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Root extracts of *Anacardium occidentale* reduce hyperglycemia and oxidative stress *in vitro*

TM Archana, K Soumya, Jesna James and Sudheesh Sudhakaran*

Abstract

Background: Hyperglycemia is the hallmark of diabetes, and the associated oxidative stress is a major concern that invites an array of diabetic complications. The traditional practices of medicare are of great, current interest due to the high cost and side effects of conventional diabetic medications. The present *in vitro* study focuses on evaluating the potential of various *A. occidentale* root extracts for their antihyperglycemic and antioxidant potentials.

Materials and methods: The four different solvent extracts petroleum ether (PEAO), chloroform (CHAO), ethyl acetate (EAAO), and 80 % methanol (80 % MAO) of *A. occidentale* roots were evaluated for their total phenolic, flavonoid, and antioxidant capacity. Using MIN6 pancreatic β-cells, the cytotoxicity of the extracts was evaluated by MTT assay and the antidiabetic potential by quantifying the insulin levels by ELISA at a higher concentration of glucose. The effect of 80 % MAO on *INS* gene expression was determined by qRT PCR analysis.

Results: Among the four different solvent extracts of *A. occidentale* roots, 80 % MAO showed the highest concentration of phenolics (437.33 ± 0.03 μg GAE/mg), CHAO to be a rich source of flavonoids (46.04 ± 0.1 μg QE/mg) and with the highest total antioxidant capacity (1865.33 ± 0.09 μg AAE/ mg). Evaluation of the free radical scavenging and reducing properties of the extracts indicated 80 % MAO to exhibit the highest activity. The MTT assay revealed the least cytotoxicity of all four extracts. 80 % MAO enhanced *INS* up-regulation as well as insulin secretion even under high glucose concentration (27mM).

Conclusions: The present study demonstrated that the *A. occidentale* root extracts have effective antihyperglycemic and antioxidative properties, together with the potential of normalizing the insulin secretory system of β-cells. Above mentioned properties have to be studied further by identifying the active principles of *A. occidentale* root extracts and *in vivo* effects. The prospect of the present study is identifying drug leads for better management of diabetes from the *A. occidentale* root extracts.

Keywords: *Anacardium occidentale*, Pancreatic β-cells, *INS* gene, High glucose-induced oxidative stress, qRT PCR

Introduction

A coordinated interaction of the physiological processes – Insulin secretion, glucose uptake by peripheral tissues, and hepatic glucose production is known to maintain glucose homeostasis in the body. Inadequacy in the maintenance of glucose homeostasis results in the chronic elevation of blood glucose levels [1]. Hyperglycemia, the forerunner and consistent marker of all types of diabetes and the concerning factor of diabetic complications has now become a challenge to the health systems. Hyperglycemia is often the result of insufficient secretion of insulin or its resistance which when prolonged results in microvascular and macrovascular complications [2]. The high glucose levels lead to altered metabolism, free radical generation, and induce apoptosis in β-cells and other cell types [3]. According to Robertson et al.'s perspectives, prolonged glucotoxicity is known to cause

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decreased insulin gene expression. A major factor being the deficiency of PDX-1(Pancreas Duodenum Homeobox-1), an important transcription factor for the insulin promoter in glucotoxic β-cells. INS gene, almost exclusively expressed in β-cells of the pancreas is known to maintain the glucose homeostasis in the body and its defect results in impaired β-cell insulin secretory machinery ending up in diabetes mellitus [1].

The supraphysiological glucose level contributes to the generation of reactive oxygen species via autooxidation of glucose, via hexosamine metabolism, or oxidative phosphorylation during anaerobic glycolysis creating oxidative stress. A critical increase in the intraislet peroxide levels was observed during hyperglycemia, resulting in the deterioration of islet cells [4]. A weak manifestation of antioxidative enzymes in the pancreatic cells makes them more susceptible to oxidative stress. Thus, exogenous supplementation of antioxidants could protect the islet cells from the deteriorative effects of excess glucose levels [1]. Oxidative stress in the cells is well handled by antioxidants. The use of natural antioxidants from spices and herbs against oxidative stress has gained much attention over synthetic antioxidants due to the carcinogenic properties reported on synthetic antioxidants [5]. Studies have proven that many plant-derived compounds especially polyphenols like phenols and flavonoids are known to scavenge ROS and prevent lipid peroxidation [6].

Though many medications like sulfonylureas, biguanides, and incretins are used to curb diabetes, their big-budget and side effects demanded the search for safer and cost-effective drugs [7]. Phytotherapy has been widely used to treat various ailments for thousands of years. Phytotherapy includes combination drugs comprising several different active compounds from plants. The traditional practice of curing disease though exhibited appreciated pharmacodynamics, the unrevealed scientific validation forms the concerning factor for promoting the use of traditional medicines [8].

Anacardium occidentale (cashew tree) is a member of the *Anacardiaceae* family. It is grown widely in tropical countries like Malaysia, India, Brazil, and Senegal. It is used as a folk remedy for treating diabetes mellitus by traditional practitioners of South Cameroon and other tropical countries [9]. The *Anacardium* plants are gaining much importance due to their nutritional and wide biological activities. *Anacardium occidentale* is considered to be versatile as every part of the tree produces resources and products. Its leaf, bark, root, and nutshell oil are used for medicinal purposes [10]. Literature supports the anti-hypoglycemic, anti-ulcerogenic, and acute toxicity effects of hydroethanolic leaf extracts of *A. occidentale* [11] and the analgesic and anti-inflammatory activity of cashew gum extract [12]. The bark of *A. occidentale* was known to be an antihyperglycemic agent as well as a detoxifier of snake-bites in Ayurveda [13]. Also, root infusion of *A. occidentale* was reported to be a notable purgative [14]. Literature also supports the crude methanolic extract of *A. occidentale* to be effective against postprandial hyperglycemia as it exhibited α-amylase enzyme inhibition around 26.39 % [15]. *In vivo* studies on the antihyperglycemic and antioxidative properties of *A. occidentale* leaves, stem bark has been reported, but not the effect of its root on diabetic parameters [16, 17]. Though the literature reports wide pharmacological activities of the plant, there are no such reports on the effect of roots of *A. occidentale* on the *INS* gene and insulin secretion. and hence, *A. occidentale* to be our plant of interest.

In this context, we have focused on providing a shred of scientific evidence for the use of this traditional medicine on diabetic parameters. The present study aims at investigating the antidiabetic potential of *Anacardium occidentale* root extracts to simultaneously attend the hyperglycemia and oxidative stress, normalizing the insulin secretory machinery of β-cells.

Materials and methodology

Chemicals

The chemicals used for the study- Folin- Ciocalteu's reagent, sodium carbonate, aluminium chloride, potassium acetate, sodium phosphate, sulphuric acid, ammonium molybdate, potassium ferricyanide, trichloroacetic acid, ferric chloride, and solvents - petroleum ether, chloroform, ethyl acetate, and methanol were purchased from Merck, India Ltd., and the Biochemicals –Gallic acid, Quercetin, Ascorbic acid, DPPH (2, 2-diphenyl-1-picrylhydrazil) (Sigma Aldrich, Germany), DMEM (Dulbecco's Modified Eagle's Medium), and MTT (3, 4, 5-dimethylthiazol-2'-yl)-2, 5-diphenyltetrazolium bromide) from Sigma Aldrich, India.

Plant Material and Extraction

The roots of *Anacardium occidentale* were used as plant material. The samples were collected from local areas of the Kannur district, Kerala. The plant material was identified and authenticated by Dr. Sujanapal P., Senior Scientist, Department of Silviculture, Sustainable Forest Management Division, Kerala Forest Research Institute, Thrissur, Kerala, India, and the voucher specimen was deposited (Acc. No. 18017). The freshly collected plant material was chopped, shade dried, and ground to an optimal coarse powder. The powder was subjected to soxhlet extraction with solvents in their increasing polarity– petroleum ether, chloroform, ethyl acetate, and 80 % methanol. The extracts were then evaporated to vacuum dryness and preserved in a desiccator for further use [18].

Determination of Total Phenolic Content

The total phenolics content in the four different solvent extracts of *A. occidentale* (PEAO, CHAO, EAAO, and 80 % MAO) was determined as per the procedure by Islam et al., A calibration curve was constructed using gallic acid as standard and the total phenolic content of the extract was determined spectrophotometrically (Shimadzu UV-1700 UV-Visible Spectrophotometer, Japan) with the Folin- Ciocalteu's reagent (FCR) and expressed as μg of Gallic Acid Equivalents/mg sample [19].

Determination of Total Flavonoid Content

Total flavonoids were estimated quantitatively by Aluminium Chloride Method [20]. Quercetin was used as the standard to make the calibration curve. Measurements were done in triplicates and the total flavonoid content of the extract was expressed as μg of Quercetin Equivalents/mg sample.

Determination of Total Antioxidant

The total antioxidant capacity of the four different solvent extracts of *A. occidentale* (PEAO, CHAO, EAAO, and 80 % MAO) was quantitatively determined spectroscopically (Shimadzu UV-1700 UV-Visible Spectrophotometer, Japan) through the formation of phosphomolybdenum complex. Total antioxidant capacity was calculated by the method described by Islam et al., [19]. From the ascorbic acid standard curve, the antioxidant capacity of samples of unknown composition was expressed as equivalents of ascorbic acid in μg per mg of the extract.

Evaluation of Free radical scavenging activity (DPPH assay)

The antioxidant activity of four different solvent extracts of *A. occidentale* (PEAO, CHAO, EAAO, and 80 % MAO) was evaluated using the 2, 2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method [21]. Ascorbic acid was used as standard. Every concentration was done in triplicate. The decrease in absorption of DPPH solution is calculated by the equation:

$$Inhibition\ (\%) = \frac{Abs\ (control) - Abs\ (extract)}{Abs\ (control)\ \times 100}$$

Reducing Power Assay

The reducing power of the samples was determined as described by Oyaizu M [22]. This method is based on the principle that an increase in the absorbance of the reaction mixtures indicates an increase in antioxidant activity. The absorbance of the samples was measured at 700 nm against a blank using UV-Visible Spectrophotometer (Shimadzu UV-1700 UV-Visible Spectrophotometer, Japan)[23].

Cell Culture Experiments

MIN6 Cell lines were purchased from NCCS, Pune, India, and maintained in 25 cm^2 tissue culture flasks with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with L-glutamine, 10 % FBS, Sodium bicarbonate, Penicillin (100U/mL), Streptomycin (100 μg/mL) and Amphotericin B (2.5 μg/mL). The culture flasks were incubated at 37°C in a humidified 5 % CO_2 incubator (NBS Eppendorf, Germany). The cells were subcultured by trypsinization and maintained in DMEM. The experiments were performed twice in triplicates.

In vitro Cytotoxicity Assay

The cytotoxicity of the four different solvent extracts of *A. occidentale* (PEAO, CHAO, EAAO, and 80 % MAO) was evaluated by MTT Assay (3, 4, 5-dimethylthiazol-2'-yl)-2, 5-diphenyltetrazolium bromide) in MIN6 pancreatic cells [24]. All absorbance values were corrected against blank wells which contained growth media alone. For each test concentration, the mean absorbance of the triplicated wells was calculated. Mean absorbance of the cells grown in the absence of test compound was taken as 100 % cell survival.

Percentage cell viability was calculated by using the following formula,

$$\%\ Cell\ viability = \frac{Absorbance\ of\ sample\ x100}{Absorbance\ of\ control}$$

β-Cell assay

MIN6 cell lines maintained in DMEM were trypsinized from the plate, splitted, and replated to maxisorb 6 well plates. The confluent cells were incubated with Krebs Ringers Buffer for 1 h and then washed cells twice with KRB. An aliquot of second wash (with KRB) is saved for baseline insulin measurement. The cells were then incubated for 1 h with KRB (negative control), 50μM Glibenclamide (positive control), 0.1 % DMSO (vehicle control), the non-cytotoxic range of concentration of the four extracts as per the MTT assay in the presence (27mM), and absence of glucose. The insulin levels of the treated solutions were quantified by Indirect ELISA [25].

RNA Isolation and Real-Time PCR Analysis

The cells were lysed and the total cellular RNA was isolated using Qiazol reagent (Qiagen, Germany). The RNA was then treated with chloroform, centrifuged (13,000 rpm for 15 minutes at 4 ^{0} C), and finally precipitated with isopropyl alcohol. The quantitative yield of RNA was determined by

Qubit®.4.0 Fluorometer. The primer used for INS was F- 5' GCCCTTAGTGACCAGCTATAATC3'and R-5' GGACCA CAAAGATGCTCTTTG 3' and F-5'CATCCGTAAAGACC TCTATGCC3' and R-5' GACTCATCGTACTCCTGCTT G3' for β-actin. cDNA synthesis was done using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit followed by real-time PCR measurements of INS and β-actin with CFX Connect Real-Time PCR Detection System (Biorad, Japan) with reaction volume – 6μL, containing 2X Real-Time PCR smart mix- 3μL, Forward primer + Reverse primer- 0.5μL, Template cDNA – 1μL and nuclease-free water – 1.5μL. The cycling conditions were Polymerase activation: 95 °C for 10 min, Denaturation: 95 °C for 15 s, and Annealing/extension: 60 °C for 1 min and the reaction was repeated to 40 cycles [26].

Statistical Analysis

Statistical analyses were performed using GraphPad Instat (version 3.05) software. All the results are expressed in mean ± SD for triplicate determinations and the data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post-test. The concentration needed for 50 % inhibition (IC_{50}) was estimated graphically by linear regression analysis.

Results and discussion

Extraction Yield of four different solvent extracts of *Anacardium occidentale* roots

No single solvent is reliable for the extraction of the whole phytoconstituents from a plant. Sequential extraction with solvents of increasing polarity (nonpolar to polar) was selected for the study. This ensures the extraction and separation of a wide range of phytoconstituents from plant materials. Solvent polarity significantly reflects the extract yield and pharmacological properties of the plant material [18]. The extract yield from different fractions was calculated by using the equation:

$$Percentage\ extract\ yield\ (w/w) = \frac{Dry\ extract\ weight\ \times\ 100}{Dry\ starting\ material\ weight}$$

The percentage extract yield from all eight samples is given in Table 1.

Amount of total phenolic and flavonoid content in the four different solvent extracts of *A. occidentale*

The total phenolic content and total flavonoid content of the four different solvent extracts of *A. occidentale* roots (PEAO, CHAO, EAAO, and 80 % MAO) determined by Folin- Ciocalteu's reagent and Aluminium chloride method revealed that the extracts contained more phenolics than flavonoids. Among the four extracts, 80 % MAO (437.33 ± 0.03 μg of GAE/mg extract) contained the highest amount of phenolics and CHAO to be a rich source of flavonoids (46.04 ± 0.1 μg of QE/mg extract) (Table 2).

Total antioxidant capacity of four different solvent extracts of *A. occidentale*

The antioxidants present in the extract reduce Mo (VI) to a green phosphate Mo (V) complex. All extracts showed notable antioxidant capacity. The CHAO extract showed the highest antioxidant capacity with 1865.33 ± 0.09 μg of ascorbic acid per mg of the extract, followed by PEAO, EAAO, and finally 80 % MAO (Table 2).

DPPH scavenging activity of four different solvent extracts of *A. occidentale*

The ability of the plant extracts as antioxidants were reckoned concerning their ability to scavenge the DPPH; the higher the percentage inhibition, the higher the antioxidant property corresponding to the increasing concentration of extracts. Among the four extracts, 80 % MAO showed the strongest scavenging activity with an IC_{50} value of 0.026 ± 0.5 mg/mL, pursued by EAAO with an IC_{50} value of 0.031 ± 0.12 mg/mL and that was comparable with the standard ascorbic acid (IC_{50} = 0.011 ± 0.3 mg/mL). PEAO showed the lowest antioxidant property with IC_{50} value 6.12 ± 1.1 mg/mL. CHAO also exhibited a significant DPPH scavenging activity (IC_{50} = 3.71 ± 0.63 mg/mL) compared to standard ascorbic acid (Fig. 1).

Reducing power activity of four different solvent extracts of *A. occidentale*

Figure 2 represents the reducing power of the four different solvent extracts of *A. occidentale* roots (PEAO, CHAO, EAAO, and 80 % MAO). The increasing absorbance indicates an increase in the reducing power of the extracts. 80 % MAO exhibited an excellent reducing power with an IC_{50} value of 9.59 ± 0.71 mg/mL that was comparable with the standard ascorbic acid (IC_{50} = 9.22 ± 1.87 mg/mL) and followed by EAAO ˃ CHAO ˃ PEAO in their decreasing order of activity.

Cytotoxic effect of four different solvent extracts of *A. occidentale* root extracts against MIN6 cells

The four different solvent extracts *of A. occidentale* roots (PEAO, CHAO, EAAO, and 80 % MAO) were screened for their cytotoxic effect by MTT assay in MIN6 cells. The extracts were tested at doses ranging from 100, 50, 25, 12.5, 6.25, 3.12, and 1.56 μg/mL, and the percentage viability of cells corresponding to each concentration was calculated (Table 3.). A control well with untreated cells was maintained taking 100 % cell viability.

Table 1 The percentage yield of extracts of *Anacardium occidentale* roots

Species	Part used	Extract yield (% w/w)	
A.occidentale	Roots	Petroleum ether	0.1
		Chloroform	0.2
		Ethyl acetate	0.4
		80 % Methanol	7.5

Effect of four different solvent extracts of *A. occidentale* root extracts on Insulin secretion in MIN6 cells

The hypoglycemic activity of PEAO, CHAO, EAAO and 80 % MAO were tested by their potential in stimulating insulin secretion from cells in the presence and absence of glucose; glucose being a key regulator for the secretion of insulin from β- cells. The cells treated with extracts at 12.5 μg/mL concentration (Non-toxic concentration as per MTT Assay) and checked for insulin secretion by indirect ELISA revealed that 80 % MAO to be a potent insulin secretagogue. Though the insulin levels were less in 80 % MAO treated cells compared to the control in the absence of glucose, the insulin levels were raised to an appreciated level in the same group in the presence of a high glucose concentration (27 mM). The standard drug glibenclamide exhibited a similar effect compared to the 80 % MAO fraction. Though 80 % MAO fraction and glibenclamide showed similar effects, comparatively higher insulin levels were observed in 80 % MAO fraction treated cells. The other fractions: PEAO, CHAO, and EAAO promoted insulin secretion to a better level than the control group in the absence and presence of glucose. But, in presence of high glucose concentration, the extracts showed a comparatively lesser amount of insulin correlated to the same treatment groups in the absence of glucose. Similarly, a decrease in insulin level was observed in the control group in presence of glucose than in its absence. This observation suggests hyperglycemia impairs the insulin secretory machinery of β-cells and that the extracts PEAO, CHAO, and EAAO could be ruled out as antidiabetic agents. (Fig. 3).

Effect of 80 % methanolic fraction of *A. occidentale* roots on INS gene expression

The effect of 80 % methanolic fraction of *A. occidentale* roots in stimulating glucose-dependent insulin secretion was quantitatively analyzed by Real-time PCR. The expression of the INS gene in effect to the standard drug Glibenclamide and 80 % MAO revealed that the 80 % methanolic fraction of *A. occidentale* roots up-regulated the gene to a greater extent than the control and Glibenclamide. The test group treated with glucose alone (27mM) exhibited down-regulation of the INS gene. (Fig. 4).

Discussion

Glucose is the key regulator of insulin synthesis and secretion. However, exposure of β-cells to supraphysiological glucose concentration results in incessant stimulation of the cells leading to insulin store exhaustion and reduced insulin secretion, impaired insulin gene expression, generation of oxidative stress, and apoptosis of the cells [27]. Thus, managing hyperglycemia has become an inevitable factor for the prevention of diabetes and its associated complications. Also, recovering the deteriorated β-cells and normalizing its function, and maintaining the gene expression even in the hyperglycemic environment offers a therapeutic means of preventing the onset of diabetes; where our study becomes substantial. In the present study, we have investigated the effect of petroleum ether, chloroform, ethyl acetate, and 80 % methanol extracts of *A. occidentale* roots to promote insulin secretion while protecting the β- cells and *INS* gene expression from hyperglycemia and associated oxidative stress. In this study, the MIN6 pancreatic β-cells when incubated in the absence and presence of a high concentration of glucose (27mM) and the four extracts of *A. occidentale* roots (PEAO, CHAO, EAAO, and 80 % MAO), 80 % MAO at concentration 12.5 μg/mL was found to be a potent insulin secretagogue. In addition, 80 % MAO exhibited a significant level of insulin in presence of 27mM of glucose and was higher than that in Glibenclamide treated group. Thus, our data demonstrate that 80 % MAO could promote insulin secretion even under hyperglycemic conditions. The present study can be correlated to the *in vitro* study by Keller et al., in MIN6 pancreatic β-cells. The study reported that the saponins momordicine II and kuguaglycoside G from the traditional plant *Momordica charantia* at concentrations 10 and 25 μg/mL respectively stimulated insulin secretion under high concentrations of glucose (27mM) [25].

The Insulin gene (*INS*) almost exclusive to pancreatic cells encodes for the pancreatic hormone insulin that is secreted uniquely by the β-cells [28]. Insulin maintains glucose metabolism and blood glucose homeostasis by binding to specific insulin receptors expressed by the hepatic, muscle, and adipose cells through a cascade of biochemical reactions. A lack of production or abnormalities in the secretion or improper response of cells to insulin can lead to the pathogenesis of all types of diabetes and mainly type I Diabetes mellitus and type II Diabetes mellitus [29]. *In vivo* study by Harmon et al., in type 2 diabetes models reported that persistent exposure of β-cells to high glucose levels would distress insulin

Table 2 Amount of total phenolic and flavonoid contents and total antioxidant capacity of Petroleum ether, Chloroform, Ethyl acetate, and 80 % Methanolic Extracts of *A. occidentale* roots

A. occidentale Extracts	TPC (μg of GAE/mg)	TFC (μg of QE/mg)	TAC(μg of AAE/mg)
PEAO	390.67 ± 0.08	13.96 ± 0.07	1315.33 ± 0.2
CHAO	404 ± 0.11	46.04 ± 0.10	1865.33 ± 0.09
EAAO	407.33 ± 0.16	32.83 ± 0.12	552 ± 0.23
80 % MAO	437.33 ± 0.03	21.51 ± 0.09	552 ± 0.12

TPC Total phenolic content, *TFC* Total flavonoid content, *TAC* Total antioxidant capacity, *GAE* Gallic acid equivalents, *QE* Quercetin equivalents, *AAE* Ascorbic acid equivalents; *PEAO* Petroleum ether extract of *A. occidentale*, *CHAO* Chloroform extract of *A. occidentale*, *EAAO* Ethyl acetate extract of *A. occidentale*, *80 % MAO* 80 % Methanol extract of *A. occidentale*
*Results are expressed as mean ± SD (*n* = 3)

gene expression and thereby insulin secretion and therefore preventing hyperglycemia could preserve insulin and PDX-1 gene expression [30]. From the Real-Time PCR analysis, a double fold up-regulation in the *INS* gene was observed in the cells treated with 80 % MAO compared to Glibenclamide. This reinforces the finding that 80 % MAO could stimulate insulin secretion both at its transcriptional and translational level while managing hyperglycemia. The downregulation of the gene in the test group treated with glucose alone (27mM), further confirms the finding of Harmon et al.

Earlier studies report that high glucose levels could induce β- cell apoptosis mainly because of oxidative stress [31] and thereby declined insulin content in the cell [32]. A weak expression of antioxidant enzymes is exhibited by the pancreatic islets, which makes them more susceptible to oxidative stress [1, 4]. The continuous exposure of cells to oxidative stress results in the generation of ROS in mitochondria and also in the formation of intracellular glycation end products that glycate the antioxidants making the β-cells further deprived of antioxidative enzymes [10]. Also, it is the single hyperglycemia that causes the overproduction of superoxides by the mitochondrial electron transport chain and ends up in diabetes-specific microvascular disease, a prime cause for retinopathy, nephropathy, neuropathy, and myocardial infarction [10]. 80 % MAO protects the β-cells from hyperglycemia-induced oxidative stress which in turn helped normalize the insulin secretory machinery of the cells. This observation is strengthened by the significant free radical scavenging (IC_{50} = 0.026 ± 0.5 mg/mL) and the reducing power (IC_{50} = 9.59 ± 0.71 mg/mL) of the extract. Besides, 80 % MAO exhibited an appreciated amount of phenolic and flavonoid content as well as total antioxidant capacity compared to PEAO, CHAO, and EAAO, which further strengthens its

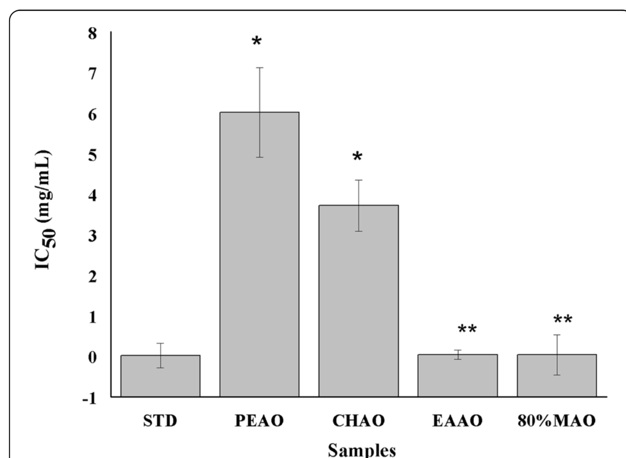

Fig. 1 DPPH scavenging activity of Petroleum ether, Chloroform, Ethyl acetate, and 80 % Methanol Extracts of ***A. occidentale.*** * PEAO: Petroleum ether extract of A. occidentale, CHAO: Chloroform extract of A. occidentale, EAAO: Ethyl acetate extract of A. occidentale, 80% MAO: 80% Methanol extract of A. occidentale. * Results are expressed as mean ± SD (*n*=3). '*' indicates values significantly differ from the standard at *P*˂0.001. '**' indicates values significantly differ from the standard at P˂0.05.

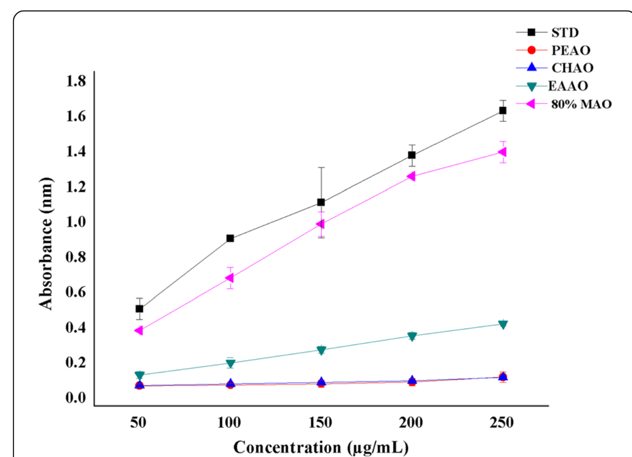

Fig. 2 Reducing power activity of Petroleum ether, Chloroform, Ethyl acetate, and 80 % Methanol Extracts of ***A. occidentale.*** * PEAO: Petroleum ether extract of A. occidentale, CHAO: Chloroform extract of A. occidentale, EAAO: Ethyl acetate extract of A. occidentale, 80% MAO: 80% Methanol extract of A. occidentale. * Results are expressed as mean ± SD (*n*=3).

Table 3 Percentage Cell Viability of Petroleum ether, Chloroform, Ethyl acetate, and 80 % Methanolic Extracts of *A. occidentale* roots against MIN6 cells

Concentration (μg/mL)	Cell Viability (%)			
	PEAO	CHAO	EAAO	80 % MAO
100	83.94 ± 0.006*	82.89 ± 0.004*	88.24 ± 0.001*	81.90 ± 0.006*
50	86.92 ± 0.004*	87.44 ± 0.005*	92.49 ± 0.007*	83.00 ± 0.001*
25	90.86 ± 0.005*	90.41 ± 0.002*	96.06 ± 0.008*	87.83 ± 0.004*
12.5	95.45 ± 0.005*	92.35 ± 0.006*	99.29 ± 0.004*	90.05 ± 0.008*
6.25	98.31 ± 0.005*	95.83 ± 0.008*	100	92.23 ± 0.003*
3.12	99.49 ± 0.003*	97.68 ± 0.003*	100	97.24 ± 0.004*
1.56	100	99.29 ± 0.002*	100	99.15 ± 0.003*

PEAO Petroleum ether extract of *A. occidentale*, *CHAO* Chloroform extract of *A. occidentale*, *EAAO* Ethyl acetate extract of *A. occidentale*, *80 % MAO* 80 % Methanol extract of *A. occidentale*
*Results are expressed as mean ± SD ($n = 3$). $P^{<}0.05$ vs. control

antioxidant efficacy. The phenolic and flavonoid compounds are known for their antioxidant property; they are used as anti-oxidative agents. The hydroxyl group in phenolics acts as a free radical scavenger and thus, a positive relationship between total phenolics and the antioxidant property is observed in plant species [33]. Kaemferol, a flavonol at 10 μM concentration protected pancreatic β-cells from apoptosis and promoted better cell viability and function under chronic hyperglycemic function [34]. The antioxidant property of *A.occidentale* is attributed to its polyphenol content. The ultrasonic extraction of cashew leaves yielded antioxidative phenolics. An increase in the total phenolic content (579.55 mg GAE/g) of the extract increased its reducing power (10.28 ± 0.21mmol TE/g) and free radical scavenging (12.14 ± 0.01mmol TE/g) properties and thus, the extract exhibited a protective effect towards DNA damage by peroxyl radical [35]. This result is consistent with our findings on the phenolic content and antioxidant properties of 80 % MAO.

Conclusions

The antidiabetic efficacy of the four sequentially solvent-extracted fractions Petroleum ether (PEAO), Chloroform (CHAO), Ethyl acetate (EAAO), and 80 % Methanol (80 % MAO) of *Anacardium occidentale* roots evaluated at concentration 12.5 μg/mL in MIN6 pancreatic β- cell lines revealed 80 % MAO to exhibit potent antidiabetic activity by promoting insulin secretion both at its transcriptional and translation

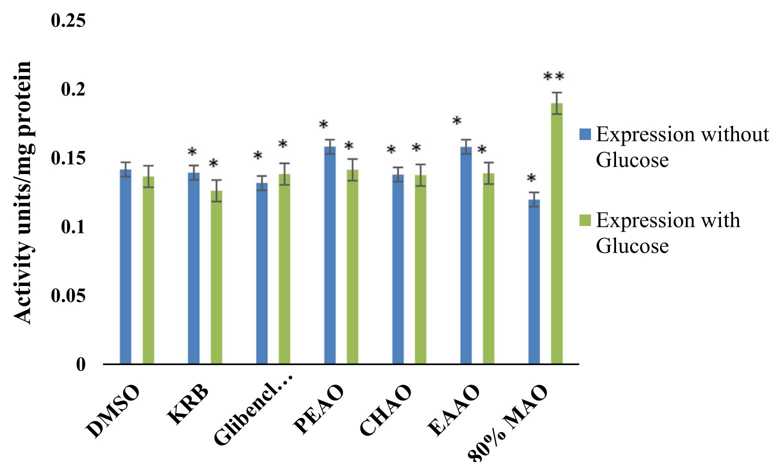

Fig. 3 Effect of four different solvent extracts of ***A. occidentale*** roots on Insulin secretion in MIN6 cells in the presence (27mM) and absence of Glucose. * DMSO: Dimethyl sulfoxide, KRB: Krebs Ringers Buffer, PEAO: Petroleum ether extract of A. occidentale, CHAO: Chloroform extract of A. occidentale, EAAO: Ethyl acetate extract of A. occidentale, 80% MAO: 80% Methanol extract of A. occidentale.'*' indicates values significantly differ from Control at $P^{<}0.05$. '**' indicates values significantly differ from the standard at $P^{<}0.01$.

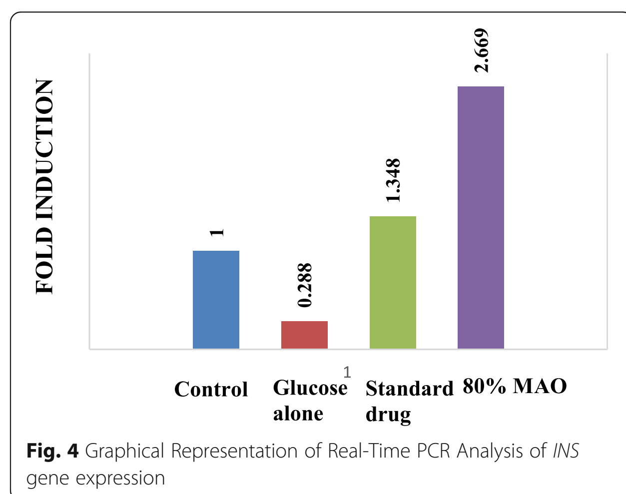

Fig. 4 Graphical Representation of Real-Time PCR Analysis of *INS* gene expression

level. The potent antioxidant activity, as well as the ability of the fraction to promote insulin synthesis and secretion even at a higher level of glucose (27 mM), indicates the capacity of the extract to protect the β-cells from oxidative stress. The 80 % methanolic extract of *A. occidentale* roots thus, offers a promising lead drug candidate in developing an antidiabetic drug that even could attend to complications associated with diabetes. Above mentioned properties have to be studied further by identifying the active principles of *A. occidentale* root extracts and *in vivo* effects. The prospect of the present study is identifying drug leads for better management of diabetes from the *A. occidentale* root extracts.

Abbreviations

PEAO: Petroleum ether fraction of *Anacardium occidentale* roots; CHAO: Chloroform fraction of *Anacardium occidentale* roots; EAAO: Ethyl acetate fraction of *Anacardium occidentale* roots; 80 % MAO: 80 % methanol fraction of *Anacardium occidentale* roots; INS: Insulin gene

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Authors' contributions

Archana T. M. executed the experimental part, analyzed the data, and drafted the manuscript. Dr. Sudheesh S. guided the study by designing and coordinating the work. Soumya K. and Jesna James helped in drafting the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during the present study are available from the corresponding author on reasonable request.

Declarations

All the authors have read and approved the manuscript and agree with its submission to the journal. This manuscript has not been published elsewhere and is not under consideration by any other journal.

Ethics approval and consent to participate

NA.

Consent for publication

NA.

Competing interests

The authors declare that they have no competing interests.

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