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Original Article

In vitro and *in vivo* study of inhibitory potentials of α-glucosidase and acetylcholinesterase and biochemical profiling of *M. charantia* in alloxan-induced diabetic rat models

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Abstract: Objectives: Diabetes mellitus is a multifactorial chronic disease that affects the human population and it is the third most common cause of death worldwide. *Momordica charantia* is used as popular folk medicine and its action against diabetes mellitus remains unclear. We investigated the inhibitory potentials of α-glucosidase, acetylcholinesterase, and biochemical profiling of *M. charantia* in alloxan-induced diabetic rat models. Methods: An *In vivo* study was carried out by using twenty male albino Wistar rats randomly divided into five groups each comprising four rats. Diabetes mellitus was induced by single intraperitoneal administration of 80 mg/kg body weight of alloxan and treatment with plant extract was conducted for a period of thirty days to check their impact on body weight and differentblood values. Biochemical profiling and characterization were performed by *in vitro assays* and HPLC, and FTIR. Histopathologic effects of *M. charantia* were examined through automated image analysis. Results were analyzed through Tukey's test, a complete randomized design and two factorial designs under CRD. Results: Methanolic extract demonstrated potent alpha-glucosidase (72.30 ± 1.17%) and acetylcholinesterase (50.12 ± 0.82%) inhibitory activities. HPLC analysis confirmed the existence of vital flavonoids, antioxidants, and saponins. FTIR revealed the presence of hydroxyl groups, esters, alkanes, alkenes, alkynes, ketones, alcohols, amines and carboxylic acids as major functional groups. Results of *in vivo* study demonstrated that co-administration of alloxan and methanolic extract of *M. charantia* significantly improved the levels of fasting blood glucose, glycated hemoglobin and insulin in diabetic rats. Conclusion: *M. charantia* can be recommended as a therapeutic adjunct for diabetic patients as it can provide favorable remedial action in the context of the diabetes continuum of metabolic syndrome.

Keywords: α-glucosidase, acetylcholinesterase, alloxan-induced diabetic rat models, enzyme inhibition, HPLC

Introduction

Diabetes mellitus (DM) is the most common metabolic syndrome that affects millions of individuals every year and is a global health problem. DM has become the third leading cause of death in the world. Globally, the prevalence of DM in the year 2019 is estimated about 9.3% [1]. About five percent of deaths are caused by DM all over the world and this number rise by 50% in the next ten years. Different factors increase the risk of developing metabolic complications. These include micro-vascular and macro-vascular syndromes, neuropathy, disturbances in cellular physiology, nephropathy, hepatopathy and Alzheimer's disease (AD) [2]. Different therapeutic options are available for the treatment of DM. Among the treatment strategies, for DM inhibition of key enzymes (alpha-glucosidase and cholinesterase) is the most common strategy for targeting and controlling the high rate of DM [3]. Although different synthetic inhibitors avert activities of AChE and α-glucosidase, few studies have warned about extreme side effects instance such as hepatotoxicity, tremors, weight loss, vomiting, and diarrhea [4, 5].

Acarbose, miglitol, and voglibose are alpha-glucosidase inhibitors that prevent the release of

glucose in the small intestine, hence reducing postprandial hyperglycemia. Alpha-glucosidase inhibitors are linked to the gastrointestinal problems in addition to their positive benefits. Similarly, glucagon-like peptide-1 (GLP-1) is an incretin hormone that regulates the blood sugar levels in the stomach [6]. This hormone is promptly inactivated by the enzyme, dipeptidyl peptidase-IV (DPP-IV), which is also found in the gut. Inhibition of the DPP-IV enzyme for the treatment of diabetes mellitus is a common strategy that has the potential to alter glucose levels through numerous mechanisms [7]. Furthermore, under circumstances of chronic food excess, beta amyloid precursor protein (APP) cleaving enzyme (BACE1) level plays a significant role in glucose and lipid homeostasis. BACE1 is involved in the regulation of the insulin receptor in the liver. Hence, considering BACE1 as a therapeutic target, efforts to discover and develop BACE1 inhibitors have been pursued in the last few years [8].

Momordica charantia L. is a member of the family Cucurbitaceae and is generally known as balsam pear, bitter gourd, karela and bitter melon [9]. It is frequently used as traditional medicine for the management of numerous syndromes *viz.* diabetes mellitus, and possesses anti-cancer, anti-inflammatory, anti-obesity, antiamnesic, antioxidant, antiulcer, tyrosine phosphatase 1B inhibition and cytotoxic potentials [10]. *M. charantia* is rich in constituents such as alkaloids, glycosides, polypeptides, resins, fatty acids, saponins, polysaccharides, phenolic and flavonoids. It is also a rich source of cucurbitacins, momorcharins, momordicin, cucurbitins, charantine, momordin, and choline [11]. *M. charantia* has laid the foundation for traditional systems of medicine which have been used for controlling various metabolic syndromes in existence for thousands of years. According to the WHO, about 80% population is dependent on herbal medicines as their prime health care resource. Before the invention of modern medicine, numerous plant herbs, trees, and shrubs have been generally used as traditional medicine i.e. in Unani, Chinese and Ayurvedic systems or in the crude form and are still extensively used for the treatment of common syndromes [12]. Alkaloids, carbohydrates, flavonoids, saponins, vitamins, terpenoids, minerals, coumarins, tannins, glycosides, inorganic and phenolic compounds enhance the pharmacologic efficacy of *M. charantia* [3].

In view of the above, an *in vivo* and *in vitro* study was conducted to investigate the antidiabetic (alpha-glucosidase inhibition) and antiamnesic (acetylcholinesterase inhibition) efficacies of different fractionations of *M. charantia* along with biochemical profiling, fasting blood glucose, glycated hemoglobin, and insulin in alloxan-induced diabetic rat models.

Materials and methods

Reagents, chemicals and kits

Alloxan monohydrate was obtained from Sigma Aldrich, United States (USA). Kits for estimation of glucose were bought from the Diasys Diagnostic Systems GmbH, Germany and for HbA1c were procured from BioHerms, United Kingdom (UK). An automatic analyzer (Siemens, USA) was used for the analysis of biochemical values.

Collection and identification

This research was carried out at the Clinico-Molecular Biochemistry Laboratory, Department of Biochemistry, University of Agriculture Faisalabad (UAF), Pakistan. *Momordica charantia* L. was collected from Faisalabad's local market and was identified and verified by the Department of Botany, University of Agriculture Faisalabad (UAF), Pakistan.

Preparation and fractionation of extracts

Plant material was dried and ground into a fine powder after washing with the tap water and then preserved in airtight containers for further usage. The extract was prepared by keeping 100 g powder in 1 L solvent i.e. methanol (CH_3OH) at room temperature for the interval of seventy-two hours. After filtration with Whatman number 1 filter paper, the filtrate was dried completely in a water bath. Later, distilled water was utilized to dissolve the extract and a separatory funnel was used for fractionation of a few polarity-based solvents extracts *viz.* ethyl acetate, chloroform, *n*-hexane, methanol, *n*-butanol and ethanol along with water at 1:10:10 ratio (extract: distilled water: solvent) [13]. The study flow chart is shown in **Figure 1**.

In vitro enzyme inhibition assays

Alpha-glucosidase inhibitory assay: Inhibitory potential of α-glucosidase was conducted by the spectrophotometric method. Briefly, an enzyme solution (500 µL) along with 100 µL of

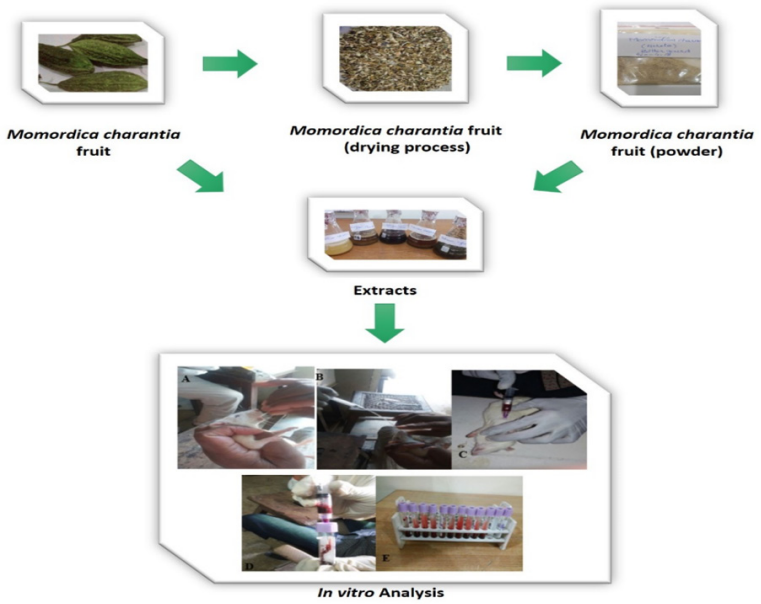

Figure 1. Study flow chart and animals for *in vitro* analysis.

plant extract was incubated for ten minutes at 25°C. About, 500 µL of substrate solution was added and again kept at room temperature for five minutes. The absorbance of all samples was recorded using a spectrophotometer at 405 nm. Extraction solvent was used to replace the extract in the negative control sample, whereas acarbose was used as a positive control [14]. Percentage inhibition was calculated by the following formula:

$$\text{Percentage Inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Acetylcholinesterase inhibitory assay: The inhibitory assay for acetylcholinesterase was accomplished by the colorimetric method. Briefly, plant extracts (30 µL) + Ellman's reagent (100 µL) + phosphate buffer (2.8 mL) + enzyme solution (30 µL) were mixed and incubated for 10 minutes at 25°C. Then (30 µL) substrate solution was added. Absorbance was measured by a spectrophotometer at 412 nm. Extraction solvent was used to replace the extract and was used as a negative control, whereas amigra (physostigmine) was used as a positive control [15]. Percentage inhibition was assessed as follows:

$$\text{Percentage Inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

In vivo study

Male albino Wistar rats were selected as model animals. Healthy rats were placed in the animal housing of the National Institute of Food Science and Technology (NIFSAT), UAF, Pakistan. Rats weighing (200-300 g) were selected for experimentation and were housed in stainless steel cages with layers of husk and given a pellet diet *adlibitum*. Prior to the beginning of a trial, animals were acclimatized for 1 week.

Experimental design

The experimental design of the *in vivo* study is shown in **Figure 2**.

Rats were randomly grouped into five categories (four animals/category). Animals were treated once a day for 30 days.

Group 1: Normal control animals fed with a normal diet.

Group 2: Alloxan induced diabetic control animals received a normal diet.

Group 3: Diabetic animals fed with glibenclamide.

Group 4: Normal animals fed with methanolic *Momordica charantia* extract (200 mg/kg body weight/rat).

Group 5: Diabetic animals treated with methanolic *Momordica charantia* extract (200 mg/kg body weight/rat).

Induction of diabetes

DM was induced in overnight-fasted rats by freshly prepared single intraperitoneal injection of alloxan (80 mg/kg body weight) dissolved in the normal saline. The transient hypoglycemia or mortality was averted by administration of a solution of 1% glucose after injection, as alloxan selectively destroys the beta cells. Glucometer was used to estimate the level of fasting blood glucose after 72 hours by the tail tipping method and rats having fasting blood

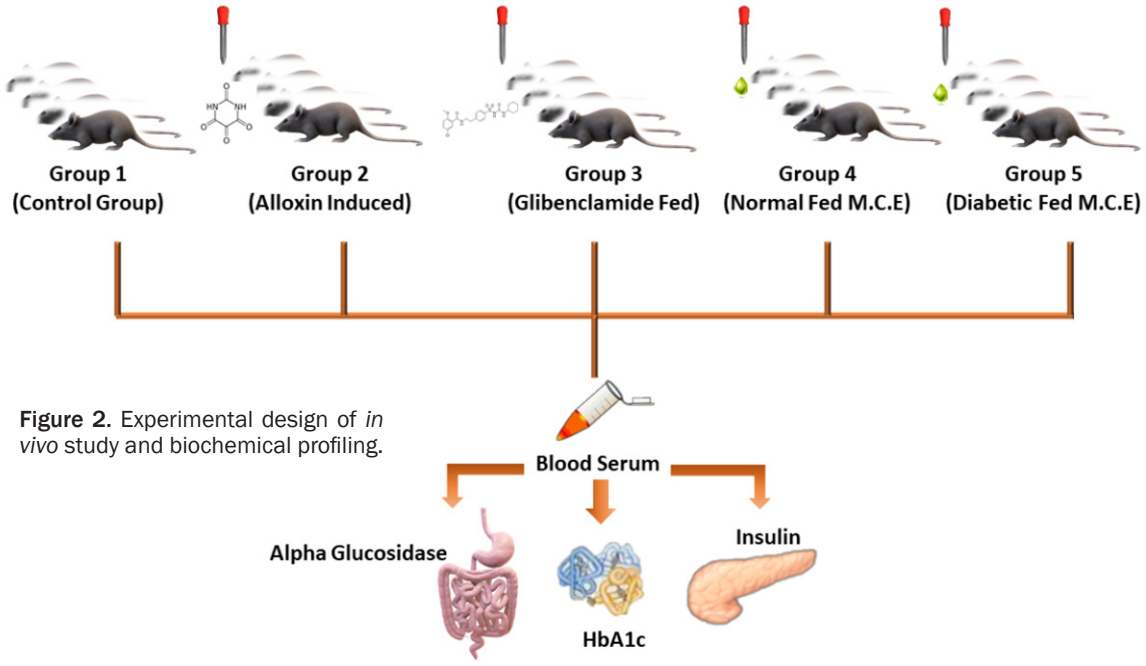

Figure 2. Experimental design of *in vivo* study and biochemical profiling.

glucose (FBG) of more than 250 mg/dL were assumed to be diabetic.

Extract administration

A force-feeding gastric gavage route was used for oral administration of methanolic extract of *Momordica charantia* and a syringe connected to an oral feeding tube was used to execute this action. Blood samples were collected into gel activators and EDTA (ethylenediaminetetraacetic acid) tubes at the start of the clinical trial and at 5 days intervals until the end of the treatment period.

Serum biochemistry

Centrifugation was performed at 3000 rpm and we separated the serum from red cells. Plasma was tested for following antidiabetic potential i.e. glycated hemoglobin, plasma glucose, and insulin. Commercial kits were used to estimate plasma glucose and glycated hemoglobin (HbA1c) respectively [16, 17]. Serum insulin was estimated by the chemiluminescent immune method [18]. Levels of glycated hemoglobin, glucose, and insulin were expressed as %, mg/dL and U/L respectively.

Histopathologic studies

On the 30th day, the last day of the trial, all animals were killed, and their liver and pancreas were taken out for histopathologic analysis. Removed livers and pancreas had blood blotted out and then were instantly put in a 10% buffered solution of formalin [1, 2].

Microscopic quantitative analysis by histopathology

Microscopic slides of the liver and pancreas of all normal and diabetic rats were further quantitatively analyzed at a 10X objective to measure the diameter of the central vein and the area and diameter of pancreatic islets of Langerhans respectively using an automated image analysis system image J® [19, 20].

Biochemical characterization

HPLC and FTIR were the techniques used for the biochemical characterization of different fragments of *M. charantia.*

HPLC analysis

Plant extract for HPLC analysis was prepared by previously reported protocol [21]. Extract of *M. charantia* (50 mg) was weighed and dissolved in methanol (24 mL) and further dilution was performed by addition of 10 mL of 6M HCl and 16 mL of dH_2O, then it was placed in an oven for 120 minutes at 95°C. Filter paper (0.45 µm) was used to filter this solution prior to the analy-

Table 1. Enzyme inhibition assays with different fractions

No.	Extracts/ Fractionations	α-glucosidase inhibition	Acetylcholinesterase inhibition
1	Water	50.44 ± 0.72[g]	1.03 ± 0.09[h]
2	Chloroform	61.00 ± 0.94[e]	12.19 ± 0.23[e]
3	Methanol	72.30 ± 1.17[b]	50.12 ± 0.82[b]
4	Ethyl acetate	68.46 ± 1.19[c]	5.18 ± 0.11[g]
5	*n*-butanol	64.51 ± 1.04[d]	17.29 ± 0.10[d]
6	Ethanol	45.48 ± 0.48[h]	32.14 ± 0.64[c]
7	*n*-hexane	56.35 ± 0.98[f]	9.03 ± 1.18[f]
8	Acarbose	79.91 ± 0.77[a]	Nil
9	Amigra	Nil	81.52 ± 1.01[a]

Small letters represent the overall mean and comparison among interaction means respectively.

sis of HPLC. Analysis was performed on the RP-HPLC which was equipped with the UV-Vis detector.

FTIR analysis

The powder form of the plant sample was used for the assessment of the different functional groups by the Agilent Cary 630 FTIR system. The range of IR recorded for studied medicinal plants was 650-4000 cm^{-1}, with scans and resolution of 20 and 4 cm^{-1} respectively [22].

Statistical analysis

Results were documented as mean ± SE. Data were subjected to statistical analysis by complete randomized design and two factorial designs under CRD using statistical 8.1 software. Furthermore, Tukey's test was applied to measure the significance level among experimental groups.

Results

Efficacy of enzymatic inhibition

Antidiabetic (alpha-glucosidase) and antiamnesic (acetylcholinesterase) efficacies of *M. charantia* checked are presented in **Table 1**. All the fractionations/extracts had variable inhibitory effects on both enzymes. High alpha-glucosidase inhibition was exhibited by methanolic extract (72.30 ± 1.17%) followed by ethyl acetate (68.46 ± 1.19%), *n*-butanol (64.51 ± 1.04%), chloroform (61.00 ± 0.94%), *n*-hexane (56.35 ± 0.98%) and water (50.44 ± 0.72%), while lowest inhibition was demonstrated by ethanolic extract (45.48 ± 0.48%). Acarbose, the synthetic inhibitor, showed 79.91 ± 0.77% inhibitory potential. Regarding antiamnesic potential, minimum and maximum restrictive activities were revealed by water extract (1.03 ± 0.09%) and methanol (50.12 ± 0.82%) respectively, while the results of other fractionations were in the range between 5.18 ± 0.11% to 32.14 ± 0.64%. Physostigmine (amigra) showed a percentage inhibition of 81.52 ± 1.01%.

In vivo study

Body weight: **Table 2** shows that the mean body weight of alloxanized rats significantly ($P < 0.01$) declined after injection of alloxan. Treatment of diabetic rats with *M. charantia* extract stabilized the body weight and a significant ($P < 0.01$) increase in body weight was noticed in comparison to rats in the diabetic control group; however, a non-significant difference was noticed in comparison to rats of the normal control group.

Antidiabetic potential: In this study, normal values of HbA1c, insulin, and FBG were exhibited in normal groups, as these parameters did not change during the entire experimental period. Results showed non-significant ($P > 0.05$) differences in overall means of FBG, insulin, and HbA1c levels between normal treated and normal control groups. However, after toxification with alloxan monohydrate, rats of diabetic groups had greater levels of FBG, HbA1c and reduced serum insulin relative to these values in normal rats (**Table 3**). In treatment groups, mean FBG and HbA1c levels were elevated, while insulin levels declined in the diabetic control group. Administration of glibenclamide and *M. charantia* significantly ($P \leq 0.01$) recovered their levels of mean FBG, HbA1c, and insulin as compared to the untreated diabetic group. Significant alterations ($P \leq 0.01$) in terms of normalization of FBG, HbA1c, and insulin concentrations were observed in the *M. charantia* treated diabetic group as compared to the diabetic control group. This group also demonstrated significant differences in levels of FBG and HbA1c as compared to the standard drug-treated group. Results also determined that overall mean serum insulin level did not vary significantly (based on $P \leq 0.01$) between the glibenclamide group and the diabetic treated group.

Table 2. Impact of methanolic extract of *M. charantia* on body weight (grams) of the diabetic and normal rats at different days

Group	Time interval (days)						
	0	5	10	15	20	25	30
Normal control	227.75 ± 0.63*	232.00 ± 1.47**	235.00 ± 1.78**	244.00 ± 2.61**	252.50 ± 2.90**	258.50 ± 3.59**	267.00 ± 3.42**
Diabetic control	185.75 ± 1.25*	180.00 ± 1.47**	176.75 ± 1.65**	170.25 ± 0.63**	164.75 ± 1.55**	157.00 ± 1.47**	152.00 ± 1.08**
Glibenclamide	186.75 ± 1.38*	193.25 ± 1.93**	199.75 ± 2.32**	208.25 ± 1.38**	215.50 ± 1.04**	221.50 ± 1.85**	228.50 ± 1.85**
Normal + *Momordica charantia*	227.50 ± 2.78ns	232.00 ± 2.42ns	237.00 ± 2.86**	241.75 ± 1.89**	249.50 ± 1.94**	254.00 ± 2.35**	257.25 ± 1.80**
Diabetic + *Momordicachr*	189.00 ± 2.45ns	196.00 ± 2.65*	201.50 ± 2.60**	206.50 ± 2.22**	214.00 ± 2.35**	221.50 ± 2.33**	228.00 ± 2.42**

All values are expressed as mean ± SE (standard error). * = Significant variation as compared to week zero ($P < 0.05$). ** = Significant variation as compared to week zero ($P < 0.01$). NS = Non-significant variation as compared to week zero ($P > 0.05$).

Table 3. Impact of methanolic extract of *Momordica charantia* on fasting blood glucose (mg/dL), insulin (U/L), and HbA1c (%) levels of diabetic and normal rats

Sr No.	Groups	Values		
Events	Control/Treated	Fasting blood glucose (mg/dL)	Insulin (U/L)	HbA1c (%)
1	Normal control	84.25 ± 1.45D	26.11 ± 0.39A	4.95 ± 0.06D
2	Diabetic control	380.83 ± 9.78A	6.97 ± 0.47C	11.17 ± 0.42A
3	Glibenclamide	247.33 ± 21.24C	13.11 ± 1.44B	8.06 ± 0.32C
4	Normal + *M. charantia*	76.58 ± 1.21D	26.24 ± 0.32A	4.36 ± 0.10E
5	Diabetic + *M. charantia*	291.92 ± 10.32B	12.41 ± 1.20B	8.58 ± 0.21B

Data is represented as mean ± S.E. Means sharing similar letters in a column are statistically non-significant ($P > 0.05$). Capital letters represent the overall mean and comparison among interaction of means respectively.

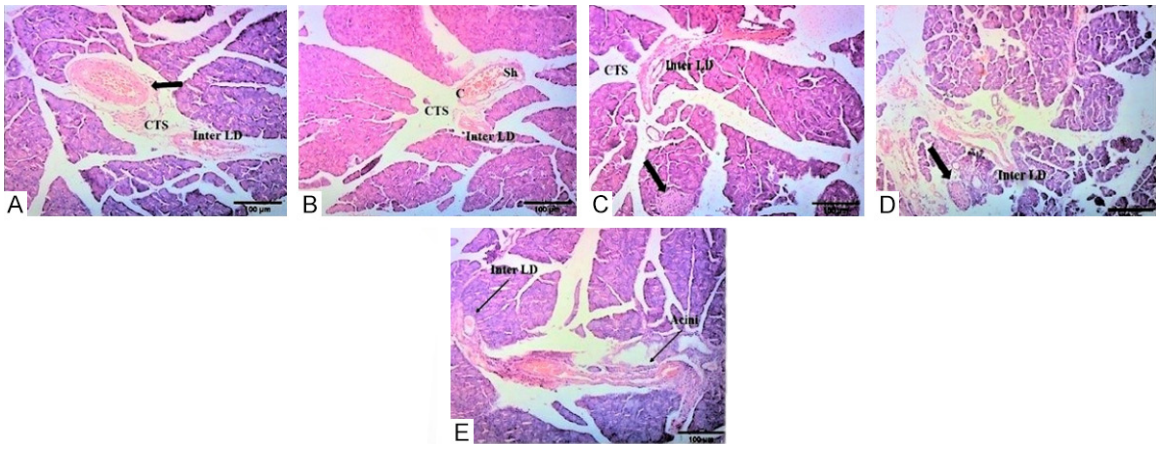

Figure 3. (A) Microphotographs (10X) displaying histopathologic alterations in pancreatic sections from normal control, (B) diabetic untreated, (C) glibenclamide treated, (D) medicinal plant treated (*M. charantia*) alloxanized rats, and (E) medicinal plant treated (*M. charantia)* normoglycemic groups. Arrow: cells of Islets of Langerhans; CTS: connective tissue septum; Sh: shrinkage; C: congestion; Inter LD: interlobular duct.

Histopathologic assessment

Histopathologic assessment of pancreas and liver of experimental animals was done and photomicrographs (**Figures 3** and **4**) that were obtained at 10X are described below.

Histopathologic examination of the pancreas

Photomicrographs of the normal control group and normal treatment groups indicated that pancreatic islets of Langerhans and circumscribed masses surrounded by the deeply-stained exocrine acini appeared normal. Islets appeared more lightly stained than the surrounding cells of acini. Prominent nuclei, normal interlobular duct, and connective tissue septa (CTS) were also seen (**Figure 3A** and **3E**).

Photomicrographs of untreated diabetic rats displayed a breakdown of micro-anatomical features, i.e. necrosis and degeneration. Destruction in islets of Langerhans cells with irregular shape and atrophy was noticed. Shrunken islet cells, hypo-cellularity, and congestion were also observed. CTS disappeared and interlobular duct lumen was also reduced (**Figure 3B**).

However, in contrast, the drug-treated group revealed that pancreatic islets had a less or more normal population of cells with the absence of degenerative changes as noticed in the positive control group. Islets of Langerhans along with interlobular ducts appeared to be normalized and CTS also partially reappeared after drug treatment (**Figure 3C**).

Pancreatic sections from the diabetic rats treated with selected medicinal plants demonstrated regenerated islets of Langerhans with normocellularity and reduced degenerative alterations as seen in the alloxanized untreated group (**Figure 3D**). Such noticeable reduction in the pancreatic injuries and enhanced restoration of impaired beta cells might be due to the suppression of further impairment to the pancreas and this is attributed to phytoconstituents in the studied plants.

Histopathologic examination of liver

Microscopic investigation of the liver of normal control (**Figure 4A**) and normally treated (**Figure 4E**) groups of rats demonstrated the normal appearance of radially arranged hepatocytic cells around the central vein. Hepatocytes have acidophilic cytoplasm and rounded central vesicular euchromatic nuclei with well-defined nucleoli. The hepatic cells are separated by thin-walled blood sinusoids lined by flat endothelial cells. Occasionally, von Kupffer cells are also observed.

Rats in the untreated diabetic group, in contrast, showed hepatocellular injury, with apoptotic hepatocytes with loss of normal architecture, congested blood vessels, congestion and dilation in the central vein, hyperplasia, and existence of pyknotic nuclei and activated

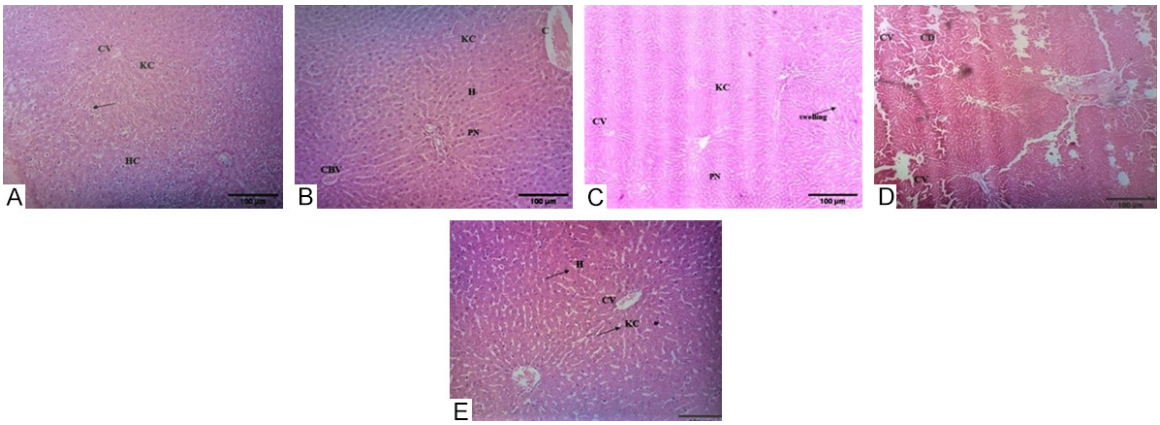

Figure 4. (A) Microphotographs (10X) showing histopathologic alterations in the hepatic sections from normal control, (B) diabetic control, (C) glibenclamide treated, (D) medicinal plant treated (*M. charantia*) alloxanized rats, and (E) medicinal plant treated (*M. charantia*) normoglycemic groups. KC: Kupffer cells; CV: central vein; PN: pyknotic nuclei; H: hyperplasia; C: congestion; HC: hepatic cells; CBV: congested blood vessel.

Table 4. Quantitative analysis of all experimental groups reflecting diameter and area of pancreatic islets

Group	Pancreas		Liver
	Area of IL of pancreas (μm^2)	Diameter of IL of pancreas (μm)	Diameter of central vein
	Mean ± S.E	Mean ± S.E	Mean ± S.E
Normal Control	2981.8 ± 2.15^c	51.34 ± 1.02^c	40.27 ± 0.81^a
Diabetic control	1274.82 ± 16.6^e	33.83 ±0.51^d	10.45 ± 0.142^d
Glibenclamide	4550.75 ± 19.26^a	71.1 ± 2.41^a	35.32 ± 1.26^b
D + MC	3533.15 ± 23.15^b	60.24 ± 1.59^b	26.74 ± 0.133^c
N + MC	2840.2 ± 19.24^d	51.05 ± 0.65^c	40.46 ± 1.209^a

All values are presented as mean ± SE (n = 3). Means within a column with different letters are significant at P < 0.05. Small letters represent the overall mean and comparison among interaction means respectively.

Kupffer cells (**Figure 4B**). Hepatocytes of diabetic rats treated with glibenclamide presented nearly normal radially arranged hepatic cells around the central vein, blood sinusoidal spaces, as well as Kupffer cells, which were just like the untreated normal group (**Figure 4C**).

Meanwhile, liver sections of the alloxanized medicinal plants treated groups of rats demonstrated almost mild to moderate dilation in the central vein and improvement in hepatic triad structure in comparison to the diabetic control group. Kupffer cells lining sinusoidal spaces were also evident (**Figure 4D**).

Quantitative analysis of histopathology

Morphometric analysis of the pancreas is demonstrated in **Table 4** and confirms histologic findings and furthermore reflects the ameliorator effect of the studied medicinal plants on the area and diameter of pancreatic islets. It indicated a non-significant difference between the normal control and normally treated groups of rats in terms of mean diameter and mean area of islets of Langerhans. On the other hand, the diabetic control group revealed a significant reduction in mean area and mean diameter as compared to the normal control group. These reductions were significantly improved upon treatment with different studied medicinal plants. Among all the diabetic treated groups, the combination-treated group demonstrated non-significance as well as significant results in comparison to the positive-treated group in regard to diameter and areas of pancreatic islets respectively.

Quantitative analysis of the central vein of the liver was also done and the results are presented in **Table 4**. The area of the central vein of hepatic tissues is significantly decreased in

Table 5. Biochemical profiling of *Momordica charantia* by HPLC

No.	Compound name	Retention time	Peak area	Amount (ppm)
1	Quercetin	3.067	473.825	25.11
2	Caffeic acid	12.600	60.327	2.77
3	Vanillic acid	13.093	88.189	5.45
4	Benzoic acid	14.813	163.052	17.27
5	Chlorogenic acid	15.673	184.321	14.37
6	p-Coumaric acid	17.713	120.048	1.56
7	m-Coumaric acid	19.907	258.264	3.19
8	Ferulic acid	22.153	78.978	5.68
9	Cinnamic acid	25.307	86.636	3.13
10	Sinapic acid	26.207	103.159	1.34

diabetic rats. A significant association was seen in diabetic control in comparison to diabetic treated and normally treated rat groups. Glibenclamide and *M. charantia* treated groups exhibited non-significance.

Biochemical characterization

Quantification of flavonoids and phenolic acids was performed by HPLC analysis. Infrared spectrometry (IR) was used for recognition of both inorganic and organic constituents.

HPLC analysis

HPLC fingerprints of *Momordica charantia* are mentioned in **Table 5** and **Figure 5**. HPLC chromatograms revealed the presence of quercetin (25.11 ppm), caffeic acid (2.77 ppm), vanillic acid (5.45 ppm), benzoic acid (17.27 ppm), chlorogenic acid (14.37 ppm), p-coumaric acid (1.56 ppm), m-coumaric acid (3.19 ppm), ferulic acid (5.68 ppm), cinnamic acid (3.13 ppm) and sinapic acid (1.34 ppm).

FTIR analysis

FTIR fingerprinting is summarized in **Table 6** and the chromatogram is shown in **Figure 6**. A band at a range of 2918.5 cm^{-1} indicated CH_2 stretching vibration, mainly lipids; however, such a peak might be due to stretching of $O\text{-}CH_3$ caused by methyl ester groups. The band at 1735.1 cm^{-1} was the characteristic feature of saturated aliphatic ester. The occurrence of a band at 1636.3 cm^{-1} was designated to alkenes, unesterified carboxylate (COO^-) ions and primary amines. Phosphate ion stretching, amine structure because of their carbon and nitrogen (C-N) bonding capabilities, sulfoxides and secondary alcohols were detected at 1013.8 cm^{-1}.

Discussion

Intestinal epithelium secretes the α-glucosidase that is responsible for the converting of complex polysaccharides into monosaccharides by the cleavage of the alpha (1-4) glycosidic linkage. These border brush alpha glucosidases may be maltase-glucoamylase, sucrase-isomaltase, trehalase and lactase [18].

In the current study, *Momordica charantia* indicated more than 50% alpha-glucosidase inhibitory potential (**Table 1**). The terpenoid rich extract of bitter gourd had the highest IC_{50} value (1.60 mg/mL) against glucosidase enzyme [23]. So, triterpenoids present in *M. charantia* are responsible for antidiabetic potential. Earlier studies also documented moderate antidiabetic efficacy in terms of inhibition of alpha-glucosidase in crude extracts as well as purified constituents from *M. charantia* [24].

Although acarbose and amigra (positive controls) exhibited maximum alpha-glucosidase and acetylcholinesterase inhibition i.e. 79.91 ± 0.77% and 81.52 ± 1.01% respectively (**Table 1**), the side effects of these as well as other antidiabetic and antiamnesic drugs argue in favor of using medicinal plants as safer natural substitutes. One investigation has reported 82% alpha-glucosidase inhibitory efficacy of acarbose, such results can be justified by the variations in experimental layout [25]. Another study also stated that a direct relationship exists between a plant's phenolic or flavonoid components and their ability to block or decrease α-glucosidase activities. It is believed that phenolic components and quercetin as polar compounds are responsible for the inhibition of alpha-glucosidase and hence participate in the management of hyperglycemia [26].

Neurodegenerative diseases are caused by a deficiency in acetylcholine. Acetylcholinesterase (AChE) blocks the action of neurotransmitters by hydrolyzing it into acetic acid and choline and influences cholinergic dysfunction coupled with AD, ultimately activity of enzymes is raised in patients of AD as it is the regulator of cholinergic neurotransmission. Hence, AChE is targeted for AD therapy. Persistent hyperglycemia interacts with receptors of acetylcholine,

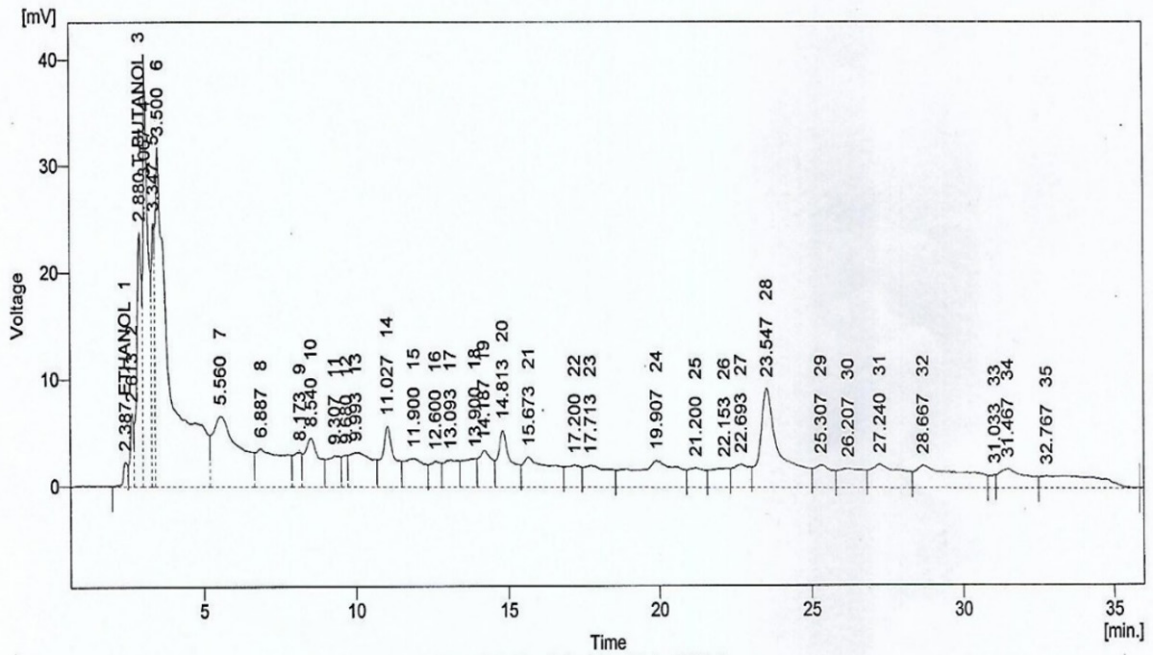

Figure 5. Representative HPLC chromatogram of *Momordica charantia.*

Table 6. Biochemical profiling of *Momordica charantia* by FTIR analysis

No.	Wave number (cm^{-1})	Functional group	Type of bond
1	2918.5	Alkane	=CH2 stretching
2	2849.5	Alkane	C-H stretching
3	1735.1	C=O, RCOOR	Carbonyl group, saturated aliphatic ester
4	1636.3	Alkene, primary amines, COO^-	C=C stretching, N-H bending, unesterified carboxylate ion
5	1395.9	Alkane	C-H stretching
6	1013.8	Amine, Phosphate ion, sulfoxide, secondary alcohol	C-N, PO_4^{3-} stretch, S=O, O-H

and affects binding affinities, leading to a rise in acetylcholinesterase and decomposition of neurotransmitters [27].

A previous study stated 77.6% AChE inhibition by *Momordica charantia* [28]. Furthermore, an earlier study declared terpenoids as effective AChE inhibitors and Kuguacin J is among those terpenoids isolated from leaves of *M. charantia* believed to possess efficacy against AChE [29, 30].

The outcome of our research work verified the therapeutic role of the tested plant in neurodegenerative diseases and the underlying mechanism is proposed by several studies. Previous studies also suggested that phytoconstituents exhibit synergism during inhibition of AChE and the mechanism of enzyme action is reinforced by molecular docking simulations [31]. Furthermore, secondary metabolites of plants either interfere with the substrate binding step by shielding the active site or interfere with the release of product [32]. However, further study in this regard is required.

According to another study, chemical profiling is a preliminary step in standardization as well as optimization of natural products. Currently, numerous techniques ranging from thin-layer chromatography (TLC) to column chromatography *viz.* HPLC are used for fingerprinting secondary metabolites of plant origin. These techniques provide a platform for comprehensive structural elucidation and quantification of phytoconstituents [33].

Generally, *M. charantia* is a rich source of flavonoids, lignans, stilbenes and phenolic acids and among them, flavonoids and phenolic acids

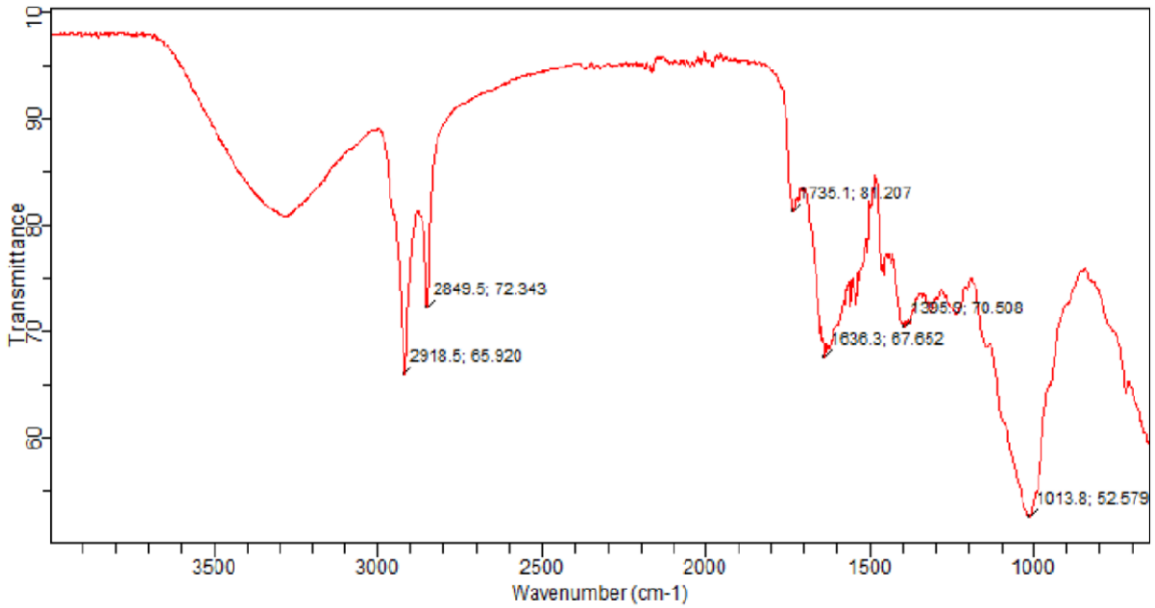

Figure 6. Representative FTIR spectrum of *Momordica charantia.*

have the strongest antioxidant potential. In the last few years, active constituents like ferulic acid, rosmarinic acids, chlorogenic acid and cinnamic acid are widely used in the cosmetics, food and pharmaceutical industries [34].

Determination of body weight in DM reflects the physiologic state of any individual or experimental animal, and variations in it are correlated with abnormal metabolism [16]. In the current study, the mean body weight of normal treated rats was gradually increased up to 30 days (**Table 2**) and such a result is in accordance with previous studies [35]. This weight gain may be due to the contents of plant extracts. Plant-based amino acids, vitamins, antioxidants and other secondary metabolites can work as growth enhancers and metabolic promoters. Contrary to this, previous studies reported that plant extracts administered to normal rats exhibited no change in body weight [36]. However, the expected decline in body weight after injection of alloxan may be due to the enhancement of hyperglycemia (**Table 2**). These findings are consistent with the results of various studies [37, 38] but disagree with other studies [39]. Due to defective cellular uptake of glucose, the blood glucose level is raised, and cells are forced to utilize the fatty acids and amino acids as an alternate energy source which ultimately leads to the degradation of tissue proteins and stored fats. Carbohydrates' inaccessibility and degradation of body proteins, as well as fats, instigates a reduction in body weight [35].

However, once a day administration of the studied plant for one month produced a significant increase in body weight. Present outcomes disagree with the results of another study that *M. charantia* extract reduced the body weight [40]. Overall mean body weight of the D + MC group showed a non-significant increase when compared to the glibenclamide group. A study also demonstrated that a rise in the weight of alloxan-induced rats confirms the antidiabetic efficacy of plants [41].

Generally, elevation in blood glucose levels during diabetes has been consistently described in both human and animal models. Insulin is one of the major anabolic hormones that elicit metabolic effects in the whole body. Therefore, any irregularity in its synthesis or secretion can cause vast physiological abnormalities [35]. The present research confirmed that elevation of FBG in alloxan-induced diabetic rats was caused by hypoinsulinemia (**Table 3**). Diminished or extra release of insulin cause hyperglycemia and hypoglycemia respectively, as secretion of glucagon from alpha cells of the pancreas is suppressed by insulin [42].

The liver and kidney are the main organs for the clearance of insulin, with the former participating to a much greater extent than the latter in

eliminating endogenously released hormones. Peripheral insulin clearance is done by extrahepatic target cells, adipocytes, and skeletal muscles [43]. Soon after its secretion from beta cells, insulin is transported quickly to hepatocytes by the portal vein. The half-life of insulin is approximately 3-5 minutes in the portal circulation, and almost 80% of the secreted insulin is cleared during the first passage through the liver. In the case of diabetes, an excessive amount of glucose reacts with the hemoglobin and results in the formation of HbA1c [44].

The results of the current study are in agreement with the previous study which showed a significant decline in the value of insulin as well as a rise in glucose in diabetic rats, while normal levels of these values are also maintained by plant samples in normal groups of rats [35]. A prominent difference in concentration of glucose was seen in diabetic and normal control rats. Glucose-decreasing potential of plants, as observed in the present study might be attributed to bioactive constituents which boost utilization as well as inhibition of glucose synthesis by elevating the ratio of $NADP^+$/NADPH, the outcome of which is a decline in the level of blood glucose in hyperglycemic rats [45].

A previous investigation suggested that the use of the methanolic extract of *M. charantia* fruit lowered the blood glucose level in both the diabetic and normal animal models [45]. Earlier, it was documented that administration of *M. charantia* powder to diabetic rats increased their insulin levels [46]. The current study inferences are also supported by a previous study that gave the *M. charantia* methanolic extract to rats, which resulted in enhancement and fall of insulin and glucose levels respectively, effects analogous to glibenclamide [44, 46]. Pathways of insulin signaling and maintenance of blood glucose are also regulated by a peptide, trypsin inhibitor from *M. charantia* [37].

The overall results of our study revealed a higher quantity of biologically active flavonoids and phenolic acids. These constituents may act singly or in combination as antioxidant agents by the use of numerous mechanisms. Flavonoids influence pleiotropic mechanisms to diminish complications of DM, hence acting either as insulin mimetics or insulin secretagogues. Sinapic acid is extensively distributed in the kingdom Plantae and various sources of this phytochemical are vegetables, spices, cereals and fruits [47]. This compound possess antihyperglycemic as well as cardioprotective [48], nephroprotective [49], anti-inflammatory, neuroprotective [50], and hepatoprotective [51] potentials.

The current study reinforced the earlier findings that extensive harm to β-cells of the pancreatic islets is caused by intraperitoneal injection of alloxan which has deleterious effects on permeability, insulin secretion, transport, and intracellular energy generation [52]. Due to persistent hyperglycemia, diabetic rats demonstrated greater oxidative stress (OS) which in turn caused depletion of the antioxidant defence system and endorsed *de novo* production of the free radicals.

Earlier studies revealed severe alterations in the pancreatic tissues of the diabetic control group, while degeneration and mild size reduction were seen in diabetic treated groups of rats [20, 35]. In the positive (+) group, the pancreas showed prominent changes such as the reduced size of islets of Langerhans and necrosis of β-cells and such findings (**Figure 3B**) were in accordance with the prior studies that also noticed the similar changes in the alloxanized untreated group.

In a previous study, administration of acetone alcoholic extract of *Momordica charantia* fruit for the period of one month recovered the beta cells [53]. This plant also elevated the number of beta cells which ultimately resulted in increased production of insulin. Earlier, another study found that the number of beta cells is increased by *Momordica charantia,* but the mechanism has not been confirmed [6, 7]. Such findings are also in support of the current research where prominent reversion of pancreatic injury has been noticed on treatment with different medicinal plant extracts.

A study conducted by a group of scientists reported that damaged beta cells of the pancreas in diabetic rats are regenerated by flavonoids [55]. It has been revealed that regeneration of β-cells can be either from the progenitor cells that are present inside the murine islets or from the stem cells in the pancreatic ducts [56].

Histopathologic examination of the liver displayed inflammation that was mild to moderate. Lipid peroxidation and ROS are the main cause of direct damage to hepatocytes by disrupting the membranes, DNA, and protein [57].

In a prior study, hepatic tissue anatomy of normal control, untreated control and different medicinal plant- treated (normal and diabetic) rat groups were accomplished. In the normal control and normal treated group, a normal structure of hepatocytes was noted. In diabetic rats, hepatocytes around the central vein demonstrated apoptosis and necrosis as well as sinusoid enlargement. On the other hand, rats of diabetic treated groups significantly reversed these degenerative changes in the hepatocytes [35].

It was previously documented that the extra space in between the hepatic sinusoids of diabetic rats disappeared after treatment with *M. charantia* and it was concluded that such extract protects the hepatic injury. his study also noticed a curative effect of medicinal plants in the hepatic tissues of diabetic rats [58]. Study analysis of the liver of diabetic control portrayed the microvesicular fatty alteration in hepatic tissue, focal fibrosis, slightly medium congestion and lymphocytic infiltrate around the portal tract, while treatment with medicinal plants demonstrated mild lymphocytic infiltrates with a definite decrease in microvesicular fatty changes [59].

Histopathologic data from the current research strongly implies that the use of medicinal plants scavenges the free radicals as flavonoids, saponins, and phenolics in plants to regenerate the damaged cells of the liver and pancreas, and might be a valuable adjuvant therapy to maintain glycemic control.

The results of current findings are inconsistent with previous studies that also studied the morphologic effect of medicinal plants on the liver and pancreas [20].

Previous research confirmed the presence of o-coumaric acid, syringic acid, gallic acid, benzoic acid, chlorogenic acid, p-coumaric acid, vanillic acid and ferulic acid in *Momordica charantia* by means of HPLC [51].

FTIR is a powerful analytical tool used for the categorization of chemical components based on molecular characteristics and is employed to distinguish medications for pharmacopeia in different countries. FTIR use reveals that this tool is a valued group belonging to components of plants i.e. polysaccharides, membranes, nucleic acids and proteins [60].

Momordica charantia displayed peaks of stretching of C-O (1240 cm^{-1}), C-C (1324 and 1548 cm^{-1}), C=C (1647 cm^{-1}), C-O (1102 and 1062 cm^{-1}) and C-H in-plane bending (1384 cm^{-1}). The presence of proteins, sterols, alkaloids, anthocyanins, flavonoids, carbohydrates and terpenoids in bitter gourd has also been investigated formerly [61]. Another study also verified the existence of alkanes at two peaks 2849.5 cm^{-1} and 1395.9 cm^{-1} which were also determined in this study [62]. Previously, it was also confirmed that the existence of carbonyls as carboxylic acids, aldehydes, esters and ketones at the frequency ranges from 1720-1820 cm^{-1} [63]. The findings of our study (**Table 6** and **Figure 6**) are also in accordance to the investigation done by other scientists; they found bands at 1596.4, 2366, 2832, 2936 and 3426 cm^{-1} [64].

Conclusion

Based on our findings, *M. charantia* seems to be safe and has high medicinal value. Among seven fractions or extracts used for *in vitro* analysis, methanolic extract was the most potent inhibitor of alpha-glucosidase and acetylcholinesterase, highlighting the hypoglycemic and anti-neurodegenerative potential of *M. charantia*. The present study reveals that co-administration of alloxan with *M. charantia extract* at a dose of 80 mg/kg body weight significantly improved the levels of fasting blood glucose, glycated hemoglobin, and insulin in diabetic rats. Structural elucidation by HPLC analysis confirmed the existence of flavonoids, phenols, tannins, and saponins. FTIR revealed the presence of hydroxyl groups, esters, alkanes, alkenes, alkynes, ketones, alcohols, amines, and carboxylic acids as major functional groups. A clinical trial with a diabetic animal model exhibited promising therapeutic efficacies for diabetes treatment and management. It is suggested that *M. charantia* can be recommended for diabetic people as a therapeutic adjunct as it can provide favorable remedial action in the diabetes continuum of metabolic syndrome.

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Disclosure of conflict of interest

None.

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