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Evaluation of phytochemical composition and antioxidative, hypoglycaemic and hypolipidaemic properties of methanolic extract of *Hemidesmus indicus* roots in streptozotocin-induced diabetic mice

Ankita Joshi, Harsha Lad, Harsha Sharma and Deepak Bhatnagar*

Abstract

Background: *Hemidesmus indicus* is an important medicinal plant and extensively used in Ayurvedic and Unani system of medicine. The aim of the study was to evaluate the antioxidant, hypoglycaemic and hypolipidemic potential of methanolic extract of roots of *Hemidesmus indicus* (HIE) in streptozotocin (STZ) induced diabetic mice.

Methods: HIE was analyzed by LC-MS to determine its phytochemical composition. The in-vitro antioxidant activity of HIE was analyzed through inhibition of free radical scavenging activity (FRSA), total antioxidant power (TAP) and reducing power. Diabetes in mice was induced by a single dose of STZ followed by HIE treatment. The antioxidative, hypoglycaemic and hypolipidemic activity were studied ex-vivo in tissues of diabetic mice.

Results: Phytochemical composition of *hemidesmus indicus* roots (HIE) revealed the presence of phenols, flavanoids, terpenoids and about 40 different phytoconstituents by LC-MS analysis. Inhibition of lipid peroxidation (LPO) and modulation in superoxide dismutase (SOD), catalase (CAT), glutathione-S- transferase (GST) activity and glutathione (GSH) content showed potent antioxidant activity of HIE in STZ induced diabetic mice, which was also substantiated by in-vitro antioxidant assays. The decrease in fasting blood glucose and serum lipid profile was also observed in mice administered HIE.

Conclusion: It is proposed that HIE modulates the oxidant/antioxidant in favor of reducing oxidative stress, hypoglycemia and improved the lipid profile in treated groups.

Keywords: Streptozotocin, Hypoglycaemic, Hypolipidaemic, Lipid peroxidation, Antioxidants, *Hemidesmus indicus*

Background

Diabetes mellitus is a complex chronic metabolic disorder characterized by alterations in carbohydrate, protein and fat metabolism. The metabolic changes are caused by the insufficiency of secretion or action of endogenous insulin [1]. Oxidative stress is suggested to be one of the mechanism underlying diabetes and diabetic complications. It results from an oxidant/antioxidant imbalance in favour of oxidants leading to damage of various intracellular components such as proteins, lipids and nucleic acid as well as extracellular matrix components such as proteoglycans and collagens. Free radicals are formed by glucose oxidation, non enzymatic glycation of proteins, oxidative degradation of glycated proteins and increased lipid oxidation (LPO), which may promote oxidative stress and lead to the development of insulin resistance [2]. The harmful effects of oxidative stress are counteracted by the cellular defense mechanism, which consists of enzymes, nonenzymatic and metabolic antioxidants. To treat diabetes various oral antihyperglycaemic agents have been developed over the past years, which include sulphonylureas, biguanides, α-glucosidase inhibitors and thiazolidinediones [3]. Hypoglycaemia, lactic acidosis and gastrointestinal

* Correspondence: bhatnagarbio@gmail.com
School of Biochemistry, Devi Ahilya University, Khandwa Road, Indore, MP 452017, India

intolerance are some of the adverse effect of these drugs. These side-effects of synthetic drugs along with drug-resistance have led to the resurgence of phytomedicine and search for novel type of antioxidant and antidiabetic from medicinal plants.

Hemidesmus indicus belongs to the family Periplocaceae and is distributed throughout India. Roots and stems of *H. indicus* act as laxative, diaphoretic, diuretic and are useful in treatment of syphilis, cough, asthma and leucoderma. *H. indicus* roots (Fig. 1) contains steroids, terpenoids, flavonoids, saponins, phenolic compounds, tannins and lignins, inulins, cardiac glycosides, proteins and carbohydrates [4]. Pregnenolone glycosides such as hemidesmosides A–C and 2-hydroxy-4-methoxybenzoic acid, 2-hydroxy-4- methoxybenzaldehyde, and 3-hydroxy-4-methoxybenzaldehyde were reported in *H. indicus* roots [5, 6]. These phytoconstituents are accountable for its different biological actions. The phenolic and flavanoid content found in aqueous extract of *H. indicus* corresponds to its reported antioxidant activity [7, 8] The present study was undertaken to investigate the effects of methanolic extract of *H. indicus* roots on lipid peroxidation, antioxidant enzyme activity, lipid profile and fasting blood glucose of STZ induced diabetic mice.

Methods

Chemicals and reagents

Streptozotocin, 2,2-diphenyl-1-p-picryl- hydrazyl (DPPH), ursolic acid and quercitrin hydrate were procured from Sigma Chemical Co. St. Louis, Missouri, USA. HPLC grade acetonitrile were purchased from Merck Chemicals, Mumbai, India. Pyrogallol, ethylenediamine tetra acetic acid (EDTA) and other chemicals and solvents of AR grade were purchased from Hi Media Co. Mumbai, India.

Fig. 1 Roots of *Hemidesmus indicus*

Plant material and preparation of the extract

H. indicus roots were procured from the local market. The plant roots were authenticated by Prof. A.B. Seerwani, Department of Botany, Holkar Science College, Indore. The dried powder of roots of *H. indicus* (HIE) (50 g) were extracted in methanol (300 ml) by the Soxhlet apparatus at 40 °C for 8 h. The extract was concentrated by evaporation at 35 °C in water bath. The yield of dried extract was 13.48% (*w*/w). The reconstitution of HIE was done in methanol to prepare stock solution of 100 mg/ml for photochemical and in vitro analysis and in 1% DMSO for in vivo dosage preparation. These extracts were stored at 4 °C for analysis.

Animals

Healthy colony bred mice of Swiss albino strain of both sex weighing 20 ± 5 g were kept in polypropylene cages at an ambient temperature. Animals had free access to feed (M.P. Livestock and Poultry Development Corporation, Indore) and water. The experiments were performed according to the guidelines of the Institutional Animal Ethics Committee (IAEC) (CPCSEA/2015/01 dt. 4/7/2015). The standard necropsies procedures were carried out at the termination of study to collect the required tissues and blood sample for various analyses from mice of both sexes, as the study does not involve any gender specific parameters.

Total phenol content

Total phenol content of HIE was determined using the Folin–Ciocalteu method by Singleton and Rossi [9]. Propyl gallate solution (1 mg/ml, 0–30 μg) was used as standard.

Total flavonoid content

Total flavonoids were estimated according to the aluminum chloride method of Zhang et al., (2011) [10]. Quercitrin hydrate (1 mg/ml in ethanol, 0–250 μg) was used as a standard.

Total triterpenoid content

Total triterpenoid content was estimated by the method of Chang and Lin [11]. Ursolic acid (1 mg/ml in methanol, 0–50 μg) was used as standard.

Liquid chromatography-mass spectroscopy (LC-MS)

LC-MS analysis of the methanolic extract of *Hemidesmus indicus* roots was carried out using Agilant (6550 iFunnel Q-TOFs) system consisting of Hip sampler, binary pump, column component, Q-TOF having dual ion source and electrospray ion generation (ESI) with Agilent Jet Stream (AJS). Chromatographic separations were performed using 5 μl of methanolic sample injected with needle wash onto an Agilent 1290 infinity UHPLC system fitted with a Zorbax Eclipse C18 column (2.1 × 150 mm, 5 μ) and flow

rate was 200 μl/min. The column was held at 95% Solvent A (water) and 5% Solvent B (acetonitrile) for 2 min, followed by an 20 min step gradient from 5% B to 95% B, then 5 min with 5% A, 95% B. Then the elution was achieved with a linear gradient from 5% A to 95% A for 4 min. The following parameters were used throughout the MS experiment: for electro spray ionization with positive ion polarity, the capillary voltage was set to 3500 V, the capillary temperature to 250 °C, the nebulizer pressure to 35 psi and the drying gas flow rate to 13 L/ min. Data acquisition and mass spectrometric evaluation were carried out using software Agilent Mass Hunter Qualitative analysis B.06.

Free radical scavenging activity using 1, 1, 2, 2-diphenyl-p-picryl hydrazyl

The method is based on the reduction of an ethanolic solution of DPPH by hydrogen donating groups of antioxidant substance [12]. The decrease in DPPH absorption at 517 nm was measured.

Total antioxidant power using ferric reducing antioxidant power

The total antioxidant capacity (TAC) of HIE was determined using the ferric reducing antioxidant power (FRAP) [13].

Reducing power

The reducing power of the test samples was determined according to the method of Oyaizu [14]. The reductive ability was measured by the reduction of $FeCl_3$ in presence of plant extracts. Ascorbic acid dissolved in distil water having concentration ranging from 0 to 17.6 μg was used as positive control.

Induction of diabetes

A single dose of freshly prepared STZ (180 mg/kg body wt.) in cold 0.01 M citrate buffer (pH 4.5) was administered intraperitonially to overnight fasted mice to induce diabetes [15]. Streptozotocin causes β-cell toxicity via mechanism involving both free radical mediated damage and alkylation of DNA [16]. Mice were orally administered 10% (*w*/*v*) glucose for 24 h after STZ injection to overcome hypoglycaemic shock and after 72 h, fasting blood glucose (FBG) was measured. Mice having FBG levels above 250 mg/dl were considered diabetic and were selected for further experiments. The FBG of the animals were estimated by glucometer (Akkiscan, Nempro Care, India) at every 3rd day, after the induction of diabetes up to the end of treatment (day 12th). The variation in the body weight during the study period was recorded (provided in Additional file 1) and the food and water intake was monitored in the animals.

Experimental design

The mice were divided into four groups of 6 animals each. The groups of animals were control mice without any treatment (Group 1), STZ induced diabetic mice (Group 2), mice treated with glibenclamide (10 mg/kg/day, orally) from 1stday of diabetes induction to 12th day (Group 3) and mice treated with HIE (35 mg/kg/day, orally) from 1st to 12th day after the induction of diabetes (Group 4). The animals were observed for the development of diabetes up to day 5 of STZ administration. Treatments in group 3 and 4 were administered to diabetic mice for 12 days after development of diabetes.

Collection and processing of biological samples

The animals were sacrificed under mild ether anesthesia. Liver and kidney homogenate (10%) was prepared using Potter-Elvehjem Homogenizer (Remi, Mumbai, India) in ice cold phosphate buffer saline (PBS) (1: 9, *v*/v) followed by centrifugation at 16000 xg for 30 min at 4 °C. Blood was collected by cardiac puncture in citrated tubes. Erythrocytes lysate was prepared as described earlier [17]. The supernatant obtained after centrifugation of tissue homogenate and erythrocytes lysate were immediately used to determine antioxidant enzymes and protein content.

Measurement of serum biochemical parameters

Blood was collected from diabetic and treated groups and the serum samples were analyzed using commercially available kits (Beacon Diagnostics, Navsari, India) for cholesterol, HDL, LDL, VLDL triglycerides by ELISA plate reader (LISA Plus, Rapid Diagnostics, China) and spectrophotometer (Shimadzu, UV-1800, Japan). The quantitative estimations were performed according to the manufacturer's protocol.

Determination of malondialdehyde levels

Malondialdehyde (MDA) content in liver, kidney and erythrocytes was measured by HPLC method [18].

Determination of antioxidant status

The activity of superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) was measured in tissue homogenates and erythrocytes lysate. The reduced GSH content was measured in tissue homogenates and blood [19–22].

Statistical analysis

The results obtained were analyzed by the SPSS software package version 20. The mean values obtained for the different groups were compared by one-way ANOVA, followed by post hoc -Tukeys (HSD) test.

Table 1 Estimation of phytoconstituents and total antioxidant power of HIE

Constituents	Content
Total phenolic[a]	12.95 ± 0.77
Total triterpenoid[b]	79.42 ± 2.35
Total flavanoid[c]	57.68 ± 1.65
Total antioxidant power[d]	77.36 ± 1.59

Values are Mean ± SE of four experiments
[a]mg GAE/g of dry wt.
[b]mg UAE /g of dry wt.
[c]mg QHE/g of dry wt.
[d]μM/g of dry wt.

Results

Determination of phytoconstituents

Phytochemical analysis of HIE was evaluated and the results are represented in Table 1.

Qualitative mass spectral analysis

HIE showed 73 unique mass signals, out of which putative empirical formulas of 62 compounds were obtained and identified by comparison with phytochemical database {developed by Sophisticated Analytical Instrument Facility - Indian Institute of Technology, Bombay (SAIF-IITB) and Pubchem} and details of the compounds are provided in Additional file 1. The MS spectrum of some of the compounds is represented in Fig. 2.

Total antioxidant power using ferric reducing antioxidant power

The results of total antioxidant power (TAP) using FRAP are presented in Table 1.

Free radical scavenging activity

HIE showed FRSA by donating the hydrogen atom or electron to stable free radical DPPH. A linear relationship was observed in the DPPH radical scavenging activity and HIE concentrations (Fig. 3a). The FRSA of butylated hydroxyl toluene (BHT, 0.18 mg/ml) was found to be 55.51 ± 0.69%. The IC_{50} value for BHT and HIE were 0.06 ± 0.02 mg/ml and 0.10 ± 0.03 mg/ml respectively.

Reducing power

The reduction of $FeCl_3$ in the presence of methanolic extract of HIE was monitored at 700 nm (Fig. 3 b). The higher absorbance of the reaction mixture indicated

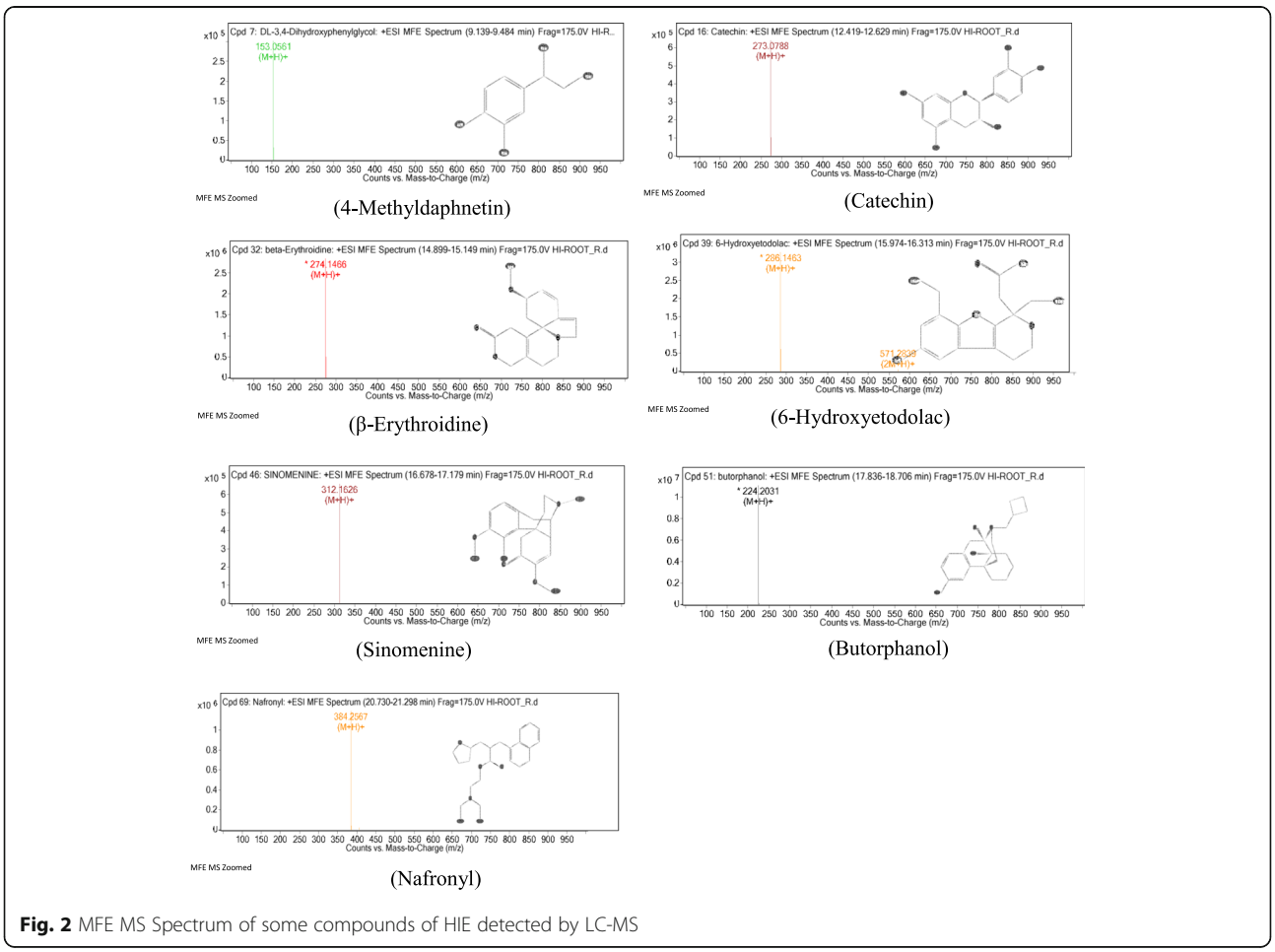

Fig. 2 MFE MS Spectrum of some compounds of HIE detected by LC-MS

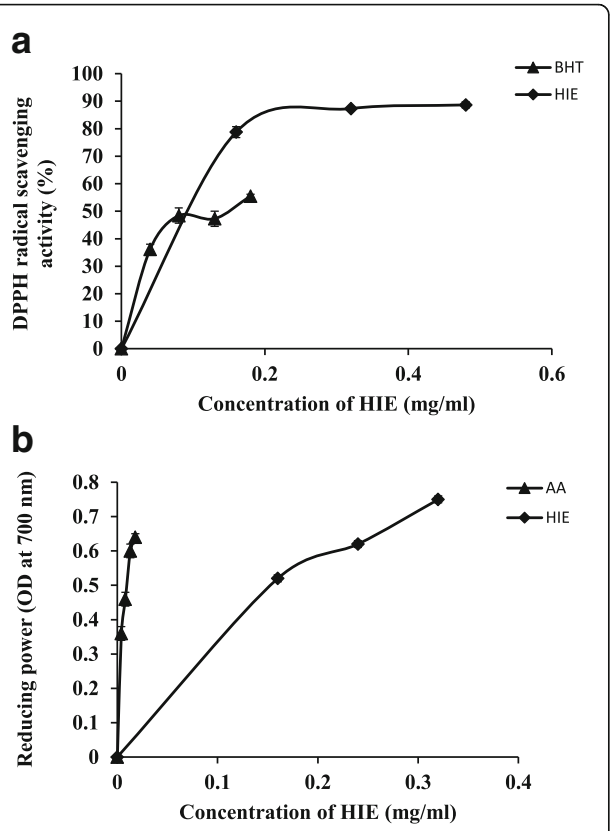

Fig. 3 Effect of different concentration of HIE on (**a**) DPPH radical scavenging activity; (**b**) Reducing power. Values are Mean ± SE of four experiments

greater reducing power. Ascorbic acid showed the absorbance of 0.64 ± 0.01 at the concentration of 176 μg/ml.

The total phenolic content of HIE showed a strong and positive correlation with reducing power (R2 = 0.997, $p < 0.05$) and DPPH radical scavenging activity (R2 = 0.851) (Table 2). The total flavanoid content exhibited correlation of 0.997 ($p < 0.05$) with reducing power and 0.921 with DPPH radical scavenging activity. It is suggested that the radical scavenging activity and antioxidant activity of HIE is due to the presence of the phenolic and flavanoid compounds.

Table 2 Correlation coefficient between phytoconstituents and antioxidant properties of HIE

Antioxidant Properties	FRSA	RP
Phyto-Constituents		
TPC	0.851	0.997*
TFC	0.921	0.997*
TTC	0.921	0.997*

FRSA Free radical scavenging activity
RP Reducing power
TPC Total phenolic content
TFC Total flavanoid content
TTC Total triterpenoid content
*Correlation coefficient is significant at $P < 0.05$

Fasting blood glucose and lipid profile

The effect of HIE on fasting blood glucose is represented in Table 3. Glibenclamide and HIE (Group 3 and 4) produced significant decrease in blood glucose level when compared to the diabetic control (Group 2) after 6 days of treatment. Food intake and body weight showed mild decrease after development of diabetes and non-significant alterations in the body weight was observed in all groups throughout the study period (Additional file 2).

The results showed that there was significant decrease in serum cholesterol, triglycerides, LDL and VLDL in the glibenclamide and HIE treated mice (Table 4; Group 3 and 4) when compared to diabetic control (Group 2).

Determination of MDA levels

MDA content was found significantly increased in the liver, kidney and erythrocytes of the diabetic group (Table 5; Group 2) as compared to control (Group 1). A significant inhibition in the liver and kidney LPO was observed with glibenclamide (Group 3) and HIE (Group 4) as compared to diabetic control (Group 2). The liver and kidney MDA content of HIE treated group of mice (Group 4) was comparable to that of control group (Group 1). Erythrocytes MDA content remained unaffected in glibenclamide and decreased in HIE treated mice as compare to diabetic control.

Determination of antioxidant status

The decrease in SOD activity was significant in the liver, whereas it was increased in kidney and erythrocytes in diabetic mice, as compared to control (Table 6; Group 1). Glibenclamide and HIE (Group 3 and 4) significantly elevated the SOD activity in the liver, while in kidney it was comparable to control and in erythrocytes SOD activity was remarkably reduced as compared to diabetic control.

A significant increase in liver, kidney and erythrocyte catalase activity was observed in diabetic animals (Table 6; Group 2) when compared against the control (Group 1). The treatment with glibenclamide (Group 3) significantly decreased the catalase activity in liver, while in erythrocytes and kidney the change was non-significant as compared to diabetic control (Group 2). However, with HIE (Group 4) treatment, liver, kidney and erythrocytes catalase activity is normalized and significantly decreased as compared to diabetic control (Group 2).

GSH content increased in liver and decreased in kidney and erythrocytes of diabetic animal (Group 2) as compared to control (Table 7; Group 1). Glibenclamide treated animal showed low GSH content in liver, while with HIE treatment, the liver GSH content was near to normal. In kidney, both the treatment resulted in recovery of GSH content to normal, whereas for erythrocytes there was no reduction in GSH content in glibenclamide treatment as

Table 3 Effects of HIE treatment on fasting blood glucose in streptozotocin induced diabetic mice

Group	Day 1	Day 3	Day 6	Day 9	Day 12
1	91.75 ± 2.78	89.25 ± 2.72	96.25 ± 1.49	82.00 ± 2.27	92.00 ± 2.55
2	368.25 ± 12.29**	294.25 ± 9.29**	320.50 ± 16.06**	312.25 ± 17.4**	321.50 ± 22.77**
3	470.00 ± 22.39**#	276.75 ± 24**	167.75 ± 5.71*##	141.50 ± 6.96*##	112.25 ± 5.76##
4	541.75 ± 11.96**##	374.57 ± 23.39**	243.75 ± 13.29**#	178.25 ± 8.12**##	121.25 ± 5.22##

Values are mean ± SE of 6 animals
*$P < 0.01$, **$P < 0.001$ = significant as compared to control (Group 1)
#$P < 0.01$, ##$P < 0.001$ = significant as compared to diabetic control (Group 2)
Values of FBG are in mg %

compared to diabetic mice. Increase in GSH content of erythrocytes was observed in HIE as compared to diabetic group (Group 2).

GST activity in tissues and erythrocytes was significantly increased in diabetic animals (Table 7; Group 2) when compared against a control (Group 1), GST activity was not improved by the treatment with glibenclamide (Group 3) as compared to diabetic mice. HIE (Group 4) significantly reduced the liver and erythrocytes GST activity when compared to diabetic control (Group 2) whereas it is restored to normal in kidney.

Discussion

ROS produced in various tissues leads to tissue injury as well as early events related to the development of diabetes mellitus and its complications [23]. HIE may have beneficial effects on type 1 and 2 diabetes, as the mechanism of development of both the diabetes involve oxidative stress. However, the animal model used represents only type 1 diabetes. Traditional plant remedies have been used in the treatment of diabetes but only a few have been scientifically evaluated [24]. Methanolic extract of *H. indicus* roots contained high quantity of flavanoid and triterpenoid than phenolic content and contributes to the observed antioxidant potential of the extract. The LC-MS analysis coupled with putative identification of compounds indicated the presence of phenolic and flavanoids such as DL-3,4-dihydroxyphenyl glycol, catechin, amiloxate (cinnamic acid derivative); 4-methyldaphnetin (coumarin), terpenoid such as punctaporin B and podocarpatriene derivative. Various alkaloids such as ecgonine, homatropine, β-erythroidine, butorphanol, securinine along with other phytoconstituents such as phytosterols, lactones, prostaglandins, amino acids, lipids and fatty acids were also identified. Cholesterol lowering triparanol and nafronyl, which enhances cellular oxidative capacity and nudifloramide an end product of NAD degradation shown to potentially inhibit PARP-1 were found in HIE. Natural compounds having anti-inflammatory activity such as safroglycol, etodolac derivative, anisodamine, β santonin, nabumetone and securinine were identified by their molecular peak (base peak) [25–28].

The results of free radical scavenging activity showed that HIE reduced DPPH free radical to non-radical DPPH-H by compounds having hydroxyl groups (catechin) or compounds, which oxidise readily (sinomenine). The phenolics, flavanoids, diterpene and sesquiterpene compounds present in HIE can donate hydrogen to terminate the odd electrons of the DPPH radical. The scavenging ability of HIE can be attributed to these bioactive compounds. The higher FRAP value represents high total antioxidant power of HIE. The phenolic compounds reduce Fe^{3+} to Fe^{2+} and interrupt free radical chain reaction, either by hydrogen atom or electron transfer process and form phenoxyl radical [29]. The reducing power of HIE was positively correlated with the total phenolic, flavanoid and triterpenoid content also evident from mass spectral analysis. *H. indicus* is an edible plant and contains significant amount of total phenolics and reported to have antioxidant activity [30]. An earlier report by Rajan et al., [31] also showed the antioxidant activity of roots of *H. indicus* having

Table 4 Effects of HIE treatment on serum lipid profile in streptozotocin induced diabetic mice

Group	Cholesterol[a]	Triglycerides[a]	HDL[a]	LDL[a]	VLDL[a]
1	125.03 ± 6.28	35.90 ± 7.68	101.82 ± 2.50	83.88 ± 3.76	7.18 ± 1.53
2	274.32 ± 15.82**	63.90 ± 2.05*	73.11 ± 3.17*	160.84 ± 5.26**	12.78 ± 0.41*
3	122.74 ± 7.09##	32.25 ± 6.24##	113.31 ± 5.59##	117.36 ± 7.59*##	6.45 ± 1.25##
4	122.77 ± 9.89##	32.99 ± 0.18##	122.74 ± 2.10##	117.84 ± 5.87*##	6.6 ± 0.04##

Values are mean ± SE of 6 animals
*$P < 0.01$, **$P < 0.001$ = significant as compared to control (Group 1)
#$P < 0.01$, ##$P < 0.001$ = significant as compared to diabetic control (Group 2)
[a]mg %

Table 5 Effect of HIE on liver, kidney and erythrocytes LPO in STZ induced diabetic mice

LPO[a]			
Group	Liver	Kidney	Erythrocytes
1	31.72 ± 1.43	70.50 ± 6.16	ND
2	308.04 ± 18.10*	340.53 ± 10.71*	37.08 ± 2.51*
3	133.75 ± 8.78$^{*\#}$	64.52 ± 6.17$^{\#}$	40.66 ± 1.23*
4	33.77 ± 1.99$^{\#}$	58.82 ± 9.17$^{\#}$	21.89 ± 0.86$^{*\#}$

Values are mean ± SE of 6 animals
$^{*}P < 0.001$ = significant as compared to control (Group 1)
$^{\#}P < 0.001$ = significant as compared to diabetic control (Group 2).
ND = Not Detected
[a]picomoles of MDA formed/mg protein for liver and kidney and nanomoles of MDA formed/gm Hb for erythrocytes

quantifiable amounts of phenolic compounds, tannins and flavonoids, as observed in the present study.

Free radicals are formed in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins [32]. Streptozotocin selectively acts on pancreatic β-cells and cause enhanced ROS in pancreas, liver and other tissues. Increased ROS results in tissue damage and enhanced LPO i.e., oxidation of membrane lipid. Increased MDA in liver, kidney and erythrocytes of diabetic animals than those of control was due to ROS mediated propagation of chain reaction. The increase in MDA content may be due to diminished activity of an antioxidant defense system to sufficiently scavenge free radicals generated in STZ induced diabetes. It has been reported that the increase in MDA associated with diabetes may be reversed by treatment with combined vitamins C, E, and β-carotene [33]. HIE treatment showed a marked inhibition in LPO and thereby reduction in MDA content probably due to inhibition of propagation of LPO. Cellular defense mechanisms, which act against free radicals, include GSH and antioxidant enzymes such as SOD, CAT and GST respectively whose activities contribute to eliminate superoxide, hydrogen peroxide and hydroxyl radicals [34]. The activity of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes. The superoxide radicals are dismutated by SOD to form hydrogen peroxide followed by its decomposition into water and oxygen by catalase. Liver SOD activity was decreased in diabetic animals as compared to control. This may be due to excessive production of superoxide radical that inhibit the activity of SOD, which consequently improved after HIE treatment. The increased activity of kidney and erythrocytes SOD in diabetic mice may be a response to combat minor generation of ROS upon STZ administration, which was normalized in HIE treated diabetic mice. The increased activity of the liver, kidney and erythrocytes catalase was observed in STZ induced diabetes that was significantly restored by HIE. The elevation of CAT activity may be endogenous compensatory mechanism for prolonged over production of free radicals and oxidative stress [35].

Glutathione (GSH) efficient antioxidant present in almost all living cells and is considered as one of the biomarker of redox imbalance at cellular level. The present study showed decreased kidney and erythrocytes GSH content in diabetic control, which may be considered to be an indicator of increased free radical scavenger in the repair of radical caused biological damage. HIE treatment restores the GSH content, whereas glibenclamide treatment does not affect GSH content of STZ induced diabetic mice. Both liver GSH content and GST activity were increased in diabetic control suggesting that cellular antioxidant defense mechanism was triggered by STZ induced generation of ROS. There was a significant decrease in both liver GSH content and GST activity upon HIE treatment probably due to the up regulation of GSH redox system in liver to counteract oxidative stress, as GSH also act as cofactor for GST in this system.

The reduction in FBG upon HIE treatment in STZ induced diabetic mice, indicates that HIE has high antidiabetic potential probably due to the insulin secretagogue and antioxidative action of the *H indicus* root extract. Hyperglycaemia stimulates ROS formation and resulting oxidative stress triggers hyperglycaemia induced diabetic complications [36]. The hypoglycaemic and antioxidative effect of HIE may prevent development and progression of diabetic complications.

Table 6 Effect of HIE on SOD and CAT in STZ induced diabetic mice

SOD[a]				CAT[b]		
Group	Liver	Kidney	Erythrocytes	Liver	Kidney	Erythrocytes
1	19.19 ± 0.50	6.34 ± 0.10	120.83 ± 4.64	199.96 ± 7.94	187.28 ± 5.13	693.76 ± 89.77
2	2.97 ± 0.05***	13.92 ± 0.21***	263.95 ± 9.96$^{***\#}$	312.66 ± 1.44***	243.86 ± 10.68*	1652.21 ± 85.34***
3	8.25 ± 0.37$^{***\#}$	5.32 ± 0.12$^{**\#}$	128.97 ± 5.98$^{\#}$	219.78 ± 6.54$^{\#}$	204.07 ± 11.69^{NS}	1444.72 ± 27.18***
4	7.95 ± 0.69$^{***\#}$	6.96 ± 0.17$^{\#}$	27.34 ± 2.28$^{***\#}$	209.79 ± 5.44$^{\#}$	168.99 ± 5.63$^{\#}$	523.57 ± 76.07$^{\#}$

Values are mean ± SE of 6 animals
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ = significant as compared to control (Group 1)
$^{\#}P < 0.001$ = significant as compared to diabetic control (Group 2). ^{NS}Not significant
[a]units/mg protein
[b]μmoles H_2O_2 decomposed/min/mg protein

Table 7 Effect of HIE on GSH and GST and in STZ induced diabetic mice

GSH[a]				GST[b]		
Group	Liver	Kidney	Erythrocytes	Liver	Kidney	Erythrocytes
1	19.27 ± 0.62	25.33 ± 0.45	25.05 ± 0.91	0.33 ± 0.06	0.36 ± 0.07	10.22 ± 0.40
2	31.60 ± 1.98*	15.78 ± 0.39*	7.31 ± 1.11*	4.24 ± 0.42*	1.37 ± 0.13*	18.10 ± 1.45*
3	13.93 ± 1.78$^{\#\#}$	25.66 ± 1.61$^{\#\#}$	7.94 ± 0.21*	1.76 ± 0.23$^{*\#\#}$	1.11 ± 0.08 *	19.94 ± 1.61*
4	22.04 ± 0.67$^{\#}$	38.26 ± 0.61$^{*\#\#}$	13.82 ± 0.56$^{*\#\#}$	0.16 ± 0.02$^{\#\#}$	0.30 ± 0.05$^{\#\#}$	6.97 ± 0.30$^{\#\#}$

Values are mean ± SE of 6 animals
$^{*}P < 0.001$ = significant as compared to control (Group 1)
$^{\#}P < 0.01, ^{\#\#}P < 0.001$ = significant as compared to diabetic control (Group 2)
[a]nanomoles of DTNB conjugated/mg protein for liver and kidney and μmoles of DTNB conjugated/gm Hb for erythrocytes
[b]μmoles of GSH conjugated/min/mg protein

Certain oxidative stress related defects in oxidative phosphorylation machinery and mitochondrial β-oxidation lead to excess accumulation of intracellular triglyceride in muscle and liver and subsequent insulin resistance [37]. As compared to diabetic control, glibenclamide and HIE treated mice, the total cholesterol, triglyceride, LDL and VLDL were significantly lowered with increased HDL indicating recovery of normal lipid metabolism in STZ induced diabetes. The results showed that HIE was found to be effective against diabetic dyslipidaemia.

Conclusion

The methanolic extract of *H. indicus* roots contains various phytoconstituents having potent antioxidant, hypoglycaemic and hypolipidaemic activity. Oral administration of HIE, lowers the FBG of STZ induced diabetic mice as well as it modulates the intracellular antioxidant defense to overcome the oxidative damage and improves the serum lipid profile. It is suggested that the roots of *H. indicus* may serve as an important hypoglycaemic and hypolipidaemic agent to protect the cells by mitigating oxidative stress induced toxicity in STZ induced diabetes.

Additional files

Additional file 1: Putative identification of compounds in HIE. (DOCX 17 kb)

Additional file 2: Effects of HIE treatment on body weight of streptozotocin induced diabetic mice. (DOCX 14 kb)

Abbreviations

AJS: Agilent Jet Stream; CAT: Catalase; DPPH: 2,2-diphenyl-1-p-picryl-hydrazyl; ESI: Electrospray ion generation; FBG: Fasting Blood Glucose; FRAP: Ferric Reducing Antioxidant Power; FRSA: Free Radical Scavenging Activity; GSH: Glutathione; GST: Glutathione-S- Transferase; HDL: High Density Lipoprotein; HIE: Methanolic extract of *Hemidesmus indicus* roots; LC-MS: Liquid Chromatography-Mass Spectroscopy; LDL: Low Density Lipoprotein; LPO: Lipid Peroxidation; MDA: Malondialdehyde; NAD: Nicotinamide-adenine dinucleotide; PARP-1: Poly ADP ribose polymerase-1; PBS: Phosphate Buffer Saline; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase; STZ: Streptozotocin; TAP: Total Antioxidant Power; VLDL: Very Low Density Lipoprotein

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Availability of data and materials

Not applicable

Authors' contributions

AJ: designed and performed the experiments, participated in data analysis and manuscript preparation. HL, HS and DB: participated in design of experiments and helped in manuscript preparation. All authors read and approved the final manuscript.

Ethics approval

The experimental mice were treated following the ethical guidelines of Institutional Animal Ethics Committee (IAEC) constituted under supervision of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). The experimental procedures were approved by the IAEC (CPCSEA/2015/01 dt. 4/7/2015) of Devi Ahilya University, India.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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