RESEARCH

Momordica charantia nanoparticles promote mitochondria biogenesis in the pancreas of diabetic-induced rats: gene expression study

Olusola Olalekan Elekofehinti[*], Olusola Christianah Ayodele and Opeyemi Iwaloye

Abstract

Background: Mitochondria dysfunction is one of the clinical features of diabetes mellitus (DM), which is a hallmark of insulin resistance (IR). This study investigates the therapeutic effect of *Momordica charantia* nanoparticles on mitochondria biogenesis in diabetic-induced rats. Forty-two adult wistar rats (average weight of 189 ± 10.32) were grouped as follows: STZ (65 mg/kg), control group, STZ + silver nitrate (10 mg/kg), STZ + *M. charantia* silver nanoparticles (50 mg/kg), STZ + metformin (100 mg/kg), and STZ + *M. charantia* aqueous extract (100 mg/kg). DM was induced intraperitoneal using freshly prepared solution of STZ (65 mg/kg), and rats with fasting blood sugar (FBS) above 250 mg/dl after 72 h of induction were considered diabetic. Treatment started after the third day of induction and lasted for 11 days. Effect of *M. charantia* nanoparticles on glucose level and pancreatic expression of genes involved in mitochondria biogenesis (PGC-1α, AMPK, GSK-3β, PPARϒ), inflammation (IL-1B, TNFα) and glucose sensitivity (PI3K, AKT, PTEN Insulin and Glut2) were quantified using reverse-transcriptase polymerase chain reaction (RT-PCR).

Results: The results showed that *M. charantia* nanoparticles promote mitochondria biogenesis, glucose sensitivity and reverse inflammation in the pancreas of diabetes rat model through upregulation of PGC-1α, AMPK, PPARϒ, AKT, Insulin and Glut2 mRNA expression and downregulation of GSK-3β, PI3K, IL-1B and TNFα mRNA expression in the pancreas of diabetic rats.

Conclusion: This study thus concludes that *M. charantia* nanoparticles may provide effective therapeutics against mitochondria dysfunction in the pancreas of diabetic model.

Keywords: Diabetes mellitus, Mitochondria biogenesis, Inflammation, Glucose sensitivity, *Momordica charantia* nanoparticles

Background

For many decades, research on finding cure or effective therapies for a number of metabolic diseases have been implemented and executed. Despite rapid advances in technologies to improve the conventional treatment of these groups of diseases, there seems no end in sight for permanent cure of these diseases. Diabetes mellitus (DM) is the most popular health condition among the groups of metabolic diseases, and it has emerged as one of the global leading cause of health concern [1, 2]. With each passing decade, global record of people living with diabetes indicated that the number has been increasing geometrically [3, 4]. The functional changes that accompany diabetes are either insufficient release of insulin from pancreatic beta cells or poor response of the cells to the effects of insulin [5]. These pathophysiological conditions could triggers tens of complications on the long-term if left untreated. Several molecular pathways underlying DM and its complications have been studied, explored and elucidated, no available satisfactory effective therapy, however, has been found to slow down or revert the pathogenesis of diabetes [6].

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Mitochondria are double-membrane bound organelles found in abundance in most of eukaryotic organisms, and they play several critical roles in metabolism [7]. In humans, their catabolic roles include oxidation of pyruvate, fats, proteins, etc. As well as their extensive documented anabolic roles in delivering chain of carbon atoms for the synthesis of sugar, amino acids, fatty acids and other biomolecules [8]. Mitochondria biogenesis is a cellular process involving the production of new mitochondria. Due to the high versatility of mitochondria to change their morphology through fission and fussion events, these series of dynamic events permit mitochondrial network to spontaneously remodel itself [9, 10].

The peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) is known as the core orchestration of mitochondria biogenesis in the context of energy demand [11]. It is implicated in a number of signaling pathways that accentuate all reactions involved in ATP generation from nutrients in response to physiological conditions, promoting adaptation to increased energy demand [12]. PGC-1α coordinates mass mitochondrial production through stimulation of nuclear respiratory factor 1 (Nrf1) and nuclear respiratory factor 2 (Nrf2). This ensures coordination between proteins produced within and outside the mitochondria needed for biogenesis, activation of estrogen-related receptor alpha (ERRα) for high capacity to breakdown fatty acid (β-oxidation). Other signaling molecules such as glycogen kinase 3β, peroxisome proliferator-activated receptor-ϒ (PPARϒ), and AMP-activated protein kinase (AMPK) are known to play major contribution to functioning of the mitochondria [13].

Insulin resistance is an important feature of type II diabetes mellitus (T2DM). The underlying mechanism of insulin resistance, however remains vague but may involve pathological chances in multiple tissues including pancreas. Loss of mitochondria functions has been implicated in the development of insulin resistance [14]. Compelling evidence suggests that boosting mitochondrial function may represent a valuable therapeutic tool to improve insulin sensitivity [14, 15]. Scientific investigation on the effects of herbal plants and dietary supplements in mitochondria function have been executed, and the reports showed that they support increased mitochondria number or mass [16–18]. In spite of the promising effects of these plants on mitochondria biogenesis as rationale for T2DM treatment, there is no FDA approved drug for improving mitochondria function. Hence, this study explored the effect of *Momordica charantia* nanoparticles on pancreas mitochondria biogenesis in STZ-induced rats. In addition to this, the anti-inflammatory and glucose modulating effects of the plant nanoparticles in the rats'

pancreatic tissue were also taken into consideration. In our previous study, we demonstrated that *M. charantia* nanoparticles (50 mg/kg) slowed down the progression of diabetic nephropathy through JAK/STAT and AKT/PI3K pathways [19]. Although studies have provided the hypoglycemic and antidiabetic properties of different *M. charantia* under in vivo and in vitro conditions [20, 21], no available study has deployed the use of nanoparticles to deliver *M. charantia* into biological system. From our previous study, we have acquired the background knowledge that *M. charantia* nanoparticles at lower dosage (50 mg/kg) reduce blood sugar level in STZ-induced diabetic rats than the plant extract. This study will unravel new therapeutic effect of *M. charantia* nanoparticles in boosting mitochondrial biogenesis and function in diabetic rats.

Methods

Collection of plant material and authentication

One kilogram (1 kg) of matured *M. charantia* leaves was collected fresh from the vicinity of Academic Building, School of Sciences (SOS), Federal University of Technology Akure (FUTA), Ondo State, and the plant sample was authenticated with voucher number: FUTA/BIO/0125.

Preparation and methanolic extraction of the plant

The leaves were washed with distilled water, before air-drying for 9 days. The leaves were dehydrated in the oven at 40 °C for 3 h. The dried leaves were ground to powdered form using an electric blender. A weight of 250 mg was measured from the powdered form of *M. charantia* and dissolved in 1250 ml of ethanol; it was allowed to stand for 48 h and filtrated. The filtrate was dried using rotary evaporator to obtain a methanolic extract of *M. charantia* leaves.

M. charantia nanoparticles synthesis from silver nitrate solution

The modified method described by Nahar et al. [22] was adopted to synthesize the bitter melon nanoparticles. The freeze dried *M. charantia* methanolic extract were dissolved in 1 mM aqueous silver nitrate solution at ratio 1: 9 (v/v), and allowed to stand for 24 h. A solution undergo a change in color (from dark green into light brown) to demonstrate the formation of nanoparticles. The bioreduction of Ag^+ ions to Ag^0 was monitored using a UV–Vis spectrophotometer. To enhance the stability and reduce the reactivity of the newly synthesized *M. charantia* nanoparticles, the solution of the nanoparticles was freeze-dried and stored at room temperature (25 °C) [23].

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Animal purchase, experimental design, grouping and treatment

For the purpose of the present study, 42 male Wistar rats (average weight of 189 ± 10.32), aged 8 to 9 weeks old, were purchased from the rat breeding colony of Department of Biochemistry, School of Sciences (SOS), Federal University of Technology Akure, Nigeria and housed in the Departmental animal house at 25 °C with a 12 h light–dark cycle. The experimental animals were given access to animal feeds and distilled water ad libitum. Before the commencement of the experimental study, an ethical approval was collected from Animal Welfare and Research Ethics Committee at the Federal University of Technology Akure, Nigeria. The rats were acclimatized for two weeks after which the animals were sorted into 6 different groups of 7 rats each. DM was induced by intraperitoneal (i.p) injection of freshly buffered (0.1 M citrate, pH 4.5) solution of STZ (65 mg/kg) to overnight fasted rats. The fasting blood sugar (FBS) of the rats was taken before STZ induction and after 72 h of DM induction, and rats with FBS ≥ 250 mg/dl were considered diabetic. There was no mortality after the STZ induction. The animal groups are as follows:

- Group 1: STZ (65 mg/kg)
- Group 2: Control group
- Group 3: STZ (65 mg/kg) + silver nitrate (10 mg/kg)
- Group 4: STZ (65 mg/kg) + *M. charantia* silver nanoparticles (50 mg/kg)
- Group 5: STZ (65 mg/kg) + metformin (100 mg/kg)
- Group 6: STZ (65 mg/kg) + *M. charantia* aqueous extract (100 mg/kg)

The treatments were given to the rats via oral gavage. The treatment of STZ induced rats lasted for 11 days after which sacrifices were made on the 12th day. The pancreatic tissue of the rats was collected and preserved in Trizol reagent.

Isolation and purification of total RNA

The Trizol-preserved pancreatic tissues were homogenized in an eppendorf tube using plastic pestle to permit thorough exposure of the cell's nucleus. The homogenized tissues were partitioned using chloroform as gradient separation medium. Isoamy alcohol was added as precipitating solution, after which the sample was treated with DNase (NEB) for 10 min before the RNA pellet was washed with ethanol to remove any DNA and phenol contamination, respectively. The obtained DNase free RNA was suspended in nuclease-free water. The purity was determined by measuring the absorbance at 260 and 280 nm, respectively [24].

cDNA synthesis: polymerase chain reaction (PCR) and amplification of gene of interest

The obtained total RNA was converted to complementary DNA (cDNA) using reverse transcriptase polymerase chain reaction (RT-PCR). The enzyme reverse transcriptase initiated the conversation of the RNA to cDNA [24]. After synthesis of the cDNA, the genes of interest were amplified using designed and optimized set of primers (see Table 1), which comprised forward and reverse primers. The PCR Master Mix catalyzes the amplification using thermocylcer (Eppendorf Mastercycler AG 22,331) Hamburg for 30 cycles.

Agarose gel electrophoresis

The amplicons from RT-PCR products were subjected to 1% agarose gel electrophoresis to enable the migration of the amplicons from the anode to the cathode at constant voltage. The relative density and intensity of the gene bands were quantified using Image J software.

Table 1 List of designed, optimized and synthesized primers specific for each gene

Gene name	Forward primer	Reverse primer
IL-1B	GCAATGGTCGGGACATAGTT	AGACCTGACTTGGCAGAGGA
TNF Alpha	ACCACGCTCTTCTGTCTACTG	CTTGGTGGTTTGCTACGAC
PGC-1 Alpha	TTCAGGAGCTGGATGGCTTG	GGGCAGCACACTCTATGTCA
Insulin rats	GTCCTCTGGGAGCCCAAG	ACAGAGCCTCCACCAGG
Glucokinase	GTGTACAAGCTGCACCCGA	CAGCATGCAAGCCTTCTTG
AMPK	ATCCGCAGAGAGATCCAGAA	CGTCGACTCTCCTTTTCGTC
Gsk3b	GGGACAGTGGTGTGGATCAG	GCCGAAAGACCTTCGTCCAA
Glut2	TAGTCAGATTGCTGGCCTCAGCTT	TTGCCCTGACTTCCTCTTCCAAC
PI3K	AACACAGAAGACCAATACTC	TTCGCCATCTACCACTAC
AKT	TCACCTCTGAGACCGACACC	ACTGGCTGAGTAGGAGAACTGG
PTEN	CCCACCACAGCTAGAAACTTATC	CGTCCTTTCCCAGCTTTACA

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Statistical analysis

Results are expressed as means for each of the groups. Statistical analysis was done using Graphpad prism 8, with values of $p < 0.05$ being considered as statistically significant.

Results

Green synthesis of *Momordica charantia* nanoparticles

The green synthesis of *Momordica charantia* nanoparticles (a) bitter melon (*M. charantia*) plant (b) silver nitrate solution (c) mixture of silver nitrate and bitter melon aqueous solution after 5 min (dark green coloration) (d) synthesis of *M. charantia* nanoparticles after 24 h indicated by light brown coloration are presented in Fig. 1.

Characterization of *Momordica charantia* nanoparticles

The graph of the UV–Visible spectrum of the *M. charantia* nanoparticles indicates that the nanoparticles have a maximum absorbance at a wavelength of about 430 nm at the three different concentrations that were evaluated. The surface morphology of the newly synthesized nanoparticles at different magnification (100 μm, 80 μm and 50 μm) using scanning electron microscopy (SEM) are described in our previous study [19].
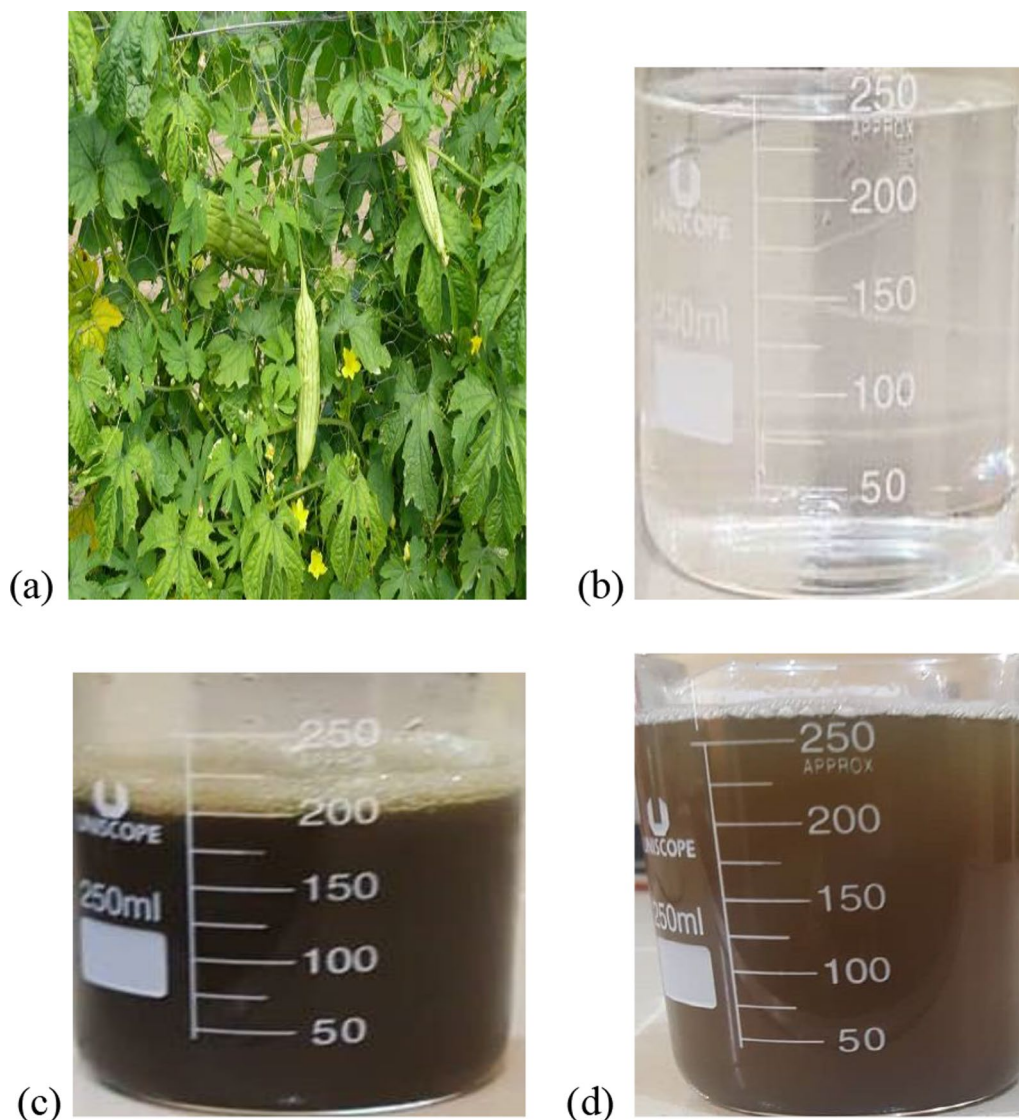

Fig. 1 Green synthesis of *M. charantia* nanoparticles. Picture of **a** *M. charantia* leave **b** silver nanoparticles (**c**) and **d** mixture of silver nitrate and bitter melon aqueous solution after 5 min

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Level of fasting blood glucose (FBS) before and after treatments

The FBS of all groups except the control, rose up to a level above 250 mg/dl after STZ (65 mg/kg) induction, which signifies the onset of diabetes (Table 2). After eleven (11) days of treatment, a significant reduction ($p < 0.05$) in the blood glucose level was observed in the *M. charantia* nanoparticles (50 mg/kg), *M. charantia* crude extract (100 mg/kg), silver nitrate (10 mg/kg) and metformin (100 mg/kg) treated groups.

Effect of oral administration of *M. charantia* nanoparticles on mitochondrial biogenesis markers

The expression of the PGC-1α gene was significantly downregulated ($p < 0.05$) in diabetic group relative to control group. Administration of metformin, *M. charantia* nanoparticles (50 mg/kg) and *M. charantia* extract (100 mg/kg) upregulated the mRNA expression of PGC-1*α* in the pancreas of STZ-induced rats (Fig. 2a). The expression of the AMPK gene was significantly downregulated ($p < 0.05$) in the diabetic rats relative to the control rats. Oral administration *of M. charantia* nanoparticles (50 mg/kg) significantly upregulated the ($p < 0.05$) AMPK expression (Fig. 2b). Figure 2c below shows the effects of oral administration of *M. charantia* nanoparticles on PPARϒ in the pancreas of STZ induced diabetic rats. A significant down regulation of PPARϒ in diabetic group compared to control group. The expression of *the* PPARϒ gene was significantly upregulated ($p < 0.05$) in treatment groups relative to the diabetic group. Figure 2d below shows the effects of oral administration of *M. charantia* nanoparticles on GSK-3β, one of the protein kinases implicated in the progression of diabetes. As seen in the bar chart, over-expression ($p < 0.05$) of GSK-3β is observed in diabetes control, relative to control. All treatment groups, however down-regulated the expression of GSK-3β in the pancreas of diabetic rats.

Effect of oral administration of *M. charantia* nanoparticles on inflammatory genes

The effects of oral administration of *M. charantia* nanoparticles, metformin, *M. charantia* extract and silver nitrate are illustrated as bar chart in Fig. 3a. There is significant over-expression ($p < 0.05$) of TNF-α in diabetic group relative to control group. The expression of the TNF-α gene was significantly upregulated ($p < 0.05$) in groups treated with *M. charantia* nanoparticles (50 mg/kg) and *M. charantia* extract (100 mg/kg) relative to control group. Figure 3b below shows the effects of administered *M. charantia* nanoparticles on the pro-inflammatory cytokines IL-1β in the pancreas of STZ-induced diabetic rats. Significantly, over-expression of IL-1β was observed in diabetic group when compared to control group. However, a significant down-regulation of IL-1β was observed in treatment groups when compared to diabetic group.
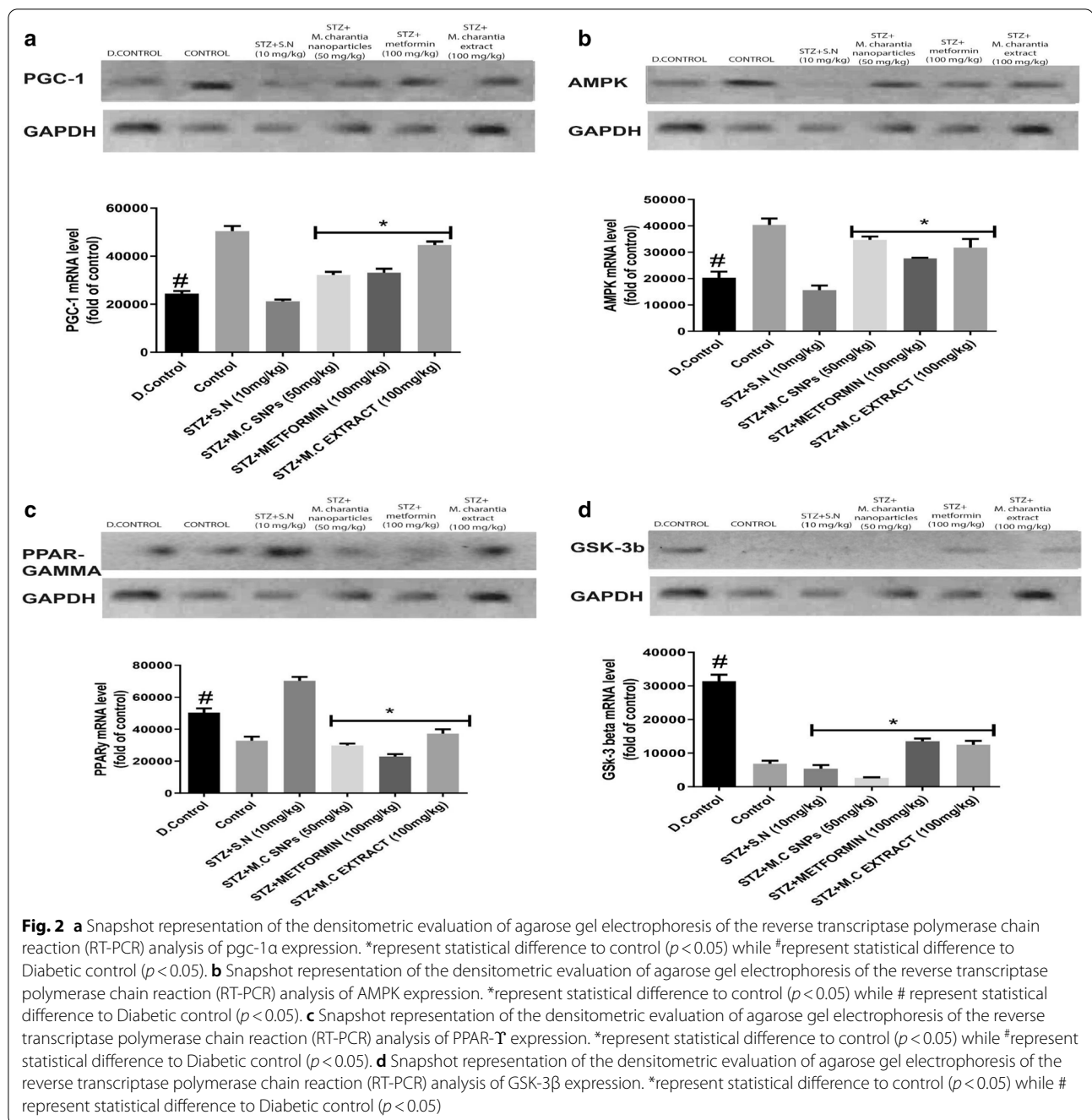
Effect of oral administration of *M. charantia* on PI3K/AKT insulin signaling pathway

Figure 4a below shows the effect of oral administration of *M. charantia* nanoparticles (50 mg/kg), metformin (100 mg/kg), *M. charantia* (100 mg/kg) on the pancreas of STZ-induced diabetic rats. There was significant downregulation ($p < 0.05$) of PI3K mRNA expression in diabetic group compared to control group. The treatment groups (*M. charantia* nanoparticles (50 mg/kg), metformin (100 mg/kg), *M. charantia* (100 mg/kg)), however significantly upregulated ($p < 0.05$) the expression of PI3K in diabetic rats. Figure 44 below shows the effects of oral administration of *M. charantia* nanoparticles (50 mg/kg), metformin (100 mg/kg), *M. charantia* (100 mg/kg) on the pancreas of STZ-induced diabetic rats. There was significant downregulation ($p < 0.05$) of AKT mRNA expression in diabetic group compared to control group. The treatment groups (*M. charantia* nanoparticles (50 mg/kg), metformin (100 mg/kg), *M.*

Table 2 Effect of *M. charantia* nanoparticles (50 mg/kg), *M. charantia* crude extract (100 mg/kg), silver nitrate (10 mg/kg) and metformin (100 mg/kg) on the glucose levels of the experimental rats

Fasting blood sugar levels of the rats on different days

Treatments	Before STZ induction	72 h After STZ induction	6th day of treatment	11th day of treatment
D. control	97 ± 5.78	271 ± 1.17	261 ± 3.44^a	258 ± 7.67^a
Control	101 ± 2.4	98 ± 1.01	103 ± 6.12	89 ± 2.53
STZ + S.N (10 mg/kg)	88 ± 8.23	275 ± 5.50	195 ± 2.11^b	170 ± 5.24^b
STZ + M.C SNPs (50 mg/kg)	115 ± 1.04	277 ± 0.45	79 ± 3.46^b	65 ± 1.73^b
STZ + Metformin (100 mg/kg)	107 ± 4.27	271 ± 4.89	88 ± 6.43^b	73 ± 1.01^b
STZ + M.C (100 mg/kg)	101 ± 4.45	272 ± 1.57	108 ± 1.88^b	84 ± 1.06^b

[a] Statistical difference to control ($p < 0.05$) while [b] statistical difference to Diabetic control ($p < 0.05$). Results are expressed in mean $\pm$ SEM, $n = 7$

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Fig. 2 **a** Snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of pgc-1α expression. *represent statistical difference to control ($p < 0.05$) while #represent statistical difference to Diabetic control ($p < 0.05$). **b** Snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of AMPK expression. *represent statistical difference to control ($p < 0.05$) while # represent statistical difference to Diabetic control ($p < 0.05$). **c** Snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of PPAR-ϒ expression. *represent statistical difference to control ($p < 0.05$) while #represent statistical difference to Diabetic control ($p < 0.05$). **d** Snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of GSK-3β expression. *represent statistical difference to control ($p < 0.05$) while # represent statistical difference to Diabetic control ($p < 0.05$)

charantia (100 mg/kg)), however significantly upregulated ($p < 0.05$) the expression of AKT in diabetic rats. The expression of the PTEN gene was significantly upregulated ($p < 0.05$) in the diabetic group relative to the control group. Drawing from observation of the results as shown in Fig. 4c. There was significant downregulation ($p < 0.05$) of PTEN gene upon oral administration of *M. charantia* nanoparticles.

Effect of oral administration of *M. charantia* nanoparticles on glucose sensitivity in STZ-induced rats

The effects of *M. charantia* nanoparticles (50 mg/kg), metformin (100 mg/kg), *M. charantia* extract (100 mg/kg) on insulin in STZ induced diabetic rats is shown Fig. 5a. Oral administration of *M. charantia* nanoparticles (50 mg/kg), metformin (100 mg/kg), *M. charantia* extract (100 mg/kg) significantly up-regulated insulin gene after the duration of treatment. In untreated
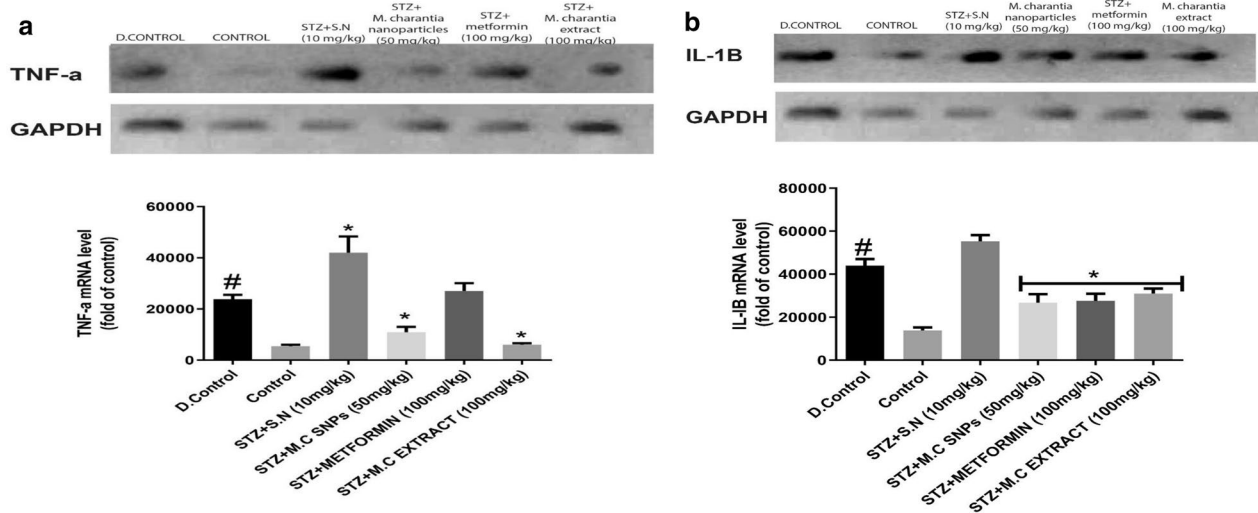
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Fig. 3 a Snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of TNF-α expression. *represent statistical difference to control ($p < 0.05$) while #represent statistical difference to Diabetic control ($p < 0.05$). **b** Snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of IL-1β expression. *represent statistical difference to control ($p < 0.05$) while #represent statistical difference to Diabetic control ($p < 0.05$)

diabetes-induced rats (diabetes control), a significant under-expression ($p < 0.05$) of GLUT-2 was observed in diabetic group when compare to control group (Fig. 5b). Oral administration of *M. charantia* nanoparticles (50 mg/kg), metformin (100 mg/kg), and *M. charantia* plant extract (100 mg/kg) significantly over-expressed ($p < 0.05$) GLUT-2 mRNA in diabetic rats.

Discussion

Diabetes mellitus (DM) is one of the major leading causes of death around the globe, and its complications continues to have devastating effect on both adults and young men. The use of nanoparticles as vehicle for drug delivery to target site in biological system has gained worldwide recognition as an effective and efficacious method of targeting specific organ in drug delivery. After the synthesis of the silver nanoparticles, its characterization using SEM analysis revealed tubular clusters of silver nanoparticles with uneven surface morphologies and dimensions. FTIR spectroscopy results revealed intense bands around 3690.1, 3280.1, 2922.2, 2851.4, 1543.1, 1625.1, 1401.5, 131.95 and 1028.7 which corresponds with O–H, N–H, C–H, C=C, C–O stretches. This indicates the possible role of phenol, flavonoids and amine functional groups in stabilizing the nanoparticles [19]. The diabetic status of the STZ-induced experimental rats was confirmed through FBS; and the oral administration of *M. charantia* nanoparticles (50 mg/kg) and *M. charantia* crude extract (100 mg/kg) to the diabetic rats was able to lower the FBS

of the treated rats over the course of eleven (11) days. With this in mind, it is obvious that the *M. charantia* has glucose lowering effects.

The significant role of mitochondria dysfunction in diabetes has attracted attention in recent years because mitochondria organelle plays a large role in maintaining cellular metabolic balance [25–27]. Several key genes have been recognised as modular of mitochondria biogenesis including PGC-1α, and other downstream proteins [28, 29]. The PGC-1α, a transcriptional co-regulation factor, is the major regulator of mitochondria biogenesis and it functions by activating different transcriptional factors including the NRFs to induce a number of genes involved in mitochondria proliferation [30]. Studies involving rats' model have demonstrated the down-regulation of PGC-1α mRNA expression in diabetic states [11], and this was also confirmed in the present studies after diabetic induction of experimental rats. In the current study, the treatment groups which include *M. charantia* extract, *M. charantia* nanoparticles and the metformin were able to modulate the dysregulated gene by significant upregulation of PGC-1α mRNA expression, which signifies the restoration of the mitochondria functions. Aside the pivotal role played by PGC-1α in maintaining the significant function of mitochondria organelles, AMPK, one of the protein kinases involved in pathogenesis of diabetes also plays essential role in mitochondria biogenesis by antagonizing decreased in cellular ATP [31, 32]. Previous study has demonstrated that an
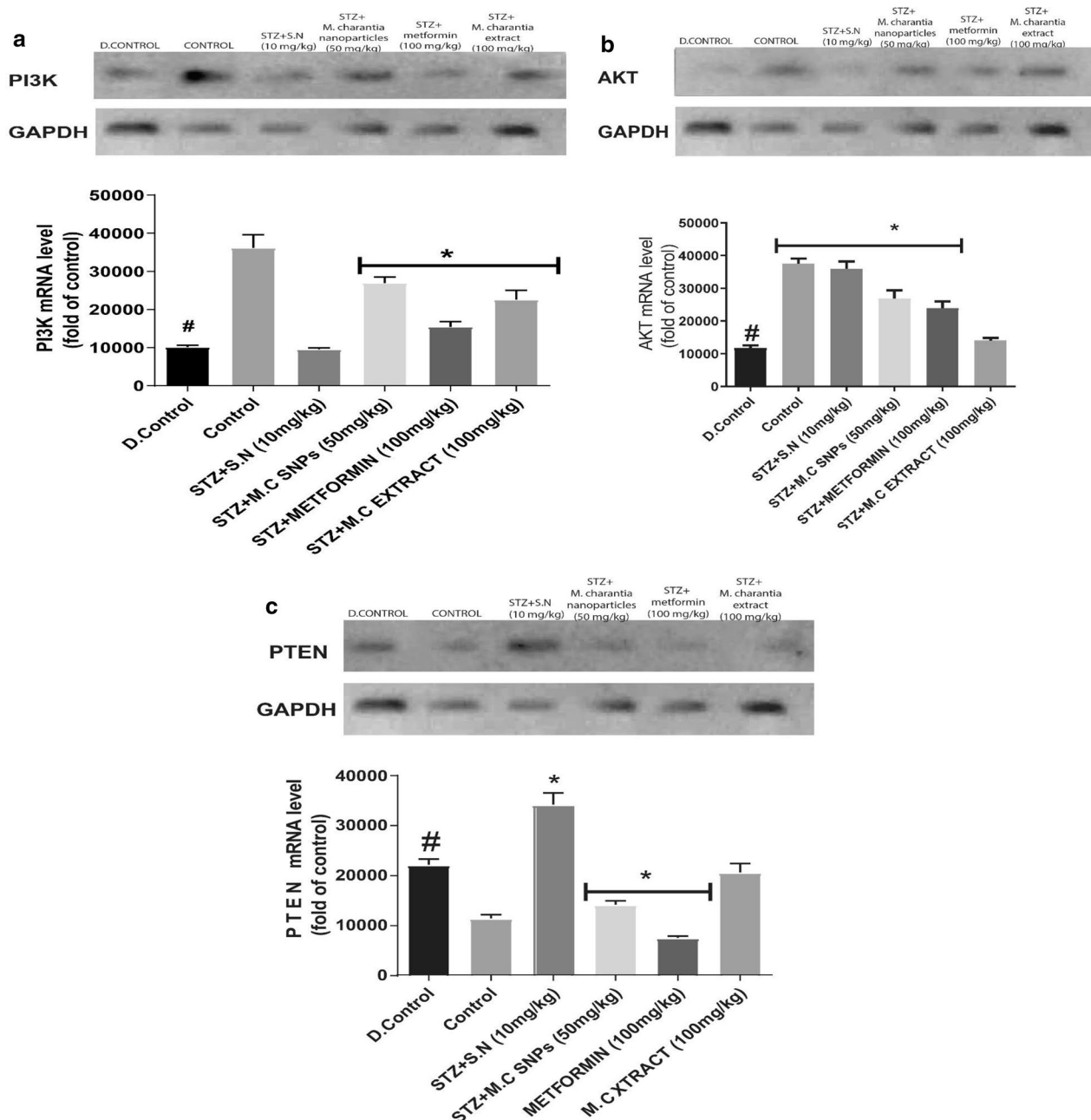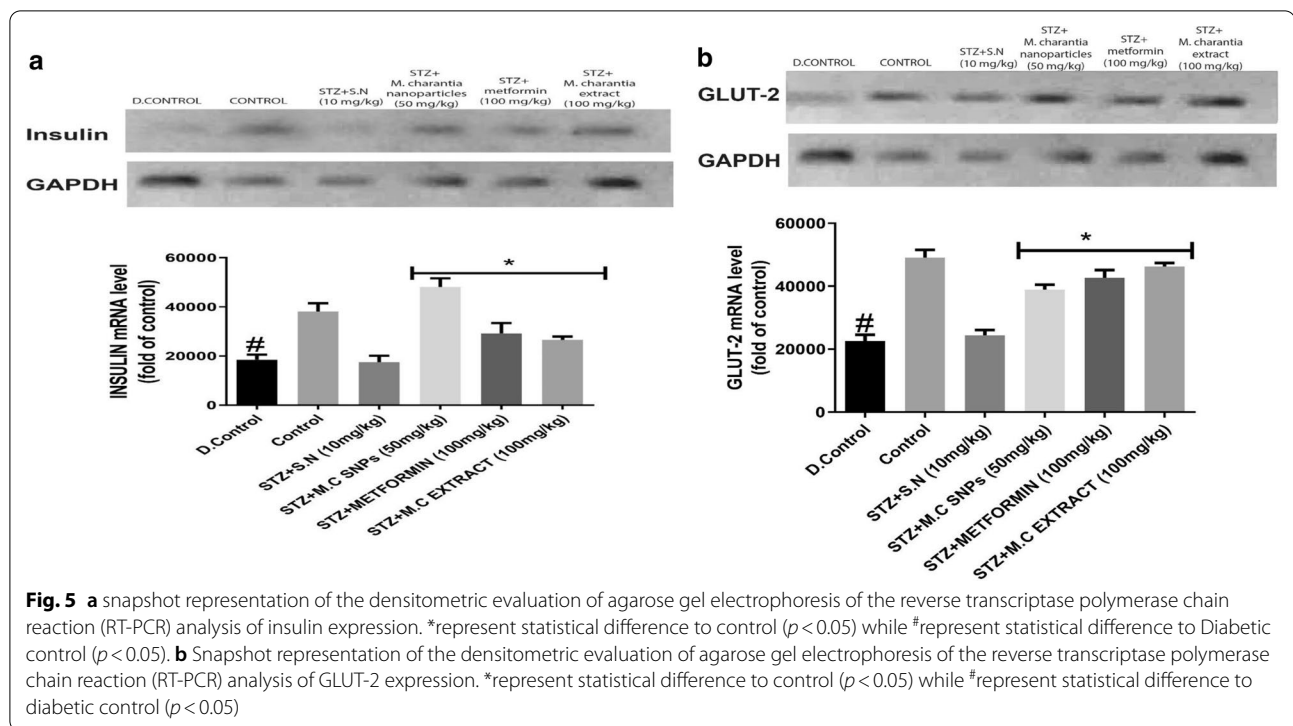
Fig. 4 **a** Snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of PI3K expression. *represent statistical difference to control (*p* < 0.05) while #represent statistical difference to Diabetic control (*p* < 0.05). **b** Snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of AKT expression. *represent statistical difference to control (*p* < 0.05) while # represent statistical difference to diabetic control (*p* < 0.05). **c** Snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of PTEN expression. *represent statistical difference to control (*p* < 0.05) while #represent statistical difference to diabetic control (*p* < 0.05)

increase in the activity of AMPK induces the expression of PGC-1α through phosphorylation [33]. Hence, investigation of the downstream regulator of PGC-1α in IR and impaired mitochondria biogenesis is of significance

important [34]. Our results showed that treatment of diabetic rats with *charantia* nanoparticles up-regulated the mRNA expression of AMPK in the pancreatic tissue. This observation corroborates with results obtained from a

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Fig. 5 a snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of insulin expression. *represent statistical difference to control ($p < 0.05$) while #represent statistical difference to Diabetic control ($p < 0.05$). **b** Snapshot representation of the densitometric evaluation of agarose gel electrophoresis of the reverse transcriptase polymerase chain reaction (RT-PCR) analysis of GLUT-2 expression. *represent statistical difference to control ($p < 0.05$) while #represent statistical difference to diabetic control ($p < 0.05$)

single study involving the treatment of T2DM and obesity with standard antidiabetic drug pioglitazone [35].

In mitochondrial dysfunction, the expression of peroxisome proliferator-activated receptor-ϒ (PPARϒ) is dysregulated; this gene is known to coordinate the energy metabolism, catabolism of fatty acid (fatty acid oxidation), induction of oxidative stress, and metabolic shift towards glucose breakdown (glycolysis) [36], and all of which are believed to make significant contribution to the development of IR and diabetes. A number of studies have shown that there is alteration of mitochondria function in patients living with diabetes as a result of increased oxidative stress [37, 38]. Previous studies have demonstrated that antidiabetic agents increase the expression of PPARϒ, replicated mitochondria DNA copy number, upregulated the regulators responsible for mitochondria biogenesis as well as modulated fatty acid oxidation. It is evidence from our current study that *M. charantia* nanoparticles restored the function of mitochondria in diabetic rats through the upregulation of PPARϒ pancreatic mRNA expression.

It is also worth mentioning that glycogen synthase kinase-3β (GSK-3β) contributes largely to biogenesis, motility, permeability of mitochondria [39]. In addition to the critical role played by GSK-3β in the onset and progression of diseases including DM [40], phosphorylation of the kinase GSK-3β acts as an upstream regulator to decrease the expression of PGC-1α [41]. Drawing from

this explanation, it is obvious that phosphor-inactivation of GSK-3β is necessary for improving the function of the mitochondria. As shown in this study, the mRNA expression of GSK-3β was upregulated in diabetic rats, and GSK-3β mRNA expression was repressed after treatment of the rats with *M. charantia* nanoparticles. The evidence that *M. charantia* nanoparticles downregulated the mRNA expression of GSK-3β validates its mitochondrial biogenesis effect through the modulation of aforementioned PGC-1α mRNA in the power house of the cells.

Some studies revealed an increase in inflammation of tissues in diabetic rats through the overexpression of both pro-inflammatory and inflammatory cytokines [42]. These cytokines are known for their unwanted effect on insulin signalling and sensitivity of glucose. The upregulation of TNF-α and IL-1β genes correlates with impaired insulin signalling pathways (through phosphorylation of protein kinase B) and T2DM [43]. The anti-inflammatory properties of *M. charantia* nanoparticles was shown through the repression of TNF-α and IL-1β mRNA. Hence, *M. charantia* nanoparticles ameliorated inflammation in the pancreas of STZ-induced rats.

To elucidate the antidiabetic effect of *M. charantia* nanoparticles, insulin, Glut2 and key genes modulating PI3K/AKT insulin signaling pathway were taken into consideration. The PI3K, AKT and PTEN genes are important modulator of PI3K/AKT pathway; while PI3K and Akt activate PI3K/Akt signalling pathway, the PTEN

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gene acts as a negative regulator of PI3K/Akt pathway. Studies have demonstrated that PI3K/AKT pathway correlate to the metabolism of glucose, which is important for the increased uptake of glucose by insulin in the tissue [44, 45]. Dysregulation of this pathway through repression of AKT, PI3K and overexpression of PTEN is peculiar to diabetic models [46]. Plant extract that activates PI3K/AKT pathway has the tendency of ameliorating IR [47]. Our study showed that *M. charantia* nanoparticles could activate the insulin signaling pathway by reversing the downregulation of PI3K and AKT, and upregulation of PTEN mRNA expression in the pancreas of diabetic induced rats. Also, it is worth noting that oral administration of *M. charantia* nanoparticles promote insulin secretion, glucose uptake and metabolism through the up-regulation of insulin and Glut2 mRNA expression in pancreatic β cells. Our result is consistent with an in vivo study demonstrating that crude extract of *M. charantia* could increase the secretion of insulin from endocrine pancreatic β cells [48].

The data obtained from this study showed that *M. charantia* nanoparticles promote mitochondria biogenesis, insulin secretion and glucose metabolism, as well as alleviate inflammation in the pancreas of STZ-induced diabetic rats through upregulation of PGC-1α, PPARϒ, AKT, PI3K, Glut2, insulin, AMPK mRNA expression and repression of GSK-3β, TNF-α, IL-1β and PTEN mRNA expression.

Conclusions

The current findings demonstrated that in addition to the already established hypoglycemic effects of *M. charantia* on model rats, the use of *M. charantia* nanoparticles significantly reduced the glucose level in STZ-induced rats at a lower dose (50 mg/kg) relative to *M. charantia* extract (100 mg/kg). Administration of *M. charantia* nanoparticle promoted mitochondria biogenesis, reversed inflammation and glucose insensitivity in the pancreas of STZ-induced diabetic rats through modulation of PGC-1α and other key genes.

Abbreviations

DM: Diabetes mellitus; IR: Insulin resistance; STZ: Streptozotocin; FBS: Fasting blood sugar; PGC-1α: *Peroxisome* Proliferator –activated receptor gamma coactivator 1 alpha; Nrf2: *Nuclear* Respiratory factor 1; Nrf2: *Nuclear* Respiratory factor 2; ERRα: *Estrogen*-Related receptor alpha; PPARϒ: Peroxisome proliferator-activated receptor-ϒ; GSK-3β: *Glycogen* Kinase 3β; AMPK: AMP-activated protein kinase; T2DM: Type II diabetes mellitus; JAK: Janus kinase; STAT: Signal transducer and activator of transcription; PI3K: Phosphatidylinositol-3-kianse; AKT: Protein kinase B; RT-PCR: Reverse transcriptase polymerase chain reaction; cDNA: Complementary DNA; SEM: Scanning electron microscopy; PTEN: Phosphatase and tensin homolog; GLUT-2: Glucose transporter 2; TNF-α: Tumour necrosis factor alpha; IL-1β: Interleukin 1 beta.

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Authors' contributions

OOE: Conceptualization, Methodology, reviewing and editing, supervision. OCA: methodology, formal analysis, writing. OI: writing—original draft preparation, writing—review and editing, formal anaylsis. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analysed during the current study are included in this published article.

Declarations

Ethical approval and consent to participate

The experiments were performed in accordance with the National Guidelines for Experimental Animal Welfare and with approval of the Animal Welfare and Research Ethics Committee at the Federal University of Technology, Akure, Nigeria. The Committee reference number is not available.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no known competing interest.

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