Amelioration of Diabetes mellitus by modulation of GLP-1 via targeting alpha-glucosidase using *Acacia tortilis* polysaccharide in Streptozotocin-Nicotinamide induced diabetes in rats

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**Background:** Polysaccharides decrease the glucose level by inhibiting α-glucosidase enzyme which further increases the level of GLP-1 (Glucagon-like peptide 1) to increase the insulin level as per earlier reports.

**Objective:** Similar hypothesis was designed in present study to investigate the α-glucosidase enzyme inhibition and involvement of GLP-1 in antidiabetic mechanism of *Acacia tortilis* polysaccharides (AEATP) in diabetic rats. Isolated polysaccharides were analyzed for their chemical nature by using HPLC and FTIR method.

**Materials and Methods:** Male albino wistar rats were divided into control, diabetic, diabetic + voglibose, diabetic + glimepiride, diabetic + 250, 500, 1000 mg/kg of AEATP, diabetic + glimepiride + voglibose, diabetic + glimepiride + 250, 500 and 1000 mg/kg AEATP, diabetic + GLP-1 antagonist + 250, 500 and 1000 mg/kg AEATP. Plasma glucose, insulin and active GLP-1 levels were measured 15 min after OGTT. Fasting blood glucose, Plasma triglycerides, glycated hemoglobin (HbA1c), Fasting insulin, pancreatic insulin content, ileum and colon GLP-1 content were assessed at 5th week. Association of alpha-glucosidase was also assessed with GLP-1 and insulin.

**Results:** AEATP significantly attenuated hyperglycemia by increasing insulin level in plasma and pancreas and increased active GLP-1 as well as insulin level in diabetic rats after OGTT. GLP-1 content was significantly increased in ileum and colon by inhibiting alpha-glucosidase. Involvement of GLP-1 in antihyperglycemic effect of AEATP was confirmed by using GLP-1 antagonist. Moreover, AEATP significantly improved dyslipidemia in diabetic rats. HPLC analysis of *A. tortilis* polysaccharide comprised four specific monosaccharides (Rhamnose, Glucuronic acid, glucose and galactose) and FTIR spectrum shown band at 3430.6 cm⁻¹ (O-H stretching), 2940.3 cm⁻¹ (C-H linkage), 1630.4 cm⁻¹ (carbonyl stretching), 1410 cm⁻¹ (uronic acid) and 1030.5 cm⁻¹ (glycosidic linkage).

**Conclusion:** It can be concluded that antidiabetic effect of AEATP is through the modulation of GLP-1 level in plasma and intestinal tissue via alpha glucosidase inhibition.

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1. **Introduction**

Abnormality of glucose homeostasis is a characteristic feature of Diabetes mellitus. Irregular metabolism of carbohydrate, fat and proteins, results in aggravation of microvascular, macrovascular, and neuropathic complications. Diabetes mellitus is extensively dispersed worldwide with prevalence from 171 million in 2000 to 366 million in 2030 [1]. Diverse range of synthetic drugs and treatment strategies are being incorporated to ameliorate diabetes mellitus but reported to have some serious side effects along with their therapeutic potential [2]. Despite availability of synthetic drugs, herbal medicines have emerged as safe and relatively economical not only as add on therapy but also in the long term management of diabetes mellitus [3].

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Role of various phyto-constituents, importantly polysaccharides in the management of diabetes mellitus has been studied earlier [4]. Increased insulin levels leads to reduction of β-cell damage in experimental diabetic animals and/or accelerate glucose metabolism in the liver. These actions of insulin may contribute to antihyperglycemic property of polysaccharides.

GLP-1 (glucagon like peptide-1) is the novel therapeutic target to maintain glycemic control in diabetes mellitus [5]. One intriguing aspect of the alpha-glucosidase inhibitor is its ability to both increase and prolong glucagon-like peptide-1 (GLP-1) secretion in normal individuals and patients with type 2 diabetes [6]. Alpha-glucosidase inhibitors delay the breakdown of carbohydrates in the small intestine and thus diminish the blood glucose levels in animals and humans [7]. Active GLP-1 (GLP-1 (7–36) amide and GLP-1 (7–37)), is secreted from gut L-cells and involved in the regulation of insulin secretion [8]. Preclinical and clinical studies have demonstrated that activating GLP-1 signals by administration of GLP-1 receptor agonists can improve diabetes in animals and humans [8]. Alpha-glucosidase inhibitors delay the absorption of carbohydrates by escalating glucose absorption in the lower gut [10].

The aqueous extract of Acacia tortilis polysaccharide has already been reported to have antidiabetic activity in streptozotocin-nicotinamide induced diabetes model in rats [4]. Our hypothesis is to explore the mode of action of antidiabetic action of A. tortilis polysaccharide in streptozotocin-nicotinamide induced diabetic Rats.

2. Material and methods

2.1. Drugs

Streptozotocin, Glimepiride, α-glucosidase, bovine serum albumin, para-nitrophenyl- α-D-glucopyranoside (pNPG), Exendin-(9–39) fragment, Acarbose and NAD were procured from Sigma–Aldrich, Milwaukee, US. GLP-1 and Insulin Elisa Kit from Merck Millipore and all other chemicals were of analytical grade.

Gum exudates from the stem and branches of A. tortilis was collected from Central Arid Zone Research Institute Campus, Jodhpur, India. The taxonomic identification was done by Ministry of Environment, Forest and Climate Change/Botanical Survey of India, India (BSI/AZRC/Tech/2016–17-(PLId.)/258) and the plant specimen has been submitted in Departmental Herbarium for future records.

2.2. Isolation and purification of polysaccharide from aqueous extract of A. tortilis

Gum exudates obtained from the stem were converted into fine powder and dissolved in distilled water and was mixed properly with vigorous shaking at 27 °C for six hours. After proper mixing, it was subjected to centrifugation to remove the water soluble and insoluble parts. Supernatant solution was decanted off and remaining solution was further diluted with three times of its volume with ethanol. After mixing with ethanol, it was stirred properly. After constant stirring the polysaccharides got precipitated. After precipitation and filtration, the precipitates were further dissolved in the distilled water. Ethanol was also mixed with above mixture for proper precipitation. After precipitation and filtration process, it was treated with ether and acetone (dry solvent). Vacuum filtration was applied over the above mixture to filter out the precipitate. Further, it was dried at 27 °C in the vacuum desiccators [11]. For purification, dried polysaccharides were dissolved in distilled water. Deionisation of this aqueous preparation was done by freshly prepared cation and anion exchange resins. The eluent so formed was further purified with dialysis process. Dialysis kit (PURG12015-Sigma) named Pur-A—Lyzer was employed. Firstly, 20 ml of ultrapure water was taken and incubated (5 min). After incubation Load sample was placed in Pur-A—Lyzer tube then place it in floating rack. Speed bar was adjusted to get the sample after 30 min and then concentrated, reprecipitated with ethanol, filtered and dried to get pure polysaccharide (amorphous powder).

2.3. HPLC analysis

A standard spectrum was prepared using monosaccharide standards like mannose, rhamnose, glucuronic acid, glucose, galactose, xylose, and arabinose [12]. These standards (2 mg) were first dissolved in distilled water (1 ml). Out of this 25 μl was labeled with 1-phenyl-3-methyl-5-pyrazolone (PMP) and then mixed properly. After proper mixing with vortex, mixture was incubated for one and half hour at 70 °C. After incubation, neutralization was done by adding hydrochloric acid (50 ml). Chloroform and distilled water was used for extraction. After extraction, aqueous phase was taken and filtered through polytetrafluoroethylene filter for final analysis in HPLC. Solution of polysaccharide of A. tortilis was prepared (25 mg/ml); 100 μl of this solution was taken and mixed with trifluoroacetic acid (2 M), and further reacted at 110 °C for 2 h. Mixture was allowed to cool at room temperature and 200 μl of methanol was added and dried with nitrogen. The residue thus obtained was dissolved in distilled water (50 ml). 25 μl of this solution was labeled with PMP as above mentioned method. Analysis was performed by HPLC system (LC-2010A Shimadzu/Japan) with C-18 column (150 mm × 4.6 mm, 5 μm) and UV detector (254 nm). Acetate buffer (0.1 M) and acetonitrile (81:19) was used as mobile phase and flow rate was kept at 1 ml/min.

2.4. FT-IR analysis

Usually polysaccharides are analysed by FT-IR to detect the glycosidic linkage. FT-IR spectra of A. tortilis polysaccharide was obtained by mixing polysaccharide with potassium bromide, further it was pressed to form pellets. The FT-IR spectra were obtained by Perkin–Elmer 2000 spectrometer equipped with MCT detector and software Spectrum. The FT-IR spectra was obtained between 4000 and 400/cm scanning range.

2.5. Experimental protocol

Male albino Wistar rats (150–200 gm) were used in this study and experimental protocol was approved by Institutional Animal Ethics Committee (1181/ab/08/CPSEA). Diabetes was induced by intraperitoneal injection of Nicotinamide (230 mg/kg) 15 min before streptozotocin (65 mg/kg, i.p) administration [13]. STZ was freshly prepared in 0.1 M citrate buffer, pH 4.5, and nicotinamide was prepared in normal saline. Diabetes mellitus was confirmed after 14 days of STZ administration when fasting blood glucose level had become constant above 250 mg/dL. Different doses of AEATP (250, 500, and 1000 mg/kg) were administered to the animals.

Further, animals were divided into groups comprising six animals in each group. Group 1: Control; Group 2: Diabetic; Group 3: Diabetic rats + Voglibose; Group 4: Diabetic rats + Glimepiride; Group 5: Diabetic rats + 250 mg/kg AEATP; Group 6: Diabetic rats + 500 mg/kg AEATP; Group 7: Diabetic rats + 1000 mg/kg AEATP; Group 8: Diabetic rats + Glimepiride + Voglibose; Group 9: Diabetic rats + Glimepiride + 250 mg/kg AEATP; Group 10: Diabetic rats + Glimepiride + 500 mg/kg AEATP; Group 11: Diabetic rats + Glimepiride + 1000 mg/kg AEATP.
Three confirmatory groups to ensure the role of GLP-1 using Exendin (9–39) fragment as GLP-1 antagonist. Group 1: Diabetic rats + Exendin (9–39) fragment + 250 mg/kg AEATP; Group 2: Diabetic rats + Exendin (9–39) fragment + 500 mg/kg AEATP; Group 3: Diabetic rats + Exendin (9–39) fragment + 1000 mg/kg AEATP.

2.6. Estimation of biochemical analysis

After 5 weeks of drug administration, blood samples were collected (Basal, 0 day and 5th week of diabetes) from retro orbital plexus under anesthesia for biochemical estimation of total cholesterol, triglycerides and HbA1c by commercially available kits (Reckon Diagnostics). Fasting blood glucose level was measured using glucometer.

2.7. In-vitro Inhibition Assay of α-glucosidase activity from \textit{Saccharomyces cerevisiae}

The α-glucosidase inhibitory activity was determined according to Kim [14]. Serial dilutions of AEATP 0.015, 0.031, 0.062, 0.125, 0.25, 0.5, 1, 2, 4 mg/mL were made and incubated with enzyme source (50 Ll) for 5 min and then with substrate (50 Ll) for 5 min at room temp. The pre and post substrate addition absorbance was measured at 405 nm on a microplate reader and percentage of α-glucosidase inhibition was calculated.

\[
\% \text{ α-glucosidase inhibition} = \left(1-\frac{A_c}{A_s}\right) \times 100
\]

where \(A_c\) = absorbance of control. \(A_s\) = absorbance of samples containing extracts. \((IC_{50})\) was calculated by regression analysis. Acarbose was used as positive control.

2.8. In vitro Inhibition Assay of α-glucosidase activity from rat small intestine

In-vitro Inhibition Assay of α-Glucosidase activity was done according to Zhang [15]. Absorbance was measured at 405 nm on a microplate reader.

\[
\% \text{ α-glucosidase inhibition} = \left(1-\frac{A_c}{A_s}\right) \times 100
\]

where \(A_c\) = absorbance of control. \(A_s\) = absorbance of samples containing extracts. \((IC_{50})\) was calculated by regression analysis. Acarbose was used as positive control.

2.9. Estimation of active GLP-1 in blood plasma and intestinal tissue

At the end of the experimental protocol, OGTT was performed and GLP-1 level was measured using an ELISA kit (EMD Millipore, ECLP-35 K) after 15 min in plasma. GLP-1 was extracted from ileum and colon by homogenization (24,000 r.p.m.) at 4 °C using ethanol/acid (5:1 v/v) solution. Further after centrifugation, tissue GLP-1 level was measured in supernatant [16].

2.10. Estimation of insulin in plasma and pancreatic insulin content

Serum insulin was measured by using ELISA kits (EMDMillipore-EZMRI-13 K). Pancreatic insulin content was extracted by taking 0.2 g pancreas portion with 5.0 ml of ice-cold acid-alcohol, homogenized, followed by sonication, stored at −20°C overnight, and finally centrifuged at 3000 rpm at 4°C for 15 min. The supernatant was transferred into a new centrifuge tube and stored at −20°C while the pellet was subjected to extraction again then insulin level was measured.

2.11. Oral glucose tolerance test (OGTT)

Glucose (2 gm/kg, p.o.) was fed 30 min after administration of extracts and GLP-1 antagonist in overnight fasted animals. Blood samples were collected at 15 min in EDTA tubes containing DPP IV inhibitor (10 ml/ml, ADL Research, USA) [17]. Plasma samples were obtained and stored at −80 °C for estimation of blood glucose, insulin and active GLP-1 levels.

2.12. Quantification of dose of exendin (9–39) fragment

The dose of GLP-1 antagonist was assessed through dose response relationship with insulin, at 15 min following a glucose load, when maximum attenuation of insulin release was achieved.

2.13. Evaluation of liver and pancreas histology

Liver and pancreas were harvested from sacrificed animals and fixed in 10% neutral buffered formalin solution for the histopathological examinations. Sections of 5 μm thickness were prepared and stained with hematoxylin and eosin (H & E) dye for microscopic observations.

2.14. Statistical analysis

Statistical analysis was performed using Graph pad Prism 6. Values are expressed as mean ± SD and statistical analysis was carried out by using one way ANOVA followed by Tukey’s multiple test.

3. Results

3.1. HPLC analysis

The results of the HPLC analysis of \textit{A. tortilis} polysaccharide comparing the retention times of peaks to reference standards, standard monosaccharide peaks were obtained i.e. mannose, rhamnose, glucuronic acid, glucose, galactose, and arabinose with retention time 19.989, 27.894, 45.287, 51.998, and 58.746 and four specific monosaccharides was also found (Rhamnose, Glucuronic acid, glucose and galactose) with retention time 27.158, 34.852, 44.895 and 51.112 (Fig. 1a & b).

3.2. FTIR analysis

The FTIR spectrum of revealed the band at 3430.6 cm\(^{-1}\) assigned for uronic acid. The strong absorption at 1030.5 cm\(^{-1}\) contributed for amide linkage (Fig. 1).

3.3. Effect of AEATP treatment (5 weeks) on plasma active GLP-1 level after oral glucose tolerance test

15 min after glucose load the Active GLP-1 level was found to be significantly high in control group (16.38 ± 0.58) as compared to diabetic animals (8.92 ± 0.38). Similarly, after 15 min voglibose, 500, 1000 mg/kg of AEATP, glimepiride + voglibose, glimepiride+250, 500 and 1000 mg/kg of AEATP significantly elevated the level of Active GLP-1 level as compared to diabetic group. Whereas, glimepiride, 250 and 500 mg/kg of AEATP did not produce any significant effect on the level of active GLP-1. The high level of Active GLP-1 produced by AEATP after 15 min of glucose

\[\text{Active GLP-1 level} = \frac{\text{Absorbance of samples}}{\text{Absorbance of control}} \times 100\]
load in confirmatory groups was suppressed by exendin-(9–39)-fragment (GLP-1 Antagonist), exendin-(9–39)-fragment +250, 500 and 1000 mg/kg of AEATP (Table 1).

3.4. Effect of AEATP treatment (5 weeks) on plasma insulin level after oral glucose tolerance test

Insulin level was significantly decreased in diabetic animals at 15 min (0.78 ± 0.028) as compared to control animals (3.57 ± 0.043) after glucose load. Whereas, after 15 min of glucose load voglibose, glimepiride, 500, 1000 mg/kg of AEATP, glimepiride + voglibose, glimepiride +250, 500 and 1000 mg/kg of AEATP shown significant increase in insulin level as compared to diabetic group. The increased level of insulin by AEATP was significantly reduced at 15 min of glucose load in confirmatory groups exendin-(9–39)-fragment +250, 500 and 1000 mg/kg of AEATP; that is, 0.80 ± 0.03, 0.81 ± 0.03 and 0.84 ± 0.03 respectively due to exendin-(9–39)-fragment (GLP-1 Antagonist) (Table 1).

3.5. Effect of AEATP treatment (5 weeks) on plasma glucose level after oral glucose tolerance test

Glucose level after glucose load in control animals as well as diabetic animals was significantly increased after 15 min (149.8 ± 6.4 mg/dL) as compared to basal level (88.8 ± 3.9 mg/dL) (478.8 ± 9.3 mg/dL) respectively. After 15 min of glucose load, voglibose, glimepiride, 500, 1000 mg/kg of AEATP, glimepiride + voglibose, glimepiride +250, 500 and 1000 mg/kg of AEATP shown significant reduction in elevated glucose level as compared to diabetic group except 250 mg/kg of AEATP, that is, 206.2 ± 11.6, 178.8 ± 7.2, 374.4 ± 6.1, 188.8 ± 8.4, 168.4 ± 9.1, 171.2 ± 8.2, 172.6 ± 7.1 and 168.4 ± 4.8 respectively. The beneficial antidiabetic effect of AEATP was significantly attenuated at 15 min of glucose load by exendin-(9–39)-fragment (GLP-1 Antagonist) in confirmatory groups and hyperglycemia in exendin-(9–39)-fragment +250, 500 and 1000 mg/kg of AEATP; that is, 4718 ± 15.9, 463.8 ± 12.4 and 462.4 ± 10.0 respectively (Table 1).

3.6. Effect of AEATP on α-glucosidase activity from S. cerevisiae

The in vitro α-glucosidase inhibitory study showed that AEATP had α-glucosidase inhibitory activity and percentage inhibition was concentration-dependent (i.e. 72.63% for 4 mg/ml). Acarbose showed maximum inhibition 89.47% at 4 mg/ml. The IC50 value of the AEATP was found 1.59 mg/ml (Fig. S1).

3.7. Effect AEATP on α-glucosidase activity from rat small intestine

AEATP inhibited α-glucosidase in small intestine and percentage inhibition was found to be concentration dependent in following manner i.e. 2.3, 5.6, 13.9, 24.3, 39.5, 47.5, 59.2, 62.2, 65.3. The IC50 value of the AEATP was found 1.92 mg/ml (Fig. 3).

3.8. Effect of AEATP on fasting blood glucose level

Fasting blood glucose level of control group ranged from 88.2 ± 2.30 to 98.8 ± 2.19 mg/dL in 5 weeks of study, while there was a significant increase in fasting blood glucose level in STZ + nicotinamide treated rat (302 ± 2.94 to 320.6 ± 3.89 mg/dL) as compared to control group. Fasting blood glucose level was measured on basal, 0, and 5th week. Different doses of AEATP (250, 500, and 1000 mg/kg) were administered continuously for 5 weeks. After 5 weeks of treatment with voglibose, glimepiride, 250, 500, 1000 mg/kg of AEATP, glimepiride + voglibose, glimepiride +250, 500 and 1000 mg/kg of AEATP shown significant reduction in elevated glucose level as compared to diabetic group, i.e. 142.2 ± 3.70, 110.6 ± 2.89, 164.6 ± 3.23, 117.2 ± 2.40, 115.8 ± 2.95, 106.2 ± 3.11, 117.2 ± 4.15, 115.2 ± 4.86 and 109.6 ± 2.07 respectively (Fig. 4).

3.9. Effect of AEATP on glycated hemoglobin (HbA1c) level

Glycated hemoglobin was significantly increased in STZ-induced diabetic group 9.94 ± 0.11% as compared to control group 4.72 ± 0.14%. After 5 weeks of treatment with voglibose, glimepiride, 250, 500, 1000 mg/kg of AEATP, glimepiride + voglibose, glimepiride +250, 500, 1000 mg/kg of AEATP shown significant attenuation in elevated glycated hemoglobin level as compared to diabetic control group (Fig. 5).
Effect of AEATP treatment (5 weeks) on GLP-1 Level, Fasting insulin Level and Blood Glucose Level at 15 min followed by Oral Glucose Tolerance Test (2 gm/kg, p.o.).

| Groups                        | Parameters               | GLP-1 Level (pMol) | Fasting insulin level (ng/mL) | Blood Glucose Level (mg/dl) |
|-------------------------------|--------------------------|--------------------|-------------------------------|----------------------------|
| Control                       | GLP-1 Level (pMol)       | 16.38 ± 0.58       | 3.57 ± 0.04                   | 149.8 ± 6.4                |
| Diabetic                      | GLP-1 Level (pMol)       | 8.92 ± 0.38***     | 0.78 ± 0.03***                | 478.8 ± 9.3***             |
| Diabetic rats + Voglibose     | GLP-1 Level (pMol)       | 13.82 ± 0.44***    | 1.23 ± 0.02***                | 206.2 ± 11.6***            |
| Diabetic rats + Glimepiride   | GLP-1 Level (pMol)       | 9.42 ± 0.31        | 3.08 ± 0.03***                | 178.8 ± 7.2***             |
| Diabetic rats + 250 mg/kg AEATP| GLP-1 Level (pMol)       | 7.78 ± 0.54        | 0.82 ± 0.02                   | 498.4 ± 9.1                |
| Diabetic rats + 500 mg/kg AEATP| GLP-1 Level (pMol)       | 9.86 ± 0.23**      | 1.19 ± 0.04**                 | 374.4 ± 6.1***             |
| Diabetic rats + 1000 mg/kg AEATP| GLP-1 Level (pMol)      | 12.92 ± 0.33***    | 3.03 ± 0.03***                | 188.8 ± 8.4***             |
| Diabetic rats + Glimepiride + Voglibose | GLP-1 Level (pMol) | 14.58 ± 0.38***   | 3.31 ± 0.04***                | 168.4 ± 9.1***             |
| Diabetic rats + Glimepiride + 250 mg/kg AEATP | GLP-1 Level (pMol) | 9.88 ± 0.33**     | 3.12 ± 0.03***                | 171.2 ± 8.2***             |
| Diabetic rats + Glimepiride + 500 mg/kg AEATP | GLP-1 Level (pMol) | 11.78 ± 0.48***   | 3.21 ± 0.06***                | 172.6 ± 7.1***             |
| Diabetic rats + Glimepiride + 1000 mg/kg AEATP | GLP-1 Level (pMol) | 14.06 ± 0.46***   | 3.39 ± 0.05***                | 168.4 ± 4.8***             |
| Diabetic rats + exendin-(9–39)-fragment + 250 mg/kg AEATP | GLP-1 Level (pMol) | 8.07 ± 0.29       | 0.80 ± 0.03                   | 471.8 ± 15.9               |
| Diabetic rats + exendin-(9–39)-fragment + 500 mg/kg AEATP | GLP-1 Level (pMol) | 8.08 ± 0.34       | 0.81 ± 0.03                   | 463.8 ± 12.4               |
| Diabetic rats + exendin-(9–39)-fragment + 1000 mg/kg AEATP | GLP-1 Level (pMol) | 8.18 ± 0.37       | 0.84 ± 0.03                   | 462.4 ± 10.0               |

*p < 0.05, **p < 0.01, ***p < 0.001.

a Versus control.

b Versus Diabetic.

Fig. 3. Effect AEATP on α-Glucosidase Activity from rat small intestine; The IC50 value of the AEATP was found 192 mg/ml.

3.10. Effect of AEATP on total cholesterol level

The present result showed that total cholesterol level in serum was significantly elevated in diabetic control group 241.2 ± 2.37 mg/dl as compared to control group 128.6 ± 2.07 mg/dl. Voglibose, glimepiride, 250, 500, 1000 mg/kg of AEATP, glimepiride + voglibose, glimepiride+250, 500, 1000 mg/kg of AEATP shown significant reduction in elevated total cholesterol level as compared to diabetic control group (Fig. S2).

3.11. Effect of AEATP on total triglycerides level

There was significant increase in total triglyceride (TG) in diabetic group when compared to control group (118.8 ± 2.35 mg/dl). The administration of Voglibose, glimepiride, 250, 500, 1000 mg/kg of AEATP, glimepiride + voglibose, glimepiride+250, 500, 1000 mg/kg of AEATP significantly attenuated diabetes induced high level of TG (Fig. S3).

3.12. Effect of AEATP on fasting plasma insulin level and pancreatic insulin content

A significant decrease was observed in fasting plasma insulin level (0.32 ± 0.01 ng/ml) and pancreatic insulin content (51.3 ± 2.8 mg/mg pancreas) in STZ-induced diabetic rats as compared to control group 0.78 ± 0.01 ng/ml and 107.9 ± 2.03 mg/mg respectively. Fasting insulin level was statistically increased after 5 weeks administration of Voglibose, glimepiride, 250, 500, 1000 mg/kg of AEATP, glimepiride + voglibose, glimepiride+250, 500, 1000 mg/kg of AEATP as compared to diabetic control (0.32 ± 0.01 ng/ml) (Table S1). Similar results were observed in pancreatic insulin content with Voglibose, glimepiride, 250, 500, 1000 mg/kg of AEATP, glimepiride + voglibose, glimepiride+250, 500, 1000 mg/kg of AEATP when compared to diabetic control group (51.3 ± 2.8 mg/mg pancreas) (Table S2).

3.13. Effect of AEATP on active GLP-1 level of rat ileum and colon

The level of active GLP-1 content was found to be significantly decreased in ileum (27.23 ± 1.08 pMol/gm vs 46.33 ± 1.00 pMol/gm) and colon (42.2 ± 0.97 pMol/gm vs 62.4 ± 1.61 pMol/gm) of diabetic animals as compared to control group after 15 min of glucose load at 5 week. The administration of Voglibose, 500, 1000 mg/kg of AEATP, glimepiride + voglibose, and glimepiride+500, 1000 mg/kg of AEATP significantly increased level of Active GLP-1 in ileum and colon i.e.41.5 ± 1.05, 34.9 ± 1.10, 39.4 ± 1.06, 40.8 ± 1.09, 35.2 ± 0.90 and 40.6 ± 0.99 pMol/gm and 56.8 ± 1.03, 51.5 ± 1.21, 58.7 ± 1.17, 57.7 ± 1.46, 52.3 ± 1.06 and 57.6 ± 1.42 pMol/gm respectively as compared to diabetic group 42.2 ± 0.97 pMol/gm (Fig. 6a & b).

3.14. Dose response curves of exendin (9–39) fragment on insulin release

Exendin (9–39) fragment inhibited glucose-induced insulin secretion and maximal inhibition achieved at 4 μg/200 gm body weight in dose dependent manner. Hence, Exendin (9–39) fragment (4 μg/200 gm body wt) was selected for oral glucose tolerance test studies (Fig. S4).

3.15. Effect of AEATP on liver histology

In normal rats, normal central vein with radiating sinusoid cords were present. There was no sinusoid congestion, swelling and necrotic cells (Fig. 7a). Whereas, in the liver of diabetic rats, changes in structure of the hepatic cells, congest was observed in central vein, fatty infiltration and cellular inflammation and distortion in hepatocytes (Fig. 7b). In glimepiride treated group, adverse changes were found to be rectified to normal architecture with reduced degenerative cells, congestion in central vein, as well as...
inflammation and fatty infiltration (Fig. 7c). Diabetic rats treated with 500 and 1000 mg of AEATP also showed similar features of normal animals like normal architecture of hepatocytes, reduced swelling, less apoptosis (Fig. 7d–f).

3.16. Effect of AEATP on pancreas histology

In control rats, pancreatic cell showed normal architecture with normal acini and islets cells. No edema and inflammation were seen in section (Fig. S5a). In diabetic rats, inflammation, disorganisation of the islets and steatosis were observed. Cell infiltration was seen in the acinar cells along with necrosis and shrinkage of islet cells (Fig. S5b). In glimepiride treated animals, pancreas showed fewer cells necrosis with mild atrophy changes (Fig. S5c). In treatment group 500 mg and 1000 mg of AEATP showed protective effect on islets of Langerhans and acinar cells as compared to diabetic rats and further reduction in edema, inflammation and shrinkage of islets (Fig. S5d–f).

4. Discussion

The main aim of the current study is to explore the role of AEATP in the secretion of active GLP-1 in blood plasma, ileum and colon accompanied by an increase of fasting insulin in diabetic rats. Like previous reports, significant increase in fasting blood glucose level was observed in STZ-NAD induced diabetic rats as compared to control group [18]. Administration of AEATP for 5 weeks resulted in significant reduction in the fasting blood glucose
level as compared to diabetic rats. It is evident from this investigation that AEATP was effective in maintaining the blood glucose levels by increasing the insulin level in plasma and pancreas in diabetic rats.

Our results showed that AEATP ameliorate elevated blood glucose, which is consistent with our previous report [4]. Moreover, present study revealed the positive correlations between antihyperglycemic effect, insulin levels in plasma and pancreas, and GLP-1 levels. This indicated that AEATP increased insulin levels and regulated glucose homeostasis via enhancing GLP-1 release. Moreover AEATP improved the insulin level in the plasma and tissue of diabetic rats. It is well-known fact that GLP-1 is one of the most potent factors for enhancement of insulin secretion, and there are some studies suggesting that GLP-1 may increase insulin secretion by improving \(\beta\)-cell function [19]. Previous studies suggested that peak insulin, blood glucose and active GLP-1 level is found at 15 min followed by glucose load [17]. Our study showed that AEATP treatment may increase active GLP-1 release in plasma and intestine. The level of active GLP-1 in blood plasma was significantly high at 15 min of OGTT in treatment group (AEATP 1000 mg/kg) after 5 weeks when compared with diabetic control and again the role of GLP-1 was reversed and confirmed by giving GLP-1 antagonist, this suggested that AEATP may improve plasma and tissue insulin via enhancing active GLP-1 release. Furthermore, after OGTT, as the plasma active GLP-1 concentration increased simultaneously there was increase in plasma insulin and decrease in blood glucose level takes place at 15 min.

Several studies have reported \(\alpha\)-glucosidase inhibitory activity of natural phytochemicals (flavonols, alkaloids, saponins and saccharides) [20]. Some polysaccharides from natural sources were reported to have inhibitory activity on \(\alpha\)-glucosidase [21], with the same line of research, our study also showed significant inhibition of \(\alpha\)-glucosidase from \(S.\) cerevisiae and rat small intestine. AEATP significantly inhibited \(\alpha\)-glucosidase which is one of the mechanisms for its antihyperglycemic activity. The inhibition of \(\alpha\)-glucosidase activity delayed the carbohydrate digestion leading to increased concentration of carbohydrate in lower small intestine (rich in GLP-1 secreting cells) [16] which further stimulated the secretion of active GLP-1 in the lower gut through osmotic action [22]. Similar results were also found to increase level of active GLP-1 in ileum and colon after 5 weeks treatment with AEATP.

Glycated hemoglobin (HbA1c) is the standard biochemical marker in assessment of diabetes. In our study, diabetic rats showed higher level of glycated hemoglobin (9.94 $\pm$ 0.11\%) indicating their poor glycemic control which is also supported by other previously reported studies [23]. Oral administration of AEATP at all the dose levels significantly reduced HbA1c to near normalcy by 5
weeks of intervention as compared to diabetic group and also analogous of our previous report on AEATP [4].

Insulin deficiency or insulin resistance may be responsible for dyslipidemia which is also seen in our study by increasing total cholesterol and triglyceride [24]. Some reports are showing beneficial interaction between dyslipidemia and α-glucoaldase i.e. Chronic consumption of mulberry juice and cake powder with α-glucosidase inhibitory activity reduced blood triglyceride and total cholesterol in STZ-induced diabetic rats [25]. It was suggested that acarbose (α-glucoaldase inhibitor) improves blood lipid profile by increasing insulin sensitivity [26]. Chronic α-glucosidase inhibitor lowers VLDL triglyceride secretion resulting in improvement of hypertriglyceridemia and hypercholesterolemia [27]. Chronic consumption of touchi with α-glucosidase inhibitory activity decreased blood triglyceride and total cholesterol in animal model of diabetes and blood triglyceride in diabetic patients [28]. Similar result of present study showed that AEATP treated group produced significant improvement in HDL level and decreased blood triglyceride and total cholesterol.

In various pathological symptoms were shown at histopathological levels in STZ induced diabetic rats like distortion of hepatic cells, congested central vein, inflammation, edema and fatty vacuolation. Previous report confirmed that changes like lymphocyte invasion, inflamed hepatic cells and dilatation of the sinusoids were seen in STZ induced diabetic rats [29]. Swollen hepatic cells with invasion of lymphocytes and characteristic fibrosis were also reported as adverse action of STZ in earlier report [30]. In our experimental protocol normal animal showed normal central vein with no congestion of sinusoids whereas STZ induced diabetic rats have shown congestion in central vein with fatty infiltration and inflammation in hepatocytes which were attenuated by standard drug and protective effects were also produced by 500 and 1000 mg of AEATP treatment. Islets structure and function alteration causes to loss of glycemic control due to decreased insulin secretion and insulin sensitivity. Various etiologies like high fat diet and cytotoxic chemicals like STZ causes atrophy and degeneration of pancreatic cells along with fat deposition, decreased β cells and amyloid deposition during diabetic condition [31]. Similar adverse effects were also found during our experimental protocol in diabetic rats like inflammation, disorganization of the islets (atrophy), steatosis [32] and these were significantly decreased by standard drugs, 250, 500 and 1000 mg dose of AEATP.

Glimepiride falls under sulfonylurea category of antidiabetic agents and when it is bound to sulfonylurea receptor (SUR) then it closes potassium ion channel. The net result is increased concentrations of intracellular potassium, which progressively depolarizes the cell. Depolarization induces voltage-dependent calcium channels and increasing calcium influx towards the beta cells which further activating cytoskeletal changes that end in degradation of insulin vesicles [33,34]. An enzyme α glucosidase hydrolyses oligosaccharides and disaccharides to glucose and other monosaccharide in the brush border of the small intestine. Voglibose comes under the category of α-glucoaldase inhibitors and allow the undigested carbohydrate to reach to distal small intestine. The anti-hyperglycaemic action of voglibose results from a reversible inhibition of membrane bound α-glucoaldase of the small intestine which further induces production of GLP-1 from the enteroendocrine L-cells of the intestine due to excess approach of undigested carbohydrates. GLP-1 is a insulinotropic hormone which is known to enhance insulin secretion and insulin sensitivity [35].

Loss of Hyperglycaemic control is again string tied by treatment with AEATP by increase the level of GLP-1 in plasma which further parallel increase the level of insulin and providing protection and improvement to β cells [19] of diabetic rats insulin level not only in plasma after glucose load but also fasting insulin level and pancreatic insulin content via increased GLP-1 level by inhibiting α-glucoaldase to attenuate dislipidemia of diabetic rats.

5. Conclusion

It can be concluded that AEATP showed antidiabetic effect by modulating GLP-1 level in plasma and intestinal tissue by inhibiting alpha glucoaldase which further increased insulin in blood plasma and pancreas.

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Conflicts of interest
None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaim.2019.06.003.

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