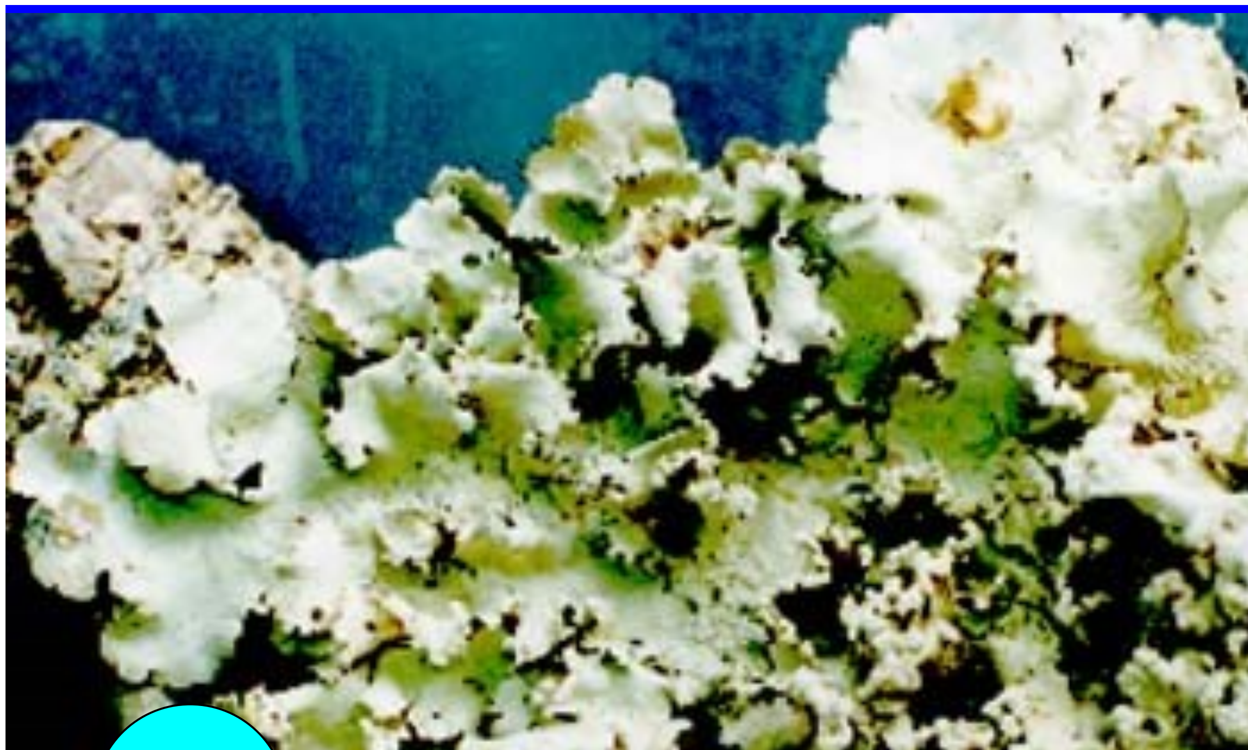

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Antihyperglycemic and antioxidant activity of various fraction of *Parmotrema hababianum* in streptozotocin-induced diabetic rat

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Abstract

The core objective of this study was to investigate the *in vitro* antioxidant and antihyperglycemic effect of the ethanol extract of *Parmotrema hababianum* in streptozocin-induced diabetic rats for 42 days. The extract showed nearly all antioxidant activities with maximum presence. The treatment with extract in diabetic rats at the dosage level of 100 and 200 mg/kg, which is compared with diabetic control and glibenclamide at a dosage level of 4 mg/kg and the biochemical parameters such as blood glucose, total cholesterol, triglycerides, HDL, LDL, insulin, total protein, urea and creatinine were assessed. The extract showed positive correlation ($p<0.001$) in reducing blood glucose level as compared to the control. Moreover, there was a significant ($p<0.01$) decrease in TC, TG, LDL, SGPT, SGOT, urea and creatinine level and significant ($p<0.01$) increase in HDL and insulin level. Thus, the results of this study show considerable efficacy in curing diabetes with potent antioxidant and antihyperglycemic activity.

Introduction

Diabetes mellitus is considered as a greater health concern all over the world. Expected result shows that in 2030, 6 out of 10 countries in Asia will having higher number of diabetic patients (Ku and Kegels, 2013). Free radicals are reactive oxygen species (ROS) that are integrated into the physiology of the human system (Shih et al., 2006). An excess production of these ROS leads to oxidative stress to the normal cellular functions. Antioxidant molecule protects the cellular components from the oxidation effect of free radicals (Jeya et al., 2015). Thus, they are important in protecting against diseases, such as arthritis, diabetes mellitus, carcinogenesis and cardiovascular diseases, developed in response to oxidative stress (Sangameswaran et al., 2009).

Searching of new drug molecule different from natural resources is gaining importance to mankind. The unexplored and unexploited plant species having a resource of therapeutic potential is a symbiont, lichen. Lichens are composite organisms consisting of fungi and other species either algae or cyanobacteria. Lichen metabolites spur diverse biological activities, such as antimicrobial, antitumor, antimutagenic, antiherbivore, and allergenic (Atalay et al., 2011). The biological activity of an extract can be determined by the presence of phenolic compounds, such as flavonoids, phenolic acids and total phenols (Brown and Rice, 1998). Lichens are also known for having higher phenolic content, which has various applications.

This study will enable researchers to understand various pharmaceutically active compounds in various lichen sample extracts (Muller, 2001). It discusses about the antidiabetic, antioxidant potential as well as phenolic content in various extracts obtained from lichen *Parmotrema hababianum*.

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Materials and Methods

Plant source and preparation of lichen extracts

Lichen sample was collected from the Shevaroy hills region of the Eastern Ghats of Tamil Nadu. The sample was identified by following standard procedures with respect to studying their morphology, anatomy, and chemistry. Thin-layer chromatography was conducted following the procedure established by Walker and James (1980), using solvent system A to identify secondary metabolites. Voucher specimens were deposited in the lichen herbarium of the K. S. Rangasamy College of Technology, Tiruchengode, for reference purposes. Approximately 8 g samples of each species were powdered and used for the extraction with different solvent systems with increasing polarity (petroleum ether, ethyl acetate, acetone, ethanol, and water) using Soxhlet extractor (Soxhlet, 1879). Using a vacuum desiccator, we subjected the extracts to evaporation in order to form a dry powder. Powdered extracts were stored at –80°C in a deep freezer for further study.

Antioxidant activity of lichen extracts

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical capturing assay

Hydrogen free radical quenching assay was performed with different concentrations of lichen extracts (20, 50, 100, 200, 400, 800 and 1,000 µg/mL) in corresponding solvents (w/v). Ascorbic acid was used as the standard (Khalaf et al., 2008). DPPH solution (2 mL, 0.002%) was mixed with 2 mL lichen extracts. The reaction mixtures were kept in the dark conditions for 30 min at 37°C. The reduction in color intensity was measured at 517 nm using a UV–visible spectrophotometer (U-2900, Hitachi, Japan).

Ferric reducing power assay (FRAP)

FRAP assay was performed on 20, 50, 100, 200, 400, 800 and 1,000 µg/mL samples of lichen extracts, with ascorbic acid as the standard. Phosphate buffer (2.5 mL; 200 mM, pH 6.6) and 1% potassium ferricyanide solution (2.5 mL) were added to each concentration. The amount of Fe^{2+} iron formation was studied by measuring the Perl's prussian blue at 700 nm. The increase in absorbance value indicates the steep reduction in power of lichen extracts (Oyaizu, 1986).

Hydrogen peroxide–reducing activity assay

Hydrogen peroxide activity was assayed by measuring 200, 400, 600, 800 and 1,000 µg/mL concentrations of lichen extracts. These extracts were added to 0.6 mL hydrogen peroxide (40 mM). The reaction mix contains hydrogen peroxide and various extracts of lichens were incubated for 10 min at room temperature and the absorbance was read at 230 nm against phosphate buffer (pH 7.4) as a blank solution. The percentage of inhibition was calculated and then compared with that of ascorbic acid (Ruch et al., 1989).

Total phenolic content of lichen extracts

The total phenolic content of the lichen extract was analyzed using Folin–Ciocalteu reagent, which was described by Slinkard and Slingleton (1997). Lichen extracts were diluted at a concentration of 1 mg/mL with respective solvents, from which 0.1 mL extracts was taken and added to 1 mL Folin-Ciocalteu reagent (1:1) and 3 mL Na_2CO_3 (2%). Color intensity was measured at 760 nm using gallic acid as a standard.

Animals used

Adult Wister albino rats weighting 120-150 g of both sex were used in the present study. The experimental animals were fed using standard food pellets with water *ad libitum* and placed in polypropylene cages for the period of study. The rats were maintained at the temperature range of 23 ± 2°C having the relative humidity of 55-60% with 12 hours of light and dark cycle.

Diabetes induction at rats

Diabetes in rats was done using intraperitoneal injection of streptozotocin at the dosage level of 50 mg/kg (prepared by dissolving in 0.1 M citrate buffer, pH 4.5) by fasting the rats to 16 hours. Induction of diabetes was identified by measuring the fasting blood glucose level (above 250 mg/dL level was considered as diabetes) after 5 days of induction period. Diabetic rats were orally administered with 5% glucose solution to avoid the hypoglycemia condition during the streptozotocin administration of diabetes.

Experimental design

The rats were divided into five groups of six rats each. The ethanolic extracts were administered orally at the doses of 100 and 200 mg/kg for a period of 42 days by dissolving it in CMC to different groups of diabetic induced rats. Group I (healthy control rats), group II (diabetic control rats), group III (diabetic treated with ethanolic extract of lichen sample -100 mg/kg), group IV (diabetic treated with ethanolic extract of lichen sample 200 mg/kg) and group V (diabetic treated with standard drug glibenclamide).

Blood biochemistry

Finally, the blood was collected from retinal orbital plexuses to all the overnight fasted rats under mild anesthesia. Serum was separated out and blood glucose level was analyzed by Erba diagnostics kit using biochemical analyzer. Lipids profile such as total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were analyzed. Blood insulin, urea, protein and

enzymes like serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were also studied in various experimental rats.

Results

The *in vitro* antioxidant effect of different solvent extracts of *P. hababianum* was shown in the Table I. All the values were calculated in the ascorbic acid equivalent antioxidant capacity ($AEAC_{DPPH}$) of per gram of lichen extracts. The petroleum ether extract of *P. hababianum* showed the maximum activity followed by the activity of ethanol and benzene extracts. Methanol, acetone and aqueous extract did not show any activity towards DPPH. But $AEAC_{FRAP}$ showed activity for all the extracts of *P. hababianum* which was in the progression from ethanol > methanol > petroleum ether > benzene > aqueous > acetone. $AEAC_{H2O2}$ activity of ethanol extract showed maximum activity in *P. hababianum* with the order followed by activity of petroleum ether, methanol and benzene extracts. But the exception was that acetone and aqueous extracts showed no activity towards $AEAC_{H2O2}$. The total phenolic content of *P. hababianum* was expressed in terms of gallic acid equivalent that showed the maximum phenolic presence in ethanol extract of about 224.7 mg of gallic acid equivalent.

Table I

Antioxidant activity of different extracts of *P. hababianum*

Extracts	µmol of ascorbic acid/g			mg gallic acid equivalent
	$AEAC_{FRAP}$	$AEAC_{DPPH}$	$AEAC_{H2O2}$	TPC
Methanol	1446 (4.5)	*NA	11.7 (0.3)	197.3 (2.7)
Ethanol	1655 (5.3)	98.3 (2.6)	25.7 (0.4)	224.7 (4.1)
Benzene	998 (4.1)	20.0 (1.1)	8.7 (0.1)	75.2 (0.7)
Acetone	620 (3.8)	NA	NA	22.5 (1.3)
Petroleum ether	1120 (2.7)	122.3 (3.4)	18.8 (0.2)	123.7 (2.7)
Aqueous	998 (2.9)	NA	NA	66.6 (0.9)

All values are expressed as mean ± SEM (n = 3); *NA indicates no activity

Blood glucose level of normal (control) and streptozotocin induced diabetic rats were shown in the Table II.

In our study, diabetic rats were treated with ethanolic extracts of two different concentrations because ethanolic extracts of *P. hababianum* showed almost all the maximum activity towards antioxidants such as DPPH, FRAP, H_2O_2 and total phenolic content. Blood glucose level of diabetic rat was significantly ($p<0.001$) higher than the treated group at 0, 7, 14, 21, 28, 35, 42 days interval. After the successive period of incubation of diabetic rats, there was a significant decrease of blood glucose level in the diabetic treated groups.

During the high concentration of administration of ethanolic extract of *P. hababianum* showed highly significant decrease ($p<0.001$) in blood glucose level as measured at 7th day (211.5 ± 0.8), 14th day (202.5 ±1.3), 21th day (190.8 ± 1.0), 28th day (181.3 ± 0.9), 35th day (174.9 ± 0.8) and 42th day (161.4 ± 1.4). To further know the role of ethanolic extract of *P. hababianum*, there was a significant increase ($p<0.01$) of total cholesterol, triglyceride and LDL level in diabetic induced rats

Table II

Effect of ethanolic extract of *P. hababianum*on on blood glucose level in streptozotocin-induced diabetic rat

Groups	Blood glucose level (mg/dL)						
	0 day	7th day	14th day	21st day	28th day	35th day	42th day
Control	90.4 (0.4)	89.6 (1.1)	88.2 (0.9)	89.4 (0.8)	91.8 (1.3)	90.4 (0.6)	88.7 (0.7)
Diabetic control	283.5 (0.5)	287.2 (0.5)	290.4 (0.8)	294.1 (1.1)	297.6 (0.9)	302.5 (1.2)	307.5 (1.1)
Diabetic + Ethanolic extract (100 mg/kg)	284.4 (0.9)	225.6 (1.4)	219.9 (1.7)	200.3 (1.0)	181.8 (1.2)	173.4 (0.5)	155.7 (1.3)
Diabetic + Ethanolic extract (200 mg/kg)	286.7 (0.7)	211.5 (0.8)	202.5 (1.3)	175.8 (1.03)	161.3 (0.9)	144.9 (0.8)	129.4 (1.4)
Diabetic + Glibenclamide (4 mg/kg)	305.3 (1.4)	256.9 (0.5)	204.4 (0.9)	171.4 (1.4)	145.8 (1.04)	119.5 (0.8)	91.7 (1.5)

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

Table III

Effect of ethanolic extract of *P. hababianum* on serum profile in streptozotocin-induced diabetic rats for 42 days

Groups	Insulin (µIU/L)	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	SGPT (IU/L)	SGOT (IU/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	15.5 (0.6)	76.8 (0.9)	101.4 (0.7)	65.2 (0.9)	47.3 (0.3)	103.7 (1.5)	116.5 (1.4)	47.7 (0.5)	1.3 (1.5)
Diabetic control	5.1 (0.3)	160.7 (1.3)	189.7 (1.4)	30.5 (0.5)	115.3 (1.2)	206.7 (1.7)	178.8 (1.8)	68.7 (1.3)	1.7 (1.0)
Diabetic + Ethanolic extract (100 mg/kg)	12.8 (0.4)	134.6 (0.9)	147.7 (1.2)	39.3 (1.2)	57.5 (0.5)	183.5 (1.0)	155.9 (1.0)	55.4 (0.5)	1.7 (0.5)
Diabetic + Ethanolic extract (200 mg/kg)	13.8 (0.7)	119.8 (1.1)	124.6 (1.2)	48.8 (1.0)	31.4 (1.2)	175.2 (1.5)	134.5 (1.3)	48.5 (1.5)	1.5 (0.9)
Diabetic + Glibenclamide (4 mg/kg)	14.4 (0.7)	122.8 (1.0)	150.3 (0.8)	59.9 (0.7)	49.3 (0.5)	162.4 (1.0)	122.7 (0.8)	49.9 (1.8)	1.4 (0.9)

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

while there was a significant reduction ($p<0.01$) in HDL level. The ethanolic extract of *P. hababianum* of different concentration showed significant decrease ($p<0.01$) in total cholesterol, triglyceride and LDL level and increased HDL level. Similarly there was a gradual increase ($p<0.01$) in insulin level compared to the diabetic control group. Moreover, the reduction ($p<0.01$) in SGPT, SGOT, urea and creatinine level were noted in diabetic treated rats which was shown in Table III.

Discussion

In current study, we have investigated the antioxidant and antidiabetic potential of *P. hababianum.* The scavenging of free radicals produced in our body is performed by the action of antioxidant molecules (Oboh et al., 2007). The ethanolic extract revealed the best activity of DPPH free radicals. The phenolic compounds existent in the plant extract can be responsible for the free radical scavenging activity, since it readily contributes hydrogen atoms to the free radical (Tung et al., 2009). The reducing capacity of plant extract was based on the capability to reduce Fe^{3+} to Fe^{2+}. The different concentration of plant extracts posses various capacity of reduction ability as well as electron donating antioxidants (Halvorsen et al., 2002).

Ethanolic extracts of *P. hababianum* was investigated for the decreased blood glucose level in the streptozotocin induced diabetic rats as compared to the diabetic control and glibenclamide treated diabetic rats. The induction of diabetes using streptozotocin leads to DNA strand breakage in β-cells of pancreas (Ponnusamy and Thangaraj, 2015). The occurrence of anti-diabetic activity is due to phenols, flavonoids, tannins and several other phytochemical compounds.

During the metabolic process, insulin activates lipoprotein lipase that hydrolyse the triglycerides (TG), deficiency or inactivation of these enzyme leads to hypertriglyceridemia (Maruthapandian and Mohan, 2011). Moreover, increase in fat diet leads to over helming of TG (Xu et al., 2005) and LDL level (Kesavalu et al., 2001) which leads to the artery hardening. Our investigation showed that was an increased HDL level that will ultimately reduce the complication of diabetes.

The reduction in the increased level of SGPT and SGOT in diabetic groups revealed that our extract may reduce the risk from liver failure linked with diabetes. Additionally, reduction in the urea and creatinine level may regress the metabolic homeostasis by crucially elicit the kidney function (Liaqat et al., 2014). The anti-diabetic activity of ethanolic extract of *P. hababianum* possibly may due to potent antioxidant activity and thus it can be effective in preventing the oxidative and cellular damage of β-cells of pancreas.

Conclusion

Extracts of *P. hababianum* showed potential antioxidant activity against the free radical damage. The significant activity of these antioxidant leads to potent anti-hyperglycemic activity of ethanolic extract of *P. hababianum* by reducing the blood glucose level thus protecting our body from liver and kidney damage.

Ethical Issue

The experimental study was approved by the committee for the purpose of control and supervision on experimental animals (CPCSEA) at animal house facility, K. S. Rangasamy College of Technology (1826/PO/EReBi/S/15/CPCSEA). The experiments were approved and done as per institutional animal ethics committee.

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Conflict of Interest

Authors declare no conflicts of interest.

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