

Original Article

Antihyperglycemic and antihyperlipidemic activities of wild musk melon (*Cucumis melo* var. *agrestis*) in streptozotocin-nicotinamide induced diabetic rats

K. Gopalsatheeskumar^{a,*}, G. Ariharasivakumar^b, V. K. Kalaichelvan^a, T. Sengottuvel^b, V. Sanish Devan^b, V. Srividhya^b

^a Department of Pharmacy, Annamalai University, Chidambaram 608002, India

^b Department of Pharmacology, KMCH College of Pharmacy, Tamil Nadu Dr. M. G. R. Medical University, Coimbatore 641048, India

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ABSTRACT

Objective: Wild musk melon (*Cucumis melo* var. *agrestis*, CMA) is one of the edible plants from Tamil Nadu. Traditionally, this plant was used as diabetic diet (leaves of CMA with *Momordica charantia* leaves), but there is no scientific report on antidiabetic action of this plant material. Hence, the current research work was designed to evaluate the antihyperglycemic and antihyperlipidemic effect of hydroalcoholic extract of CMA leaves (HALEC) in streptozotocin (STZ)-nicotinamide (NIC)-induced diabetic rats.

Methods: Diabetes was induced by administration of STZ (60 mg/kg, i.p.) after 15 min of NIC (120 mg/kg i.p.) administration. The diabetic rats were treated with HALEC (300 and 600 mg/kg, p.o., respectively) for 21 d.

Results: After the management with HALEC, blood glucose, HbA1c levels, total cholesterol, LDL cholesterol, triglycerides levels, glycogen phosphorylase and glucose-6-phosphatase levels were significantly diminished in diabetic rats. However, haemoglobin level, HDL cholesterol, liver glycogen, total protein, hexokinase, glucose-6-phosphate dehydrogenase levels were significantly increased in HALEC treated diabetic rats. The histopathological studies of the pancreas in HALEC-treated diabetic rats showed almost normal appearance. L6 cell line study revealed the increased glucose uptake activity of HALEC. High performance thin layer chromatography (HPTLC) analysis confirms the presence of active principles such as rutin, gallic acid and quercetin in HALEC.

Conclusion: The results indicated that HALEC possess significant antihyperglycemic and antihyperlipidemic activity in STZ-NIC-induced type II diabetic rats with protective effect. This research work will be useful for the isolation of active principles and development of herbal formulation in phytopharmaceuticals.

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1. Introduction

Diabetes is a metabolic disorder which is categorized by high levels of glucose in blood (hyperglycemia). Diabetes is affected by shortcomings in biosynthesis of insulin or insulin exploit, and overdue function in the breakdown of starches, lipids and proteins which motivates extended health difficulties (Maitra & Abbas, 2009; Wild, Roglic, Green, Sicree, & King, 2004). Hyperlipidemia is a grouped disorder commonly categorized by augmented level of unrestricted fatty acids, preminent triglycerides (TGs), low-density lipoprotein-cholesterol, and also diminished plasma high-

density lipoprotein (HDL)- cholesterol concentration (good cholesterol) (Kolovou, Anagnostopoulou, & Cokkinos, 2005). Diabetes is a globally fastest growing disease. Nowadays various man-made drugs commercially exist to control diabetes and hyperlipidemia; However, these man-made drugs accompanying with many side effects, adverse effect and economically high budget. The herbal materials and herbal extracts are the alternative therapies with less side effects, toxic effects and economically low cost. Herbs and herbal derivatives contain wide-ranging phytochemicals responsible for antihyperglycemic and antihyperlipidemic activities, like alkaloids, flavonoids, glycosides, etc., (Patlak, 2002; Switi, Krishna, & Sandhya, 2014). *Cucumis melo* L. var. *agrestis* Nuad. (CMA) is under the family of Cucurbitaceae, this aromatic plant is normally widespread in countryside and seaside areas. This

* Corresponding author.

E-mail address: gskpungai@gmail.com (K. Gopalsatheeskumar).

aromatic plant is commonly called as wild musk melon, kachari, small guard (Kripa et al., 2015). Many Cucurbitaceae species of aromatic plants were reported as effective antidiabetic, antihyperlipidemic activity (Kameshwar, Kesavulu, & Appa, 2003; Kameswara, Kesavulu, Giri, & Appa, 1999; Pc Fernandes, Lagishetty, Panda, & Naik, 2007). CMA is available in some commercial markets of Tamil Nadu with the name of mithukkuvathal as a food to control the diabetes and also the leaves of CMA has been used with *Momordica charantia* leaves to cure diabetes in Tamil Nadu traditional system. Due to lack of evidence for its antidiabetic activity, the aim of current research is to study the antidiabetic and antihyperlipidemic activities with protective effect of CMA.

2. Materials and methods

2.1. Chemicals and reagents

Streptozotocin (STZ), nicotinamide (NIC), glibenclamide (GBN) and metformin were purchased from Sigma Aldrich, USA. All other reagents and chemicals were used under analytical grade.

2.2. Plant materials and sample preparation

The leaves of CMA were procured from villagers of Pungavaratham (Thoothukudi region, Tamil Nadu, India) on December 2017. The collected CMA leaves were authenticated and the specimen was submitted to the Government Siddha Medical College, Chennai, and Voucher specimen No. GSMC/MB-87/18. The CMA leaves were cleaned, washed carefully with purified water, dried in sunshade at dark condition for 15 d, and pulverized using mechanical mixer. After pulverization, CMA leaves were separated between mesh no. 40 & no. 60, and stored in sealed container at room temperature. Then coarse powder obtained was defatted with petroleum ether and extracted with hydroalcohol (60% methanol) using soxhlet apparatus and concentrated by distillation. The obtained crude extract was dissolved in water and again concentrated for removal of alcoholic traces. Then the extract was marked as hydroalcoholic leaf extract of CMA (HALEC) and stored in sealed container at 4 °C for experiment (Mirunalini & Vaithyanathan, 2015).

2.3. Experimental animals

Totally 30 male Wistar rats (6–8 weeks old and 160–180 g body weight) were purchased from KMCH College of Pharmacy, Coimbatore. Rats were housed and maintained under standard conditions of temperature (25 ± 5 °C), relative humidity (55 ± 10%), and 12/12 h light/dark cycle. Institutional Animal Ethical Committee (IAEC) approval No. is KMCHRET/M.Pharm/03/2018-19.

2.4. *In vitro* glucose uptake capacity of HALEC

The L6 cell line was the optimal cell line for the estimation of glucose assay *in vitro* which was acquired from National Centre for Cell Sciences (NCCS), Pune, India. The HALEC was prepared in different concentrations (100–500 µg/mL) with DMSO for the estimation of glucose uptake in L6 cell line and metformin was used as standard. The experiment was followed the standard procedure as per literature (Mangesh, Somnath, Dheeraj, & Ganesh, 2017).

2.5. *In vivo* antihyperglycemic and antihyperlipidemic activity

2.5.1. Oral glucose tolerance test (OGTT) in normal rats

OGTT was performed for the estimation of acceptance capacity of the glucose in experimental animals. The OGTT was accom-

plished in 6 h starved rats. Totally 30 animals were separated into five groups, six animals for each. Group I: Receives normal saline; Group II: Receives normal saline; Group III: Administrated with standard glibenclamide (2 mg/kg, p.o); Group IV: Administrated with HALEC (300 mg/kg p.o); Group V: Administrated with HALEC (600 mg/kg p.o).

The above grouped animals were served with glucose (2 g/kg) after 30 min treatment except for Group I. The blood glucose concentrations were examined at 0, 30, 60, 120, and 180 min after glucose administration from tip of rat tail vein using Accucheck glucometer.

2.5.2. Experimental induction of diabetes

The STZ was prepared in cold 0.1 mol/L citrate buffer solutions for the induction of diabetes and NIC in normal saline for the partial prevention of pancreas. Before the induction of diabetes, all the rats were abstained feed for 16 h. Fasted rats were injected with 120 mg/kg of NIC 15 min before the injection of 60 mg/kg, i.p. of STZ for the induction of diabetes. Diabetes was established by the increased levels of blood glucose checked at 72 h. The animals with blood glucose concentration more than 200 mg/dL will be used for the study (Kesari, Gupta, Singh, Diwakar, & Watal, 2006). After confirmation of diabetes, all the rats were divided into five groups excluding normal group. Group I: Vehicle (normal saline) for 21 d; Group II: STZ 60 mg/kg (i.p) + NIC 120 mg/kg (i.p) rats receives normal saline for 21 d; Group III: STZ 60 mg/kg (i.p) + NIC 120 mg/kg (i.p) rats treated with GBN 2 mg/kg, p.o dissolved in normal saline for 21 d; Group IV: STZ 60 mg/kg (i.p) + NIC 120 mg/kg (i.p) rats treated with HALEC 300 mg/kg p.o for 21 d; Group V: STZ 60 mg/kg (i.p) + NIC 120 mg/kg (i.p) rats treated with HALEC 600 mg/kg p.o dissolved in normal saline for 21 d.

The fasting animal body weight and blood glucose concentrations were estimated every week in the treatment of respective groups for 21 d from tip of rat tail vein using Accucheck glucometer. The treatment dose of HALEC was fixed as per OECD-423 guidelines conducted by our lab.

2.5.3. Serum biochemical analysis

After the treatment period, rats in all groups were anaesthetized using ketamine hydrochloride (87 mg/kg, i.p) and the blood was collected from Retro-orbital sinus by using capillary into a centrifugation tube which contains ethylene diamine tetraacetic acid (EDTA) for haematological parameters (haemoglobin and glycosylated hemoglobin levels) (Nayak & Pattabiraman, 1981; Reitmann & Frankel, 1957) without EDTA for serum biochemical parameters. The estimation of serum glutamic pyruvate oxaloacetate (SGOT), serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum urea (Fawcett & Scott, 1960), uric acid, creatinine (Kammcraft, 1978), serum total cholesterol, total triglyceride, high-density lipoproteins (HDL)-cholesterol level and low-density lipoproteins (LDL) cholesterol level were done by using standard enzymatic methods (Span Diagnostics, India) (Albert, Bennie, & Albert, 1953; Foster & Dunn, 1973; Marudamuthu & Leela, 2008).

2.5.4. Analysis of antioxidant and hepatic key enzyme levels in liver

After treatment with HALEC, the rats were euthanized by cervical dislocation, liver were isolated and washed with normal saline and stored for 12 h for *in vivo* antioxidant studies. A total of 10% of liver homogenate was prepared with 0.1 mol/L tris-HCl buffer (pH 7.4) using Teflon covered homogenizer. The supernatant was used for the study of total protein (Lowry, Rosebrough, Farr, & Randall, 1951), total glycogen (Nicholas, Robert, & Joseph, 1956), glycogen phosphorylase (Fiske & Subbarow, 1925; Garcia et al., 2001; Gilboe, Larson, & Nuttall, 1972), hexokinase (Brandstrup, Kirk, & Bruni, 1957; Pari & Srinivasan, 2010; Teiichi & Sanae, 1972),

glucose-6-phosphatase (G6P) (Singh & Kakkar, 2009), glucose-6-phosphate dehydrogenase (G6PD), catalase (CAT) (Sinha, 1972), reduced glutathione (GSH) (Ellman, 1959) and lipid peroxidation (LPO) (Okhawa, Ohishi, & Yagi, 1979) levels. The standard procedure was followed as per literature.

2.5.5. Histological examinations

At the completion of the experimental period (after 21 d of treatment with respective groups), the pancreas of all rats were dissected for the analysis of histopathology. The haematoxylin-eosin (H&E) dye was used for the observation of histopathological changes (Danish, Vikas, Amita, Girija, & Manju, 2015).

2.6. Estimation of antioxidant biomarkers using HPTLC analysis

About 100 mg of standard quercetin, gallic acid, rutin and 1 g HALEC were separately dissolved in 100 mL flask with 100 mL of methanol, allowed 30 min for sonication and filtered using Whatman filter paper for HPTLC analysis. A CAMAG HPTLC system prepared with Linomat V Automatic Sample Spotter (Camag Muttenz, Switzerland) and CAMAG-TLC scanner III through winCATS planar chromatography director software version 1.4.3 was used for the experiment. Toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) was used for the mobile phase (Mukherjee, Kumar, Khatua, & Mukherjee, 2010).

2.7. Statistical analysis

Statistical calculations [mean ± standard error mean (SEM)] were analyzed by one-way analysis of variance (ANOVA), and significant differences were determined by Dunnett's post hoc test using Graphpad Instat version 3.06 computer software ($P < 0.05$).

3. Results

3.1. In vitro glucose uptake capacity of HALEC

The glucose utilization in L6 cell line showed that HALEC was found to be prominent over control. The L6 cell line enhanced the glucose uptake by (41.80 ± 0.25) at 500 µg/mL concentration (Table 1). This result was compared with metformin, which was

Table 1
In vitro glucose uptake study of HALEC in L6 cell lines (mean ± SEM, n = 6).

| Samples No. | Concentrations/ (µg·mL ⁻¹) | Percentage glucose uptake of Metformin/% | Percentage glucose uptake of HALEC/% |
|-------------|--|--|--------------------------------------|
| 1 | 100 | 42.33 ± 0.22 | 22.36 ± 0.20 |
| 2 | 200 | 53.82 ± 0.12 | 27.17 ± 0.14 |
| 3 | 300 | 63.50 ± 0.31 | 33.25 ± 0.29 |
| 4 | 400 | 72.25 ± 0.42 | 38.20 ± 0.38 |
| 5 | 500 | 79.84 ± 0.23 | 41.80 ± 0.25 |

Table 2
Blood glucose levels of OGTT in normal rats.

| Groups | Blood glucose levels / (mg·dL ⁻¹) | | | | |
|--------|---|-----------------------------|------------------------------|------------------------------|-----------------------------|
| | 0 min | 30 min | 60 min | 120 min | 180 min |
| I | 58.42 ± 6.60 | 56.42 ± 7.18 | 57.78 ± 1.20 | 59.33 ± 0.82 | 59.00 ± 3.85 |
| II | 67.66 ± 5.78 ^{###} | 99.67 ± 1.90 ^{###} | 97.67 ± 3.33 ^{###} | 106.66 ± 3.80 ^{###} | 98.30 ± 1.96 ^{###} |
| III | 55.67 ± 1.38 ^{***} | 97.00 ± 1.11 ^{***} | 106.00 ± 3.01 ^{***} | 70.00 ± 6.60 ^{***} | 56.55 ± 5.98 ^{***} |
| IV | 71.57 ± 0.65 [*] | 95.14 ± 1.62 ^{**} | 88.33 ± 1.93 [*] | 66.33 ± 7.85 [*] | 72.00 ± 1.13 [*] |
| V | 78.66 ± 0.85 ^{***} | 96.67 ± 6.36 ^{***} | 105.00 ± 3.60 ^{***} | 81.65 ± 3.60 ^{***} | 77.31 ± 4.60 ^{***} |

Note: Group I is normal group, Group II is disease control group, Group III is standard GBN-treated group, Group IV is HALEC 300 mg/kg treated group, and Group V is treated with HALEC 600 mg/kg. Data is expressed as mean ± SEM; n = 6, One-way ANOVA followed by Dunnett's test; ns = non-significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs disease control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs disease control.

used as the standard antidiabetic drugs. Metformin at a concentration of 100 µg/mL was found to enhance the glucose uptake over control.

3.2. In vivo antidiabetic activity

3.2.1. Effect of HALEC in OGTT

For OGTT, the blood samples were analyzed for glucose content at 0, 30, 60, 120 and 180 min, individually. In glucose loaded normal rats, significant decreased concentration of blood glucose existed at 120 min later administration of the HALEC. (Table 2).

3.2.2. Effect of HALEC in blood glucose level and body weight analysis

The STZ-NIC induced type 2 diabetic rats showed significant increased level of blood glucose compared with normal rats, oral administration of HALEC for 21 d displayed a significant decrease in blood glucose level compared with group II (Table 3). The STZ-NIC induced diabetic rats showed bodyweight loss. After the treatment of HALEC and GBN for three weeks, the body weight of animal was significantly improved when compared with the diabetic control rats.

3.2.3. Effect of HALEC on haematological parameters

The STZ-NIC induced diabetic rats showed a significant increase in HbA1c and decrease in hemoglobin levels when compared with group I. The oral administration of HALEC and GBN exhibited a significant decrease in HbA1c concentration and significantly raised concentration of haemoglobin when matched to group II.

3.2.4. Effect of HALEC on liver glycogen and hepatic key enzymes

The STZ-NIC persuaded diabetic rats exhibited a significant declined level of liver glycogen. The diabetic rats treated with HALEC and GBN for 21 d significantly improved liver glycogen concentrations in a dose dependent manner (Table 4). In the current research, a significant raised concentration of glycogen phosphorylase and G6P were noticed in STZ-NIC persuaded diabetic control rats. These raised concentrations were significantly decreased in HALEC-treated diabetic rats in a dose dependent manner (Table 4). The STZ-NIC persuaded diabetic rats significantly exhibited decreased concentration of hexokinase and G6PD. These elevated levels were significantly augmented in HALEC and GBN-treated diabetic rats (Table 4).

3.3. In vivo antihyperlipidemic activity

The lipid profile, STZ-NIC persuaded diabetic rats displayed elevated level of serum triglycerides, cholesterol and LDL levels (Fig. 1) and reduced HDL level matched with group I rats. After treatment with HALEC and GBN for 21 d, the diabetic rats showed a significant decrease of these changed serum lipid variables in a dose dependent manner.

Table 3
Effect of HALEC on blood glucose levels in normal and diabetic rats.

| Group | Before diabetes induction of diabetes | After induction of diabetes with STZ and nicotinamide (3rd day) | Blood glucose levels / (mg·dL ⁻¹) | | | |
|-------|---------------------------------------|---|---|------------------------------|------------------------------|------------------------------|
| | | | (7th day of induction of diabetes) 0th day | 7th d | 14th d | 21st d |
| I | 81.67 ± 1.99 | 80.67 ± 2.02 | 81.67 ± 1.98 | 83.66 ± 5.85 | 82.00 ± 7.50 | 81.00 ± 2.89 |
| II | 88.48 ± 3.47 | 300.63 ± 3.52 | 319.63 ± 7.95 ^{###} | 361.00 ± 3.80 ^{###} | 370.33 ± 3.98 ^{###} | 348.00 ± 3.98 ^{###} |
| III | 85.55 ± 2.05 | 330.00 ± 1.85 | 327.00 ± 3.80 | 252.00 ± 1.78 ^{**} | 182.33 ± 9.36 [*] | 103.66 ± 6.99 ^{***} |
| IV | 76.25 ± 3.35 | 328.00 ± 1.08 | 318.00 ± 3.75 | 271.67 ± 2.07 [*] | 167.33 ± 3.27 ^{**} | 122.67 ± 8.91 ^{**} |
| V | 80.69 ± 5.07 | 338.33 ± 5.57 | 350.33 ± 1.67 | 258.67 ± 5.90 ^{**} | 158.33 ± 6.66 ^{***} | 98.12 ± 5.67 ^{***} |

Note: Group I is normal group, Group II is disease control, Group III is standard GBN-treated group, Group IV is HALEC 300 mg/kg treated group and Group V is treated with HALEC 600 mg/kg. Data is expressed as mean ± SEM; n = 6, One-way ANOVA followed by Dunnett's test; ns = non-significant; *P < 0.05, ^{##}P < 0.01, ^{###}P < 0.001 vs group I. ^{*}P < 0.05, ^{**}P < 0.01, ^{***}P < 0.001 vs group II.

Table 4
Estimation of carbohydrate metabolizing enzymes and antioxidant parameters in liver homogenate.

| Parameters | Group I | Group II | Group III | Group IV | Group V |
|---|----------------|------------------------------|------------------------------|-----------------------------|------------------------------|
| Hexokinase/ (U/mg of protein) | 0.4867 ± 0.06 | 0.2784 ± 0.05 ^{###} | 0.4147 ± 0.06 [*] | 0.3475 ± 0.01 [*] | 0.4693 ± 0.05 [*] |
| Glycogen Phosphorylase (U/mg of protein) | 43.07 ± 7.34 | 120.67 ± 3.65 ^{###} | 56.12 ± 2.98 ^{***} | 72.67 ± 5.03 [*] | 60.35 ± 0.99 ^{***} |
| Glucose-6-phosphatase (U/mg of protein) | 0.0418 ± 0.005 | 0.0665 ± 0.007 ^{##} | 0.0537 ± 0.009 [*] | 0.0552 ± 0.008 [*] | 0.0506 ± 0.005 [*] |
| Glucose-6-phosphate dehydrogenase (U/mg of protein) | 0.0760 ± 0.001 | 0.0404 ± 0.003 ^{##} | 0.0625 ± 0.001 ^{**} | 0.0562 ± 0.001 [*] | 0.0637 ± 0.007 ^{**} |
| Total protein mg/g | 165 ± 2.03 | 15.94 ± 2.38 ^{###} | 92.67 ± 1.05 ^{***} | 43.97 ± 2.39 ^{**} | 108 ± 2.76 ^{***} |
| Glycogen/ (mg/dl) | 72.4 ± 1.08 | 39.6 ± 3.15 ^{###} | 56.8 ± 5.59 ^{**} | 52.8 ± 1.27 ^{**} | 62.4 ± 2.03 ^{***} |
| GSH (μg/mg protein) | 9.98 ± 0.06 | 7.33 ± 0.05 [#] | 9.45 ± 0.06 [*] | 9.48 ± 0.07 [*] | 9.57 ± 0.04 [*] |
| CAT (μmol of H2O2 consumed/ min/mg/protein) | 127.80 ± 3.34 | 27.30 ± 1.65 ^{###} | 91.90 ± 2.98 ^{***} | 50.47 ± 3.03 ^{**} | 95.35 ± 0.99 ^{***} |
| LPO (nmol MDA/ mg protein) | 14.14 ± 0.04 | 31.71 ± 0.56 ^{###} | 15.42 ± 0.08 ^{**} | 17.58 ± 0.38 ^{**} | 15.14 ± 0.95 ^{**} |

Note: Group I is normal group, Group II is disease control, Group III is standard GBN-treated group, Group IV is HALEC 300 mg/kg treated group, and Group V is treated with HALEC 600 mg/kg. Data is expressed as mean ± SEM; n = 6, One-way ANOVA followed by Dunnett's test; ns = non-significant; *P < 0.05, ^{##}P < 0.01, ^{###}P < 0.001 vs group I. ^{*}P < 0.05, ^{**}P < 0.01, ^{***}P < 0.001 vs group II.

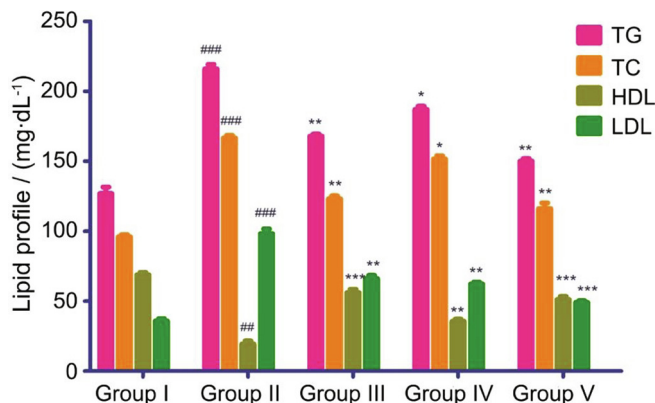


Fig. 1. Lipid profile of diabetic rats with respective treatment (Group I is normal group; Group II is disease control; Group III is standard GBN treated group; Group IV is HALEC 300 mg/kg treated group, and Group V is treated with HALEC 600 mg/kg. Data is expressed as mean ± SEM; n = 6, One-way ANOVA followed by Dunnett's test; ns = non-significant; *P < 0.05, ^{##}P < 0.01, ^{###}P < 0.001 vs group I. ^{*}P < 0.05, ^{**}P < 0.01, ^{***}P < 0.001 vs group II).

3.4. Protective effect of HALEC

3.4.1. Effect of HALEC on liver and kidney functional parameters

The STZ-NIC persuaded diabetic rats exhibited increased levels of serum urea, uric acid, creatinine, SGOT and SGPT significantly compared with group I rats. After the treatment with HALEC 300 mg/kg and 600 mg/kg for 21 d, the diabetic rats exhibited significant decreased levels of these values when compared with diabetic rats. The 600 mg/kg HALEC-treated diabetic rat showed higher activity than standard GBN-treated diabetic rats. The high dose of HALEC showed the similar effect with the standard GBN.

3.4.2. Effect of antioxidant parameters on liver homogenate

The total protein (TP) levels were estimated in the liver homogenate of normal, diseased, GBN and HALEC-treated groups. The STZ-NIC persuaded diabetic rats observed that TP was significantly declined when matched with group I. After the treatment with HALEC and GBN, the TP was increased (Table 4). STZ-NIC diabetic rats exhibited a significant declined level of CAT, GSH levels and improved level of LPO when compared with group I. After the treatment with HALEC and GBN, the STZ-NIC induced diabetic rats showed significant increased levels of CAT and GSH and reduced LPO level (Table 4).

3.5. Detection of antidiabetic responsible phytochemicals

The preliminary qualitative phytochemical test confirms the occurrence of alkaloids, flavonoids, glycosides, saponins, tanins, proteins and amino acids in HALEC. The chromatogram of HPTLC for antidiabetic biomarkers was shown in Fig. 2. The comparison of standard R_f value (Rutin 0.19, Gallic acid 0.79 and Quercetin 0.88) with sample R_f value the quercetin, gallic acid and rutin were identified and quantified (Rutin 22.83, Gallic acid 9.70 and Quercetin 19.73 mg/g of plant extract) in HALEC by HPTLC.

4. Discussion

This study was conducted for the providing of traditional claim (antidiabetic activity) of CMA, the STZ-NIC induced animal model was used for this goal. The male rats were commonly resistant to the toxicity so that male Wistar rats were used for this study. The STZ-NIC partially destructs the beta cells of pancreas thereby elevating blood glucose level, which was significantly decreased by oral administration of HALEC, it may be the reason for antidiabetic activity of HALEC (Manish et al., 2016). In diabetes rats, the body weight of rats was declined due to degradation of protein

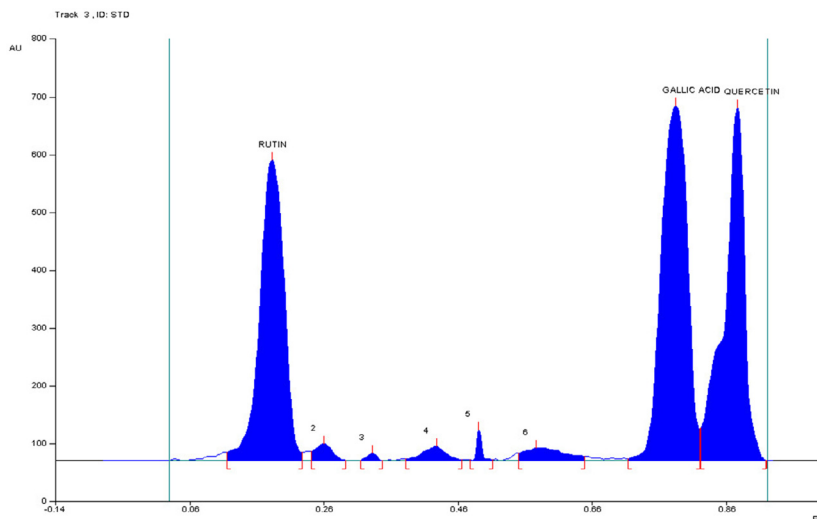


Fig. 2. Chromatogram of rutin, quercetin and gallic acid (chromatogram was scanned at 254 nm and R_f value of rutin was 0.19, R_f value of gallic acid was 0.79 and R_f value of quercetin was 0.88).

(unavailability of carbohydrate for metabolism) and muscle degeneration, which was normalized by HALEC, it may be the reason for preventing of the muscle wasting of HALEC during diabetes condition.

The carbohydrate metabolic pathways such as glycolysis, glycogenolysis, gluconeogenesis and Hexose Mono Phosphate (HMP) shunt play important role in controlling of blood glucose levels (Grover, Vats, & Yadav, 2002). Hexokinase involved in glycolysis (Bouche, Serdy, Kahn, & Goldfine, 2004; Laakso, Malkki, & Deeb, 1995; Roden & Bernroider, 2003), G6P involved in glycogenolysis and gluconeogenesis and G6PD involved in HMP shunt pathways (Gancedo & Gancedo, 1971), which are the enzymes not properly secreted in liver during diabetic condition. These levels were normalized in HALEC-treated rats which confirms the antidiabetic activity, it may be the reason for glucose uptake capacity of HALEC (Mithievre, Vidal, Zitovn, & Miriasian, 1996). The HALEC and GBN treated group animal demonstrations nearly regular morphology

in histopathological studies which confirms the antidiabetic activity by regeneration of pancreatic beta cells (Fig. 3).

This study also demonstrated the hypolipidemic effect of HALEC through reducing the lipid profile (triglycerides, cholesterol and LDL levels) and increasing the HDL cholesterol level. The diabetic complication and STZ causes liver and kidney toxicity so that the levels of urea, uric acid, creatinine, SGOT, SGPT and ALP were increased in diabetic rats (Sheela, Jose, Sathyamurthy, & Kumar, 2013). These increased parameters were normalized in HALEC-treated diabetic rats which confirms the protective effect of HALEC (Brodsky, 1998; Demerdash, Yousef, & Naga, 2005). The prolonged hyperglycemia and STZ cause hepatocytes toxicity and create free radicals, which can deplete antioxidant defense system (CAT, SOD, GSH), and increase oxidative stress through elevation of LPO (Arivazhagam, Thilagavathy, & Pannerselvam, 2000; Murugan & Pari, 2006). HALEC acts as antioxidant by scavenging free radicals that significantly reduces the LPO and increases CAT

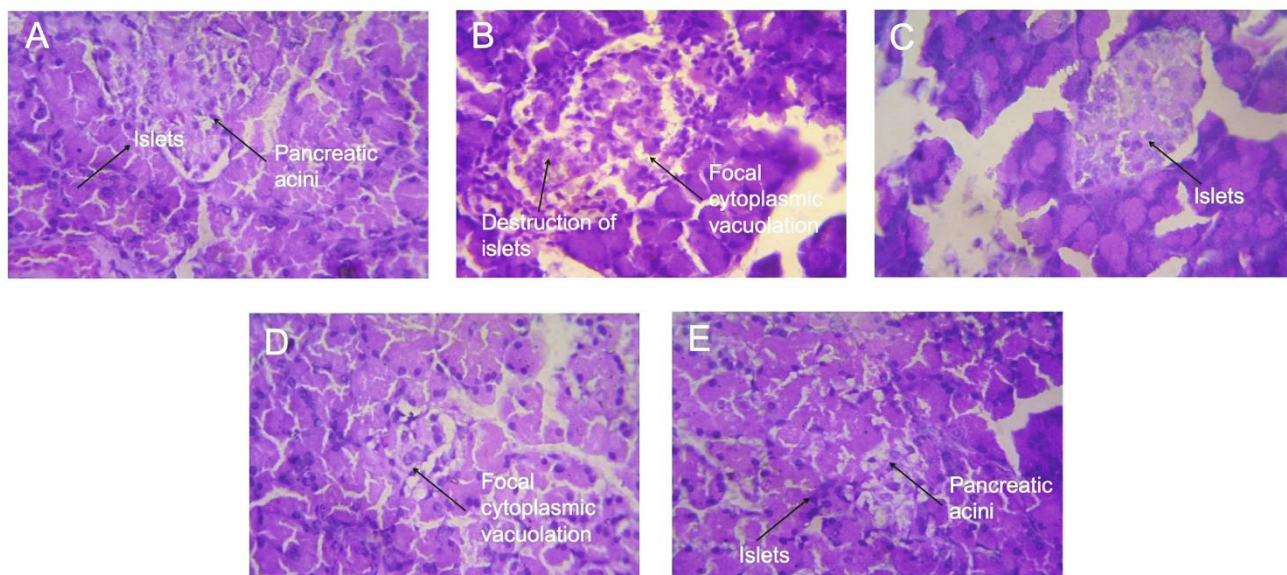


Fig. 3. Histopathological studies of pancreas. Group I: Pancreas shows normal pancreatic acini, no evidence of destruction (A); Group II: Islets are reduced in number and size with focal cytoplasmic vacuolation with destruction of islets (B); Group III: Islets are normal in number (C); Group IV: Islets are normal in number and size with few showing cytoplasmic vacuolation (D); Group V: Pancreas shows normal pancreatic acini, islets are normal in number and size (E).

and GSH in STZ-NIC persuaded diabetic rats which confirm the antioxidant and protective capacity of HALEC.

The type II diabetes is non-insulin dependent diabetes mainly caused by defects in glucose uptake. The L6 cell line was usually used cell line for estimation of glucose uptake. The L6 cell line is a skeletal muscle cell line, the skeletal muscle is mainly involved in glucose uptake by insulin induction. The results of current research revealed that the HALEC has the major glucose uptake activity in L6 cell line.

Herb and herbal products contain an extensive range of active principles, such as alkaloids, flavonoids, glycosides which are thought to have an antihyperglycemic role. In literatures, quercetin, gallic acid and rutin were evaluated for antidiabetic activity. The mechanism of quercetin increased the function of glucose transporter 4 (GLUT 4) and insulin receptor which in turn results in elevated glucose uptake (Nuraliev & Avezov, 1992), rutin suppressed the glucolipotoxicity through activating insulin receptor substrate 2 and adenosine monophosphate-activated protein kinase signaling and gallic acid scavenges the free radicals and reduces oxidative stress during diabetic (Rauter, Martins, Borges, Mota, & Pinto, 2010; Switi et al., 2014).

The CMA leaves with *Momordica charantia* are traditionally used for the management of diabetes. This study confirms the antidiabetic activity of CMA leaves scientifically. Furthermore, its active principles such as rutin, gallic acid and quercetin might be the reason for its antidiabetic activity.

5. Conclusion

The overall results of current research work concluded that HALEC has potent antidiabetic activity, antihyperlipidemic activity and also having protective effect. The future direction of this research work is isolation of active principles, development of suitable formulation and commercialization in market.

Author Contribution

All authors contribute equal to this work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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