatic antioxidants

At the end of the study, all animals were sacrificed by administering thiopental sodium (20 mg/kg, i.p) and pancreas was collected. The part of pancreas sample from each animal was used to determine the amounts of lipid peroxidation a oxidant marker, antioxidant enzymes such as superoxide dismutase (SOD), catalase peroxidase (CAP), Glutathione peroxidase (GPX) and glutathione-s-transferase (GST) by the standard protocols using commercially available reagent kits (Agappe Diagnostics). Tissue non-enzymatic parameters substances glutathione (reduced), vitamin C and vitamin E were also estimated by standard procedures described by previous investigators [18–20].

Histopathological analysis of pancreas

Each animal's entire pancreas was extracted after it was sacrificed and cleaned in ice-cold saline right away. For histological examinations, a portion of pancreas tissue was fixed in 10% neutral formalin fixative solution. Solid slices were cut at a thickness of 5 m after tissues had been fixed, embedded in paraffin, and stained with hematoxylin and eosin [21].

In vitro antidiabetic activity against glucose utilization by rat hemidiaphragm

The utilization of glucose by isolated skeletal muscle of rat (hemidiaphragm) was assayed according to methods described in previous investigations. The study involves the incubation of skeletal muscle with normal saline consisting of known amount of glucose for prescribed period and estimation of glucose left over after incubation and hence can determine amount of glucose by tissue. The presence of insulin sensitizers can enhance utilization of glucose by tissue during incubation[25]. The study consisting of four categories, with each group containing 6 graduated test tubes, were regarded as follows:

- Group I: Containing 2 mL of glucose (2%) in Tyrode solution.
- Group II: Containing 2 mL of glucose (2%) in Tyrode solution and regular insulin suspension.
- Group III: Containing 2 mL of glucose (2%) in Tyrode solution and 1.38 mL of TPME (0.1% v/v).
- Group IV: Containing glucose (2%) in Tyrode solution and regular insulin (0.62 mL of 0.4 U/mL) solution and 1.38 mL of TPME (0.1% v/v).

The total volume of the test tubes was created by individually combining the amounts of each assay tube to create a total volume of 4 mL. A total of 100 wistar species albino rats in good health were slaughtered while fasting the entire night. Animals used in experiments had their diaphragms rapidly and painlessly separated into two equal halves. Two diaphragms from the same animal were not used for the same set of studies. In each study category, about xix diaphragms were used. The collected skeletal muscles (diaphragm) were shook at a rate of 140 CPM while being incubated at $37^{0\,C}$ for around 30 min in a 100% oxygen atmosphere. The amount of glucose left over in tyrode solution after incubation was determined by benedict's quantitative reagent method. The difference between the concentrations of starting and final glucose in the incubated medium was used to calculate the quantity of glucose utilisation per gram of tissue [22].

Statistical analysis

The outcomes of the study were subjected to statistical analysis by one-way ANOVA followed by Tukey's multiple comparison test using Graphpad Prism software version 9. All the results are expressed as mean plus/minus standard error of mean.

Results

Preparation of extract

The ethanol extract of *R. humilis* was prepared and its percentage yield was found to be 8.12 and it was light green in colour.

Table 2 Results of primary phytochemical analysis on EERH

Sl. No	Phytoconstituent present	Presence
1.	Carbohydrates	++
2.	Alkaloids	++
3.	Flavonoids	+++
4.	Glycosides	+++
5.	Tannins	+
6.	Polyphenols	+++
7.	Proteins	++

Preliminary phytochemical study

The analysis for preliminary phytochemicals conducted for the ethanol extract of *R. humilis* (EERH) has shown following phytoconstituents in the plant (Table 2).

Estimation of phenolic content in EERH

The estimation of amount total phenols in the ethanol extract of *R. humilis* was 115.20 mg pyrocatechol per gram of EERH.

Evaluation of in vivo antidiabetic activity of EERH

Determination of potentials of EERH against OGTT

In OGTT, when compared to the normal group of rats, diabetic control rats administered with a vehicle showed a significant increase in plasma glucose range during the course of the study. However, by enhancing the utilisation of oral glucose after 60 and 120 min, the treatment of glibenclamide and EERH (at both the doses) was able to considerably lower blood glucose levels in therapeutic mice ($P < 0.001$). (Fig. 2).

Chronic antidiabetic potentials of EERH

Effect of EERH on blood glucose

In the long term anti-diabetic study for the extract, the development of diabetes in disease control rats resulted in considerably ($P < 0.001$) higher blood glucose levels than the normal group throughout the study of 21 days. When compared to the diabetic control group, experimental animals treated with both the doses of EERH and glibenclamide have shown significant reduction in plasma glucose on day 14 and 21 while there was no considerable blood glucose reduction was observed in the same animals on day 7 and 14. (Table 3).

Effect of EERH on lipid profile

The concentrations of plasma total cholesterol and triglycerides were significantly ($P < 0.001$) raised in diabetic control rats treated with streptozotocin alone compare to normal rats. Administration EERH at both medium and high dose and glibenclamide significantly ($P < 0.001$) declined blood cholesterol and triglycerides when comparing to diabetic animals. The results were comparable to that of normal rats (Table 4).

Effect of EERH on kidney function tests

In kidney function tests, the concentrations of blood creatinine, urea, blood urea nitrogen and uric acid were significantly ($P < 0.001$) raised in diabetic control rats injected with strepto streptozotocin alone in comparison with normal groups. Experimental rats given with both the doses of EERH and glibenclamide significantly ($P < 0.001$) declined

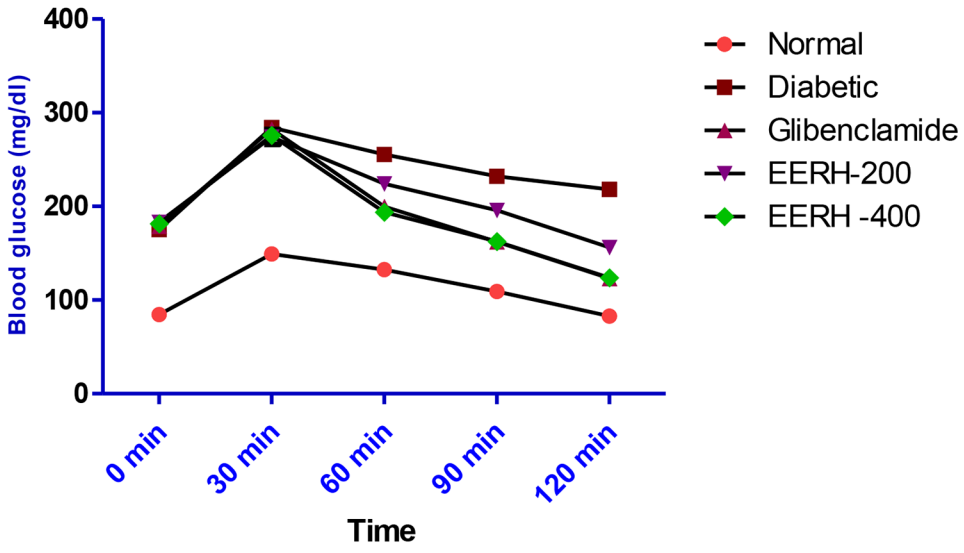

Fig. 2 Effect of ethanol extract of *R. humilis* on blood glucose in OGTT

Table 3 Effect of ethanol extract of *R. humilis* on blood glucose in chronic study

Treatment	Concentration of Blood Glucose (mg/dl)			
	DAY 1	DAY 7	DAY 14	DAY 21
Normal Control	147.4 ± 0.642	124.6 ± 1.01	127.3 ± 0.839	124.4 ± 2.18
Diabetic Control	251.1^{+++} ± 1.06	242.7^{+++} ± 1.14	238.1^{+++} ± 1.16	239.7^{+++} ± 1.26
Standard	242.7^{ns} ± 1.78	219.8^{ns} ± 1.35	171.5^{***} ± 1.08	142.2^{***} ± 1.33
EERH 200 mg/kg	247.1^{ns} ± 1.37	229.3^{ns} ± 1.67	180.7^{***} ± 0.848	167.8^{***} ± 1.98
EERH 400 mg/kg	245.7^{ns} ± 1.39	225.9^{ns} ± 1.23	175.5^{***} ± 0.754	151.2^{***} ± 0.754

Values are mean ± S.E.M, n = 6 symbols represent statistical significance

$^{+++}p < 0.001$ Normal control vs Diabetic control

***p < 0.001, Diabetic control vs Therapeutic groups. ^{ns} p > 0.05

Table 4 Effect of ethanol extract of *R. humilis* on Lipid Profile in chronic study

Treatment	Lipid Profile (mg/dl)				
	Total Cholesterol	Triglycerides	LDL	VLDL	HDL
Normal Control	83.3 ± 4.33	106.7 ± 2.53	33.0 ± 3.28	6.99 ± 1.01	36.22 ± 2.49
Diabetic Control	124.8^{++} ± 8.95	134.6^{++} ± 3.49	58.3^{++} ± 3.43	12.6^{+++} ± 1.42	23.71^{++} ± 2.07
Standard (Glibenclamide 5 mg/kg)	82.9** ± 4.15	104.2* ± 5.17	36.2** ± 3.44	7.79** ± 1.21	36.57** ± 2.21
EERH 200 mg/kg	98.9* ± 4.12	105.3* ± 2.69	50.3* ± 4.82	10.7 ± 1.32	33.04 ± 3.23
EERH 400 mg/kg	92.9** ± 2.56	99.3**0.2 ± 3.64	35.9** ± 3.88	7.77** ± 1.24	37.16** ± 2.07

Values are mean ± S.E.M, n = 6 symbols represent statistical significance. $^{++}$ p < 0.01, $^{+++}$p < 0.001 Diabetic control vs. Normal control

***p < 0.001, Diabetic control vs. Therapeutic groups. ^{ns} p > 0.05,

Table 5 Effect of ethanol extract of *R. humilis* on Kidney Function test in chronic study

Treatment	Blood Parameters					
	Creatinine (mg/dl)	Urea (mg/dl)	BUN	Uric acid (mg/dl)	Sodium (mE/L)	Chlorides (mE/L)
Normal Control	0.734 ± 0.094	32.3 ± 2.50	8.35 ± 1.62	2.92 ± 0.23	138.9 ± 4.34	81.6 ± 0.84
Diabetic Control	2.37^{+++} ± 0.097	69.5^{+++} ± 3.29	26.1^{+++} ± 1.48	4.83^{+++} ± 0.36	237.7^{+++} ± 3.87	142.8^{+++} ± 2.16
Standard (Glibenclamide 5 mg/kg)	0.84*** ± 0.13	28.8*** ± 1.45	8.60*** ± 1.79	2.09** ± 0.15	137.9*** ± 3.43	82.3*** ± 1.79
EERH 200 mg/kg	0.82*** ± 0.14	42.3** ± 2.26	13.4*** ± 1.58	2.92*** ± 0.23	144.9*** ± 4.08	87.4*** ± 2.70
EERH 400 mg/kg	0.81*** ± 0.12	34.5*** ± 2.04	12.0*** ± 1.48	2.31*** ± 0.24	141.1*** ± 4.20	86.3*** ± 3.40

Values are mean ± S.E.M, n = 6 symbols represent statistical significance

$^{+++}p < 0.001$ Diabetic control vs Normal control, ***p < 0.001 & **p < 0.01 Diabetic control vs Therapeutic groups

above said blood parameters to check kidney function parameters when compare to diabetic rats. The results were comparable to that of normal rats (Table 5). Nephropathy is a common secondary complication of diabetes which is characterized by the accumulation of nitrogenous waste urea, BUN, Creatinine and uric acid in the blood. In our study, ethanol extract could significantly reduce concentrations of above constituents in blood samples obtained from therapeutic groups indicating its ability to reverse renal complication due to diabetes.

Effect of EERH on liver function parameters

In this study, the amounts of serum ALT, AST, ALP, ACP and γ-GTP were significantly (P < 0.001) enhanced in diabetic alone animals injected with streptozotocin alone in comparison with normal groups. Experimental rats given with both the doses of EERH and glibenclamide significantly (P < 0.001) lowered plasma liver function parameters when compare to diabetic rats. The results were comparable to that of normal rats (Table 6). The disturbance in the function of liver is another secondary complication of diabetes and hence increased concentrations of liver enzymes is another feature in diabetes. In the present study, administration of streptozotocin could cause diabetes followed by elevated levels of above enzymes in the diabetic control group while administration of our ethanol extract could reduce their concentrations.

Effect of EERH on insulin

There was significant (P < 0.001) reduction in plasma insulin level was seen in animals treated with streptozotocin alone when compare to normal animals. Experimental rats treated with EERH at both medium and high doses and glibenclamide have shown considerably (P < 0.001) enhanced

Table 6 Effect of ethanol extract of *R. humilis* on Liver Function test in chronic study

Treatment	Liver Function Tests				
	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ACP (IU/L)	Total Protein (mg/dl)
Normal Control	65.7 ± 4.91	129.8 ± 2.46	80.92 ± 3.7	91.25 ± 8.24	5.95 ± 0.62
Diabetic Control	155^{+++} ±3.20	237.6^{+++} ±5.78	209.1^{+++} ±8.2	197.7^{+++} ±5.15	3.42^{+++} ±0.72
Standard (Glibenclamide 5 mg/kg)	66.4*** ±6.73	129.1*** ±3.23	87.54*** ±5.08	96.7*** ±5.28	5.85*** ±0.63
EERH 200 mg/kg	97** ±3.85	137.0*** ±5.38	134.6*** ±2.38	133.2** ±3.56	4.47** ±0.51
EERH 400 mg/kg	71.8*** ±4.02	135.9*** ±4.75	87.25*** ±3.24	91.32*** ±4.89	5.73*** ±0.85

Values are mean ± S.E.M, n = 6 symbols represent statistical significance

$^{+++}p < 0.001$ Diabetic control vs Normal control

***p < 0.001 & **p < 0.01 Diabetic control vs Therapeutic groups

Table 7 Effect of ethanol extract of *R. humilis* on insulin, hemoglobin and HbAc1 in chronic study

Treatment	Blood Parameters			Lipid Peroxidation products		
	Insulin (IU/L)	Haemoglobin (g %)	HbAc1 (g %)	Basal	H_2O_2	FeSo4
Normal Control	139.0 ± 3.96	15.2 ± 1.06	6.33 ± 0.42	1.82 ± 0.37	3.83 ± 0.41	3.08 ± 0.31
Diabetic Control	69.0^{+++} ± 3.92	7.21^{+++} ± 0.73	13.5^{+++} ± 0.76	3.23^{+++} ± 0.58	6.84^{+++} ± 0.47	5.61^{+++} ± 0.81
Standard	140.2*** ± 4.48	14.63*** ± 0.51	6.67*** ± 0.42	1.62*** ± 0.24	3.52*** ± 0.42	3.43*** ± 0.6
EERH 200 mg/kg	104.5*** ± 3.21	13.25*** ± 0.73	6.16*** ± 0.47	2.24** ± 0.21	4.08** ± 0.51	3.31*** ± 0.51
EERH 400 mg/kg	135.8*** ± 4.85	15.4*** ± 1.03	6.34*** ± 0.49	2.15** ± 0.54	3.17*** ± 0.54	3.19*** ± 0.48

Values are mean ± S.E.M, n = 6 symbols represent statistical significance. $^{+++}p < 0.001$ Diabetic control vs. Normal control

***p < 0.001, Diabetic control vs Therapeutic groups

concentrations of insulin in plasma in comparison with diabetic control rats. The results were comparable to that of normal rats (Table 7).

Effect of EERH on haemoglobin and glycosylated haemoglobin

Diabetic control animals have shown significant reduction in free haemoglobin and concurrent increase in HbAc1 when compare to normal rats. The rats treated with both medium and high dose of EERH and glibenclamide have significantly shown considerably ($P < 0.001$) enhanced concentrations of insulin and reduction of glycosylated haemoglobin in plasma when comparison with diabetic control rats. The results were comparable to that of normal rats (Table 7).

Effect of EERH on levels of lipid peroxidation products

Lipid peroxidation was significantly increased ($p < 0.001$) in streptozotocin induced diabetic control rats, thereby indicating oxidative degradation of lipids. Chronic administration of EERH and glibenclamide significantly attenuated the oxidative stress response in diabetic rats, as noted by reduced lipid peroxidation level ($p < 0.001$). Further, administration of EERH led to significant attenuation of oxidative stress simultaneously reduced basal, hydrogen peroxide, and ferrous sulphate induced lipid peroxidation in comparison with the streptozotocin induced diabetic rats (Table 7).

Effect of EERH on liver antioxidant enzymes

Administration of Streptozotocin produced diabetes in control rats which caused significant lowering of liver antioxidant enzyme ($p < 0.001$). But administration of EERH led to significant enhancement of activities of antioxidant enzymes when compare to streptozotocin control group ($p < 0.001$). Diabetic control animals have also shown significant decline in the non-enzymatic antioxidant molecules such as vitamin C and vitamin E while comparing to normal rats. The medium and high dose of EERH and glibenclamide administration could lead to significant enhancement of non-enzymatic antioxidant substances in therapeutic animals comparing to diabetic control group. The results were comparable to standard and normal group of animals (Table 8).

Effect of EERH on histopathology of pancreas

The islets of Langerhans were embedded with in acinar cells, uniformly dispersed, frequently and abundantly distributed

and varied in size in the sane of pancreatic tissue taken from normal group exhibited normal architecture. The pancreatic tissue from diabetic control group exhibited extensive damage which was characterized by reduction of size of beta cells and lymphocyte infiltration. But in the pancreatic tissue collected from standard group, we have noticed initial reduction in size and number of beta cells which were back to normal when compare to diabetic control due to the administration glibenclamide for 21 days. The low volume but still high percentage of islets cells was seen in pancreatic tissue taken from rats treated with medium and high dose of ethanol extract comparing to diabetic control group. Though there were little disturbances in the structure of pancreas, significant regeneration of islets of beta cells was noticed in therapeutic groups due to administration of glibenclamide and ethanol extract for 21 days (Fig. 3).

Evaluation of in vitro antidiabetic activity of EERH

In the current investigation, EERH greatly boosted the rat hemidiaphragm's use of glucose, and its effect was comparable to that of the standard agent Insulin. EERH and insulin work well together and have demonstrated synergistic effects. According to the findings, either insulin or EERH alone for 30 min significantly increased glucose absorption by 3.34 and 2.88 times, respectively. Rats' consumption of glucose in their hemidiaphragms increased by 3.54 times when insulin and EERH were added to the incubation medium compared to the group that of normal (Table 9).

Discussion

Diabetes and its comorbidities have grown to be a serious issue in health management. Given the connection between diabetes and other conditions like cancer, cardiovascular risk, fatty liver events, and neurodegenerative illnesses, controlling the glycaemic load and associated stress is essential. Glycemic load and insulin resistance are the two main risk factors for type-2 diabetes mellitus [23]. Therefore, increasing insulin sensitivity is the aim of anti-diabetic drugs. However, the effects of diabetes are frequently connected to the polyol pathway and the consequent oxidative stress cascades. An anti-diabetic medicine must have the ability to limit oxidative damage in order to successfully treat the difficulties brought on by secondary diabetes [24].

Previous studies have shown that streptozotocin can successfully produce type 2 diabetes mellitus in rats within an experimental setting. It demonstrated that giving an experimental animal a single dose of 60 mg/kg STZ could increase blood glucose levels [25]. When STZ is administered, the pancreatic beta cells are damaged by free radicals,which reduces the amount of insulin secreted and causes diabetes and its complications. A decrease in the amount of free haemoglobin in the blood is caused by increased blood glucose occupying haemoglobin and forming glycosylated haemoglobin. Onset of diabetes mellitus can also cause disturbances in the lipid metabolism results in hyperlipidemia characterized by the altered lipid profile which includes total cholesterol, triglycerides, LDL, VLDL and HDL. Reduced renal and liver functions are other complications of diabetes mellitus. Hence increased creatinine, urea, blood urea nitrogen, sodium and chlorides is the feature of renal disturbance and increased alanine transferase, aspartate transferase, alkaline phosphatase, GTP and reduced serum total protein are the characteristics of liver dysfunction in diabetes. One of the best approaches to reverse diabetes is to increase antioxidant mechanisms in the liver which can inactivate scavenging effects of streptozotocin lead to protection of beta cells [26, 27].

In the present study, oral glucose tolerance test was performed to check the capacity of the tissues to utilize glucose administered orally. Administration of EERH could significantly reduce blood glucose in therapeutic rats indicating the effect of *R. humilis* promote glucose utilization by the cells. In the chronic study of 21 days, EERH could significantly reduce blood glucose in therapeutic group of animals on day 14th and 21st indicating the ability of the plant extract to reverse the effects of streptozotocin induced diabetes.

Administration of our ethanol extract could significantly decline the concentrations of total cholesterol, triglycerides, LDL, VLDL and increased HDL in the therapeutic groups.

Table 8 Effect of ethanol extract of *R. humilis* on liver antioxidant enzymes in chronic study

Treatment	Liver Antioxidants						
	GPX	CAP	SOD	GST	Vitamin C	Vitamin E	GSH
Normal Control	12.08 ± 0.91	57.29 ± 2.18	9.33 ± 0.66	7.43 ± 0.39	3.66 ± 0.33	6.84 ± 0.71	7.6 ± 0.31
Diabetic Control	6.26^{+++}±0.60	29.71^{+++}±1.6	4.52^{+++}±0.42	3.95^{+++}±0.45	1.73^{+++}±0.54	3.27^{+++}±0.33	3.52^{+++}±0.33
Standard	11.56***±0.95	49.29***±1.27	8.16***±0.30	7.05***±0.62	3.57***±0.47	5.74***±0.54	7.26***±0.29
EERH 200 mg/kg	8.92**±0.83	35.65**±2.91	7.66±***0.49	5.42**±0.53	2.87**±0.61	6.29***±0.49	7.38***±0.49
EERH 400 mg/kg	10.67***±0.69	48.69***±2.76	8.34***±1.36	6.77***±0.56	3.84***±0.30	5.48***±0.67	7.56***±0.25

Values are mean ± S.E.M, n = 6 symbols represent statistical significance

$^{+++}p < 0.001$ Diabetic control vs. Normal control

***$p < 0.001$ & **$p < 0.01$ Diabetic control vs Therapeutic groups

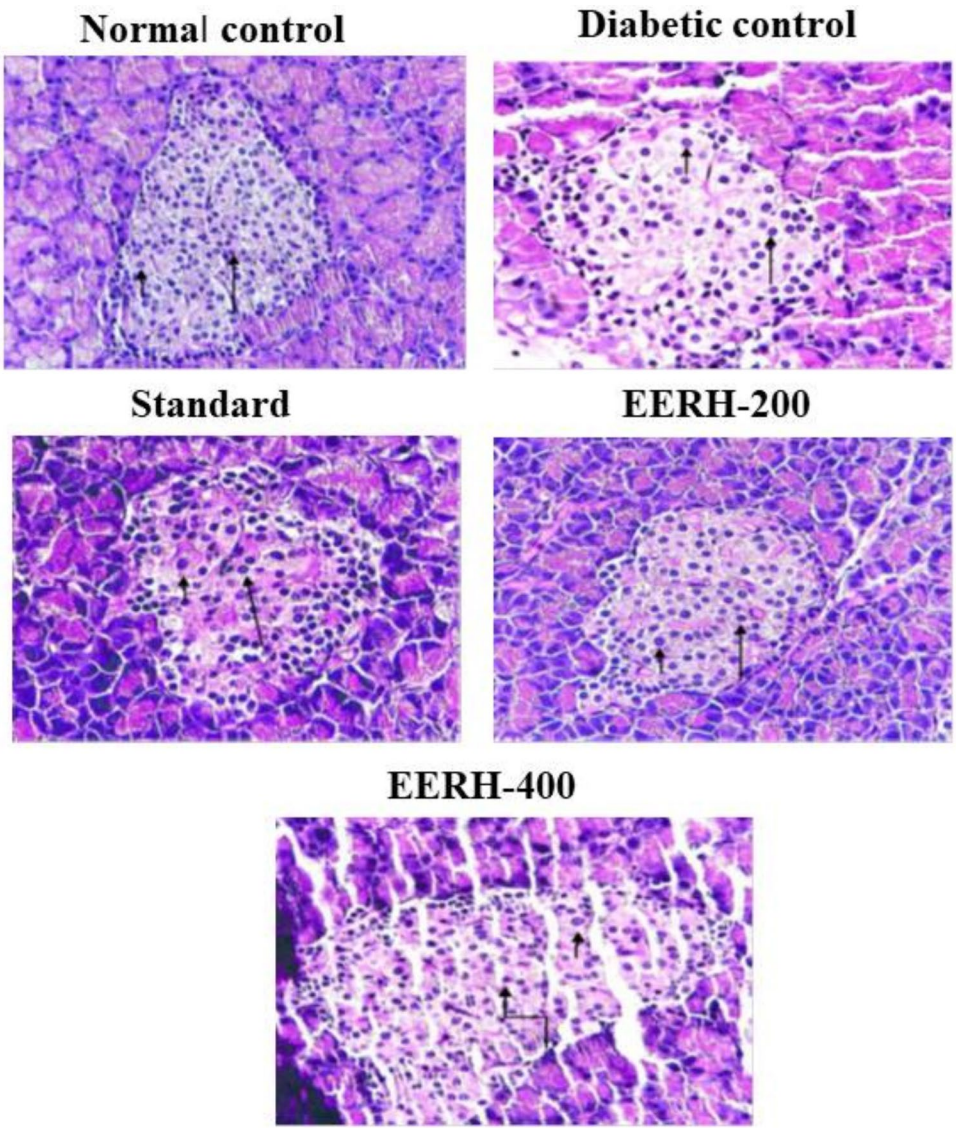

Fig. 3 Effect of EERH on histopathology of pancreas

Table 9 Effect of ethanol extract of *R. humilis* on utilization of glucose in vitro by rat hemidiaphragm muscle

S.No	Glucose uptake for 30 min (mg/g)
Control	81.35 ± 2.34
Insulin	271.8 ± 4.62***
EERH	234.22 ± 3.54***
EERH + Insulin	288.72 ± 5.66***

Values are mean ± SEM (n < 6)

*** $p < 0.01$ as compared with control

The animals treated with EERH could improve the function of kidneys characterized by the reduced concentrations of creatinine, urea, blood urea nitrogen, sodium and chlorides. There was a significantly decreased level of ALT, AST, ALP, GTP and increased concentration of total protein was found in the therapeutic animals administered with ethanol extract of *R. humilis.* The above results could explore the ability of *R. humilis* to restore the function of pancreas and reverse the complications of diabetes mellitus such as hyperglycaemia, hyperlipidemia, nephropathy and liver disease.

Several researches have been conducted to determine the mechanisms of action of their antidiabetic potentials of agents from the plant origin. Besides insulin sensitization and reducing postprandial blood glucose, restoration of the beta cell function is an important approach for the management of diabetes mellitus. The logical therapy options for diabetes are to maintain beta cells' survival and function in patients with early-stage diabetes and to restore beta cells' mass/function in patients with advanced-stage diabetes. Previous studies with isolated pancreatic islets, insulin-producing cell lines, and diabetic animals have shown evidence that phytoconstituents such as flavonoids and plant phenols help to maintain beta cell survival and function due to their antioxidant properties. The antioxidant and anti-inflammatory properties of flavonoids as well as their ability

to stimulate pro-survival pathways and to inhibit proapoptotic proteins expression contribute to the protective action of these natural compounds against beta cell death induced by cytokines, glucotoxicity, and lipotoxicity in diabetes. Also, improving mitochondrial bioenergetic function and stimulating amplifying pathways of insulin secretion (e.g., cAMP/PKA and PLC/PKC signaling) are likely the main mechanisms by which flavonoids preserve the secretory capacity of beta cells.

Phytochemical investigation performed in the present work explored medicinally active phytoconstituents and significant amounts of plant phenols in the *R. humilis* which possess antioxidant properties [28, 29]. In the current investigation, we also found that administration of ethanol extract of *R. humilis* could stimulate the antioxidant system in the liver. EERH increased the production of antioxidant enzymes glutathione peroxidase, catalase peroxidase, superoxide dismutase and glutathione s transferase and also increased the synthesis of non-antioxidant substances such as vitamin C, vitamin E and reduced glutathione in the experimental rats belongs to therapeutic groups. Stimulation of liver antioxidant system by the liver could neutralize scavenging effects and protected the beta cells of pancreas against STZ. This was supported by the results of histopathological examination of pancreas. Administration of EERH could repair the beta cells and promoted insulin release in the present research.

It was observed that ethanol extract of *R. humilis* potentially stimulate the utilization of blood glucose by rat hemidiaphragm in vitro which is also contribution to reduce blood glucose in diabetic animals. The active constituents of plant itself antioxidants and also enhanced antioxidants system in the liver which may be the main reasons for the beneficial effects of extract of *R.* in the present investigation.

Conclusion

The findings of the current research work suggesting that ethanol extract of *R. humilis* possess considerable benefits for the management of diabetes and its complications against streptozotocin induced diabetic rats. However further study should be conducted to determine specific active constituent present in the plant responsible antidiabetic properties and also should be tested clinically for its efficacy for the management of diabetes and its complications.

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Author contributions All authors have equal contributions in completing the present research work.

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Declarations

Ethics approval and consent to participate The present experimental protocol was designed according to ethical principles of CPCSEA, New Delhi and the study was approved by the Institutional Animal Ethics Committee, East West College of Pharmacy, Bangalore (Ref No.-EWCP/CPCSEA/IAEC/V/02).

Competing interests We are by declaring that there is no conflicts interest.

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