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Salacia oblonga aqueous root extract restores β-cell function in experimental diabetes via antioxidant effects and MAFA upregulation

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ABSTRACT

Optimizing the function of the existing β-cells is a potential strategy for managing hyperglycemia in diabetes. Musculo aponeurotic fibrosarcoma oncogene A (MAFA) is a β-cell-specific transcription factor crucial for binding to insulin enhancer elements to promote insulin secretion. Cytotoxic stress from chronic hyperglycemia diminishes the β-cell potential, which can be mitigated using natural antidiabetic agents. In this study, oxidative stress was induced in β-cells using streptozotocin in male Wistar rats to assess the antioxidant properties of aqueous root extract of *Salacia oblonga* (ARSO). Diabetic animals received oral doses of glibenclamide (2 mg/kg) and ARSO (200 mg/kg) for 8 weeks. Blood glucose levels, ultrastructural changes in β-cells, histopathological assessments, MAFA immunohistochemistry, transcription and translation processes, and antioxidant status within pancreatic tissues were evaluated. The group treated with ARSO exhibited a significant 54% reduction in hyperglycemia and demonstrated enhanced antioxidant status, indicating the antidiabetic and antioxidant properties of *S. oblonga*. Histopathological analysis confirmed the functional restoration of β-cells, as indicated by an increase in cellular organelles in the ARSO-treated group. Additionally, there was a 63% increase in MAFA-expressing cells, along with a 2.2-fold increase in MAFA gene expression and a 1.8-fold increase in protein expression in ARSO-treated diabetic animals compared with the control. These findings suggest that *S. oblonga* enhances the expression of β-cell-specific transcription factors and has potential as an effective therapeutic agent for diabetes management, warranting further investigations.

1. INTRODUCTION

Pancreatic β-cells, which make up 60%–70% of the islet cell mass, are responsible for secreting insulin, a hormone crucial for maintaining blood glucose homeostasis [1]. Impaired insulin secretion and peripheral resistance contribute to the pathogenesis of various types of diabetes. Therefore, elucidating β-cell epigenesis, function, regulation, and dysfunction is crucial for diabetes management. β-cells originate from progenitor cells of the dorsal pancreatic bud derived from the endoderm of the foregut, which are initially common for both exocrine and endocrine cells, and consequently differentiate into endocrine cells and ultimately into insulin-producing β-cells [2]. Thus, understanding developmental and epigenetic modulators will facilitate advances in regenerative medicine and research.

Several transcription factors, such as neurogenic differentiation 1, pancreatic and duodenal homeobox-1 (PDX-1), and musculo aponeurotic fibrosarcoma oncogene A (MAFA), regulate glucose-dependent insulin gene expression [3]. These transcription factors are classified based on their DNA-binding domains into categories, such as zinc finger, homeobox, basic helix-loop, and basic leucine zipper proteins [4]. MAFA, a basic leucine zipper protein, binds to the insulin enhancer element and acts with other regulators to activate insulin gene expression.

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MAFA is a transcription factor specific to β-cells that is expressed during the later stages of maturation and is crucial for the survival and function of β-cells [5]. MAFA knocked out mice have shown a deficiency in insulin-producing cells and altered islet cell populations [6]. Thus, a thorough examination of MAFA provides a more comprehensive understanding of β-cell dysfunction and potentially elucidates novel therapeutic targets.

Despite advancements in diabetes management, current therapeutic interventions are associated with adverse effects such as gastrointestinal disturbances, weight gain, hypoglycemia, and cardiovascular risks, and are unable to maintain long-term glycemic control [7]. Moreover, treatments that specifically target β-cell dysfunction and oxidative stress with minimal adverse effects are limited. Globally, individuals utilize alternative medicines for the management of diabetes, under the assumption that these therapies have fewer side effects than conventional pharmacological treatments. The prevalence of such utilization varies across demographic and geographical segments. Oxidative stress plays a crucial role in the pathogenesis of diabetes and its associated complications. Therefore, the investigation of effective antioxidants is crucial. In this context, *Salacia oblonga*, recognized for its peroxide scavenging and antioxidant properties [8,9], might offer a significant advancement in antidiabetic therapeutics by directly addressing oxidative stress-induced β-cell dysfunction. Traditionally, it has been used to treat ailments such as obesity, asthma, rheumatism, hemorrhoids, skin diseases, gonorrhea, and amenorrhea. This woody climbing shrub has been widely studied for its antidiabetic effects. *In vivo* studies have demonstrated that *S. oblonga* exhibits significant inhibitory activity against alpha-glucosidase and alpha-amylase, resulting in decreased levels of blood glucose, HbA1c, insulin, bilirubin, creatinine, and lipids, and increased levels of high density lipoprotein cholesterol in nicotinamide- and streptozotocin (STZ)-induced type II diabetic rats. The aqueous methanol extract of *S. oblonga* effectively reduced sucrose- and maltose-induced postprandial hyperglycemia in rats. Furthermore, an aqueous extract of *S. oblonga* containing mangiferin increased glucose transporter-4 (GLUT4)-mediated glucose uptake in L6 rat myotubes. These findings substantiate the potential of *S. oblonga* in managing diabetes and regulating lipid levels. Clinical trials involving the administration of 480 mg *S. oblonga* extract with meals and a beverage containing a mixture of *S. oblonga* yielded promising outcomes in controlling postprandial hyperglycemia and insulin levels, respectively, suggesting a mechanism similar to that of α-glucosidase inhibition [10,11]. Preliminary investigations conducted by our research group demonstrated the presence of phenols, flavonoids, tannins, and flavanols in the aqueous extract of *S. oblonga* roots. High-performance liquid chromatography analysis revealed the presence of 16 metabolites, including ponkoranol, salasol, mangiferin, and kotalanol, consistent with previous studies [12].

Despite its established antidiabetic and antioxidant properties, the effect of *S. oblonga* on pancreatic transcription factors, such as MAFA, has not been extensively studied. Therefore, this study aimed to examine the antioxidant properties of the aqueous root extract of *Salacia oblonga* (ARSO) in modulating MAFA expression to elucidate its mechanism of action.

2. MATERIALS AND METHODS

2.1. Plant collection and ARSO preparation

The roots of wild *S. oblonga* procured from the local market were verified and validated by the Pharmacognosy Department at the Siddha Central Institute, Chennai (PCOG003-TRP). Finely ground root powder (100 g) was subjected to aqueous extraction using 3 l of distilled water at 50°C for 15 minutes. Subsequently, the solution was filtered and lyophilized. The powder obtained (yield: 3.28%) was stored in a desiccated container.

2.2. Animal study design

The study was conducted after obtaining permission from the Institutional Animal Ethics Committee and adhered to the regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals. Male Wistar rats (~220 g) were housed in an institutional animal housing facility under standardized conditions. The sample size was calculated using G*Power software (version 3.1.9.7) for one-way ANOVA with five groups. Assuming a medium effect size (f = 0.25), significance level of 0.05, and standard statistical power level, it was determined that a minimum of 11 animals per group would be required to detect a statistically significant difference. However, the number of animals was limited to six per group due to ethical and practical considerations. Therefore, 30 rats were equally allotted to five groups, as described below.

Group – I: Control
Group – II: Normal rats + ARSO
Group – III: Untreated diabetes mellitus (DM) group
Group – IV: DM + glibenclamide
Group – V: DM + ARSO

2.3. Induction of DM

The euglycemic status of the animals was determined by measuring the fasting blood glucose (FBS) levels using a glucometer (Accu-Chek Active device; Roche Diabetic Care, USA) with venous samples obtained from the tail vein. Animals in Groups III to V were administered a single intraperitoneal dose of 40 mg/kg body weight STZ (SRL, Mumbai, India, #14653) prepared in 50 mM cold citrate buffer (pH 4.5). To prevent hypoglycemia, animals were administered 10% sucrose water for 2 days. Three days after the induction of DM, animals with FBS levels ≥250 mg/dl were included in the study [13].

2.4. Administration of experimental drugs

ARSO (200 mg/kg) was administered to animals in groups II and V using distilled water as the vehicle, whereas animals in group IV received 2 mg/kg glibenclamide (TCI Co. LTD, Tokyo, Japan, #G0382) in a 0.5% carboxymethyl cellulose solution orally for a duration of 8 weeks [14,15]. Equal volumes of distilled water were administered to groups I and III.

2.5. Sample collection

Blood collected from the tail vein was used for weekly FBS measurements. The animals were euthanized with ketamine hydrochloride (100 mg/kg body weight; NEON Laboratories Limited, Mumbai, India) administered intraperitoneally [13].

The splenic part of the pancreatic tissues was dissected and preserved in 10% formalin (pH 7) and 3% glutaraldehyde for histopathological and electron microscopic examinations. Unfixed tissues were kept at −80°C to study gene and protein expression, as well as to conduct antioxidant analyses.

2.6. Measurement of antioxidant markers

Pancreatic tissue homogenized with Tris-HCl buffer (0.1 M, pH 7.5) was centrifuged for 5 minutes at 4,000 rpm, and the supernatant was used for antioxidant analyses. Thiobarbituric acid reactive substances (TBARSs) were quantified spectrophotometrically (532 nm) following the procedure outlined by Kılıç *et al.* [16] to assess lipid peroxidation. The activities of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were evaluated spectrophotometrically using Ellman's (412 nm), Xanthine/Xanthine Oxidase (560 nm), and dichromate-acetic acid (570 nm) methods, respectively [17–19].

2.7. Histopathological and immunohistochemical (IHC) analysis

For histopathology, 4 µm-thick pancreatic sections were stained with hematoxylin and eosin (H&E). For IHC, 3 µm-thick paraffin sections were incubated at 60°C–70°C for 20 minutes. Antigen retrieval and endogenous peroxidase blocking were performed using Tris-ethylenediaminetetraacetic acid buffer in a pressure cooker (10–15 minutes) and H_2O_2 (10 minutes). The sections were treated with polyclonal anti-MAFA antibody (1:100 dilution; Novus Biologicals, USA, #NBP2-24636) for 45 minutes and horseradish peroxidase-labeled secondary antibody (Biogenex, USA) for 12 minutes. The sections were then treated with diaminobenzidine (Biogenex, USA) for 5 minutes and counterstained with Harris hematoxylin (30 seconds). The H&E- and IHC-stained slides were photographed under a microscope [20].

2.8. Morphometric analysis

The fractional volume of MAFA-immunopositive cells was quantified using a reticule with 121 intersections on immunohistochemically stained slides (400× magnification), as described by Subramanian *et al.* [21]. In each group, five fields were randomly selected from 10 different sections of each animal, resulting in a total of 50 fields examined per animal.

2.9. Ultrastructural study

Following post-fixation with 1% osmium tetroxide, the glutaraldehyde-fixed tissues were embedded in propylene oxide and epoxy resin. Ultrathin sections (70 nm) were routinely stained, visualized, and photographed using a transmission electron microscope (Philips Tecnai T12 Spirit, Netherlands) [22].

2.10. Gene expression analysis

Pancreatic tissue stored at −80°C was processed for reverse transcription-quantitative polymerase chain reaction using SYBR Green, following the manufacturer's guidelines [22]. The MarabanX RNA extraction kit (ImmuGenix Biosciences, India; #IGB-R-27017-B) was used for RNA extraction, and the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA, #K1622) was used to synthesize cDNA. The following primers were designed using NCBI primer-BLAST: *Mafa* forward: 5′ GCTTCAGCAAGGAGGAGGTCAT 3′, reverse 5′ TCTCGCTCTCCAGAATGTGCCG 3′; *Gapdh* forward: 5′ AGTTCAACGGCACAGTCAAG 3′, reverse 5′ TACTCAGCACCAGCATCACC 3′. Gene expression levels were measured using the 2-ΔΔCt method, with *Gapdh* serving as the reference. Each experiment was conducted thrice, and the average results were used for analysis [21,23].

2.11. Western blot analysis

Proteins were isolated from cryopreserved pancreatic tissues following standard procedures. The proteins were separated on a 10% SDS-PAGE gel and transferred to polyvinylidene fluoride membranes. Blocking was performed using Tris-buffered saline with Tween-20 containing 5% non-fat milk, followed by incubation with GAPDH (1:1,000 dilution) and unconjugated polyclonal MAFA antibodies (Novus Biologicals, USA, #NBP2-24636, 1:1,000 dilution). The membranes were then incubated with horseradish peroxidase-labeled secondary antibodies at a 1:500 dilution for 1 hour. The presence of MAFA was detected using an electrochemical luminescence reagent [24], and band densities were analyzed using computer software (Adobe Photoshop CS6, version 12.0).

2.12. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Differences between groups were assessed using ANOVA, followed by Tukey's multiple-comparison test using statistical software (SPSS, version 29, IBM, USA) with $p < 0.05$.

3. RESULTS

3.1. Antioxidant activity

Table 1 displays the findings related to the antioxidant markers assessed at the end of the study. GSH, SOD, CAT, and TBARS levels in the pancreatic tissue were significantly improved in the glibenclamide- and ARSO-treated groups than in the untreated group ($p < 0.001$). The levels of GSH, SOD, and CAT in group V animals were approximately equivalent to those in groups I and II, suggesting that the antioxidant properties of ARSO effectively counteracted the STZ-induced oxidative damage. Group V ($p < 0.001$) demonstrated superior antioxidant activity compared to group IV ($p < 0.01$), indicating that *S. oblonga* possesses greater antioxidant potential than glibenclamide.

3.2. Fasting blood glucose

Figure 1 illustrates the trend in FBS levels across various groups. In untreated diabetic rats, FBS levels were consistently and significantly elevated throughout the experiment than the control. By the end of the eighth week, a significant reduction in FBS levels was observed in group V (158.83 ± 1.37 mg/dl) and group IV animals (155.66 ± 2.01 mg/dl) ($p < 0.001$). Although a downward trend was observed in the FBS levels, they remained outside the normal range, indicating the necessity for an extended course of treatment with ARSO. Rats

in group II, treated with ARSO, exhibited a modest decrease (4 mg/dl) in FBS levels, suggesting a potential effect of *S. oblonga* on maintaining normoglycemic conditions. No significant difference was observed in FBS levels between animals treated with glibenclamide and those treated with ARSO.

3.3. Histopathological changes

3.3.1. H&E staining

Group I animals displayed normal histological features of their pancreas. Group II showed minimal changes in the exocrine portion, with serous acini exhibiting an increased eosinophilic cytoplasm and more prominent zymogen granules. In contrast, group III animals demonstrated notable atrophic and inflammatory alterations in the islets. The islet diameter was reduced, and the cell mass was lower than that in the control. Lymphocytic infiltration was observed without a distinct boundary between exocrine and endocrine tissues. These pathological alterations were significantly diminished in diabetic rats that received treatment (Groups IV and V) (Fig. 2).

Table 1. Antioxidant markers in pancreatic tissue of different groups.

Group	GSH(mU/mg)	SOD(U/mg)	CAT(µM/mg)	TBARS (nM/mg)
I	2.73 ± 0.01	72.60 ± 0.06	42.28 ± 0.23	0.52 ± 0.01
II	2.78 ± 0.01*	73.33 ± 0.16	43.32 ± 0.16	0.53 ± 0.00
III	1.16 ± 0.01$	41.69 ± 0.38$	27.94 ± 0.35$	1.11 ± 0.03$
IV	2.36 ± 0.02$#	65.54 ± 1.07$#	38.70 ± 0.16$#	0.61 ± 0.01$#
V	2.54 ± 0.02$#@	70.22 ± 0.53$#@	42.57 ± 0.15#@	0.59 ± 0.01$#

GSH – Glutathione, SOD - Superoxide dismutase, CAT - Catalase, TBARS - Thiobarbituric acid reactive substances. The results are expressed as the mean ± SEM, $n = 6$. The superscript indicates a significant difference based on Tukey's multiple-comparison test following one-way ANOVA ($p \leq 0.05$): * - comparison with Group I; $ - comparison with Groups I & II; # - comparison with Group III; @ - comparison with Group IV.

3.3.2. Immunohistochemistry

IHC analysis of pancreatic sections using the MAFA antibody demonstrated nuclear immunoreactivity in cells located within islets (Groups I and II). The number of MAFA-expressing cells was considerably reduced in untreated diabetic animals. However, in Groups IV and V, MAFA-immunopositive cells with varied intensities were relatively increased and more centrally distributed within the islets, indicating a positive response to treatment and their localization within the β-cells that occupied the central part of the islets (Fig. 2).

3.4. Morphometric analysis

Quantitative analysis of the IHC slides revealed a 63% reduction in the fractional volume of MAFA-expressing cells (Fig. 3A) in the untreated STZ-induced diabetic group (III). In contrast, in the glibenclamide- and ARSO-treated groups, there

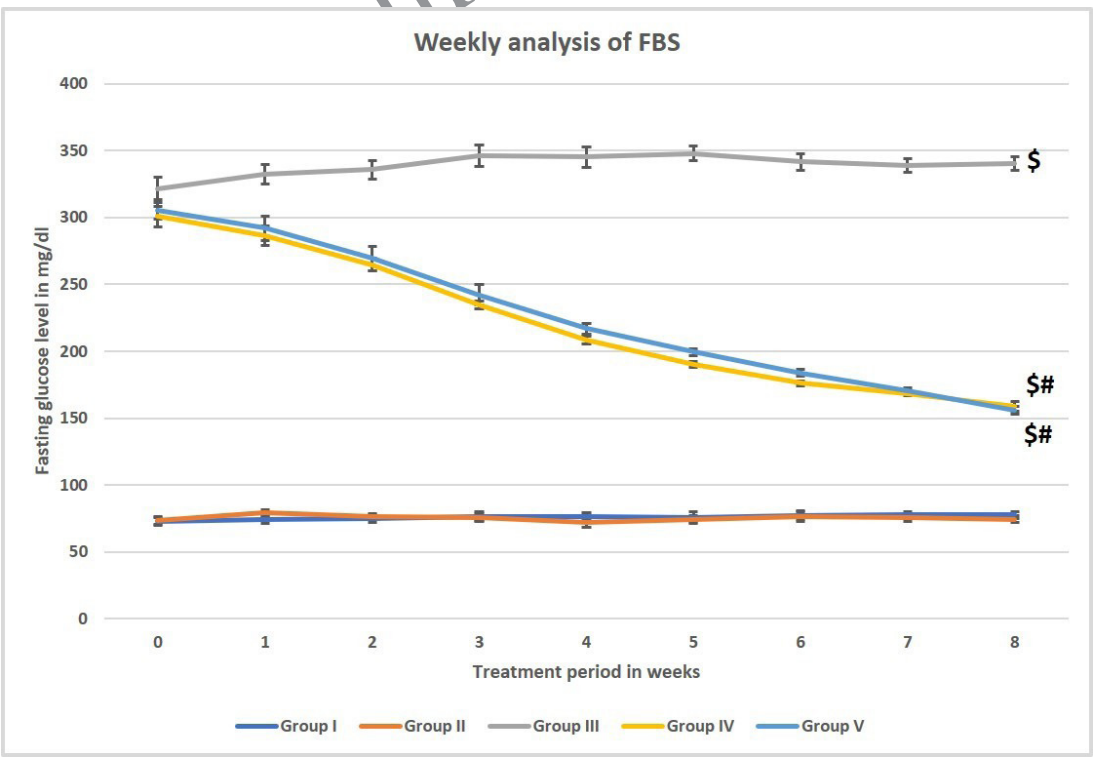

Figure 1. FBS of different groups from the beginning to the end of study. The results are expressed as the mean ± SEM, $n = 6$. The superscript indicates a significant difference based on Tukey's multiple-comparison test following one-way ANOVA ($p \leq 0.05$): $ - comparison with groups I and II; # - comparison with group III.

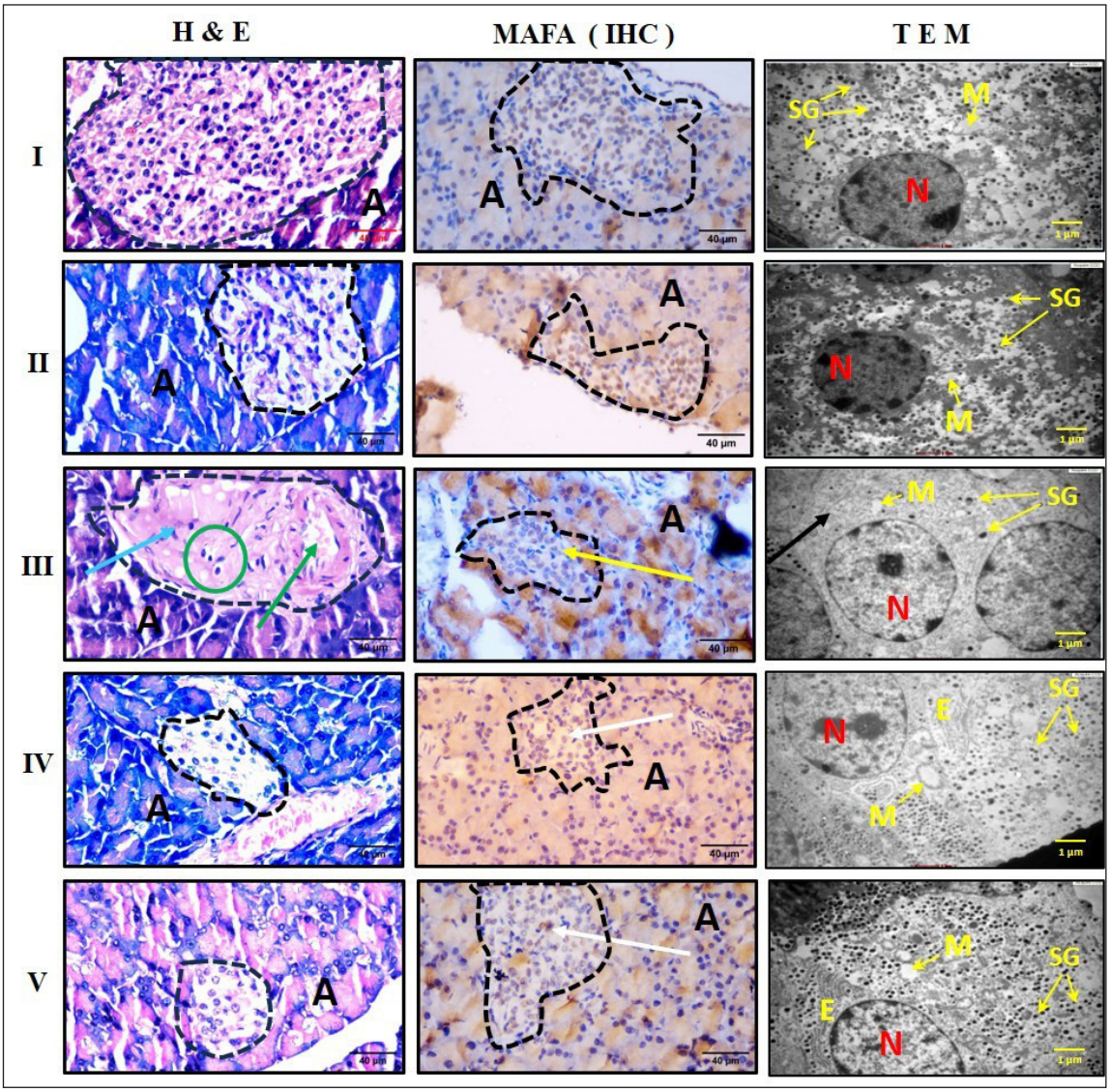

Figure 2. H & E (40×), IHC (40×) and electron micrographs (6,000×) of pancreas. H & E – Hematoxylin and Eosin, IHC – Immunohistochemistry, TEM – Transmission electron microscopy. I, II, III, IV, V – Experimental groups. A – Acini, Dotted outline – Islet of Langerhans, Green arrow – Atrophic changes, Blue arrow – Nuclear karyolysis, Green circle – Lymphocytic infiltration, Yellow arrow – Non-immunopositive cells, White arrow – Immunopositive cells, N – β-cell nucleus, SG – Secretory granules, M – Mitochondria, E – Endoplasmic reticulum.

was an increase in the fractional volume of MAFA-expressing cells (30% ± 2.25% and 47.67% ± 3.25%, $p < 0.001$).

3.5. Ultrastructural changes

The ultrastructure of β-cells in groups I and II showed secretory granules with a typical halo around them (Fig. 2). Group III exhibited atrophic changes, including colloidal masses, a marked reduction in typical secretory granules, empty vacuoles, and scanty cell organelles in β-cells, indicating sluggish activity possibly due to STZ-induced oxidative stress. In groups IV and V, β-cells exhibited enhanced secretory granules and cell organelles. Immature and mature β-cells coexisted in the islet cell populations of groups IV and V, containing proinsulin, which eventually will form insulin-secreting granules. In particular, the ARSO-treated group (V) demonstrated numerous mitochondria and endoplasmic reticulum, indicative of a functionally active state. Additionally, an increase in alpha and delta cells and prominent zymogen granules in the acinar regions was observed in group V animals, indicating the effect of ARSO on other endocrine cells and exocrine secretion (not shown).

3.5.1. Mafa gene expression

Figure 3B illustrates the relative quantification of *Mafa* gene expression among the different groups using *Gapdh* as a housekeeping gene. *Mafa* gene expression was downregulated 2.5-fold in group III untreated diabetic rats compared to that in control animals. Conversely, *Mafa* expression was 1.8 and

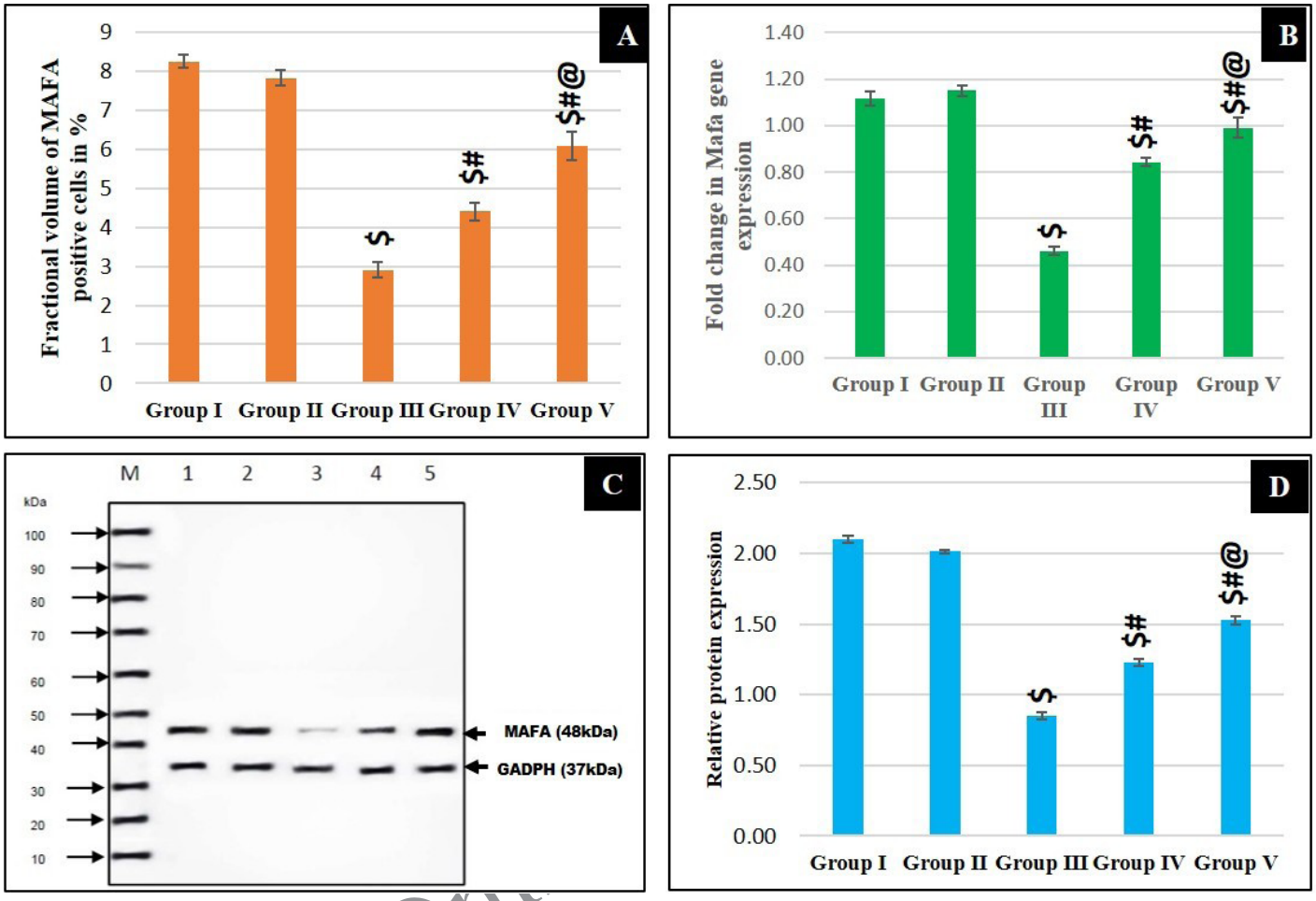

Figure 3. Fractional volume of MAFA positive cells, Mafa gene expression, Western blot protein expression gel image and graph. (A) shows fractional volume of MAFA positive cells (IHC), (B) shows relative *Mafa* gene expression, (C) shows a representative gel image of MAFA protein expression in the various groups. The *y*-axis indicates the molecular weight of the protein in kilo Dalton (kDa). M - Marker Lane, 1: Group I, 2: Group II, 3: Group III, 4: Group IV, 5: Group V, (D) shows the relative MAFA protein expression in various groups. Values are represented as mean ± SEM, $n = 6$. The superscript indicates a significant difference between groups based on Tukey's multiple-comparison test following one-way ANOVA ($p \leq 0.05$): $ - comparison with Groups I and II. # - comparison with Group III: @ - comparison with Group IV.

2.2 fold upregulated in diabetic rats treated with ARSO and glibenclamide, respectively, compared to group III. Although statistically insignificant, the observed increase in *Mafa* expression in group II suggests that ARSO may play a role in *Mafa* expression under normoglycemic conditions.

3.6. MAFA protein expression

Qualitative analysis of MAFA protein expression via western blotting revealed faint bands on SDS-PAGE for group III (diabetic untreated rats), which were 2.6 fold lower than those of the control (Fig. 3C). In contrast, MAFA expression increased 1.5 and 1.8 folds in the diabetic-treated groups IV and V, respectively, suggesting the presence of mature and functional β-cells. Notably, ARSO-treated group V animals showed higher MAFA expression (1.53, $p = 0.02$) than group IV (glibenclamide-treated rats), indicating that *S. oblonga* has a more pronounced effect on MAFA expression than glibenclamide (Fig. 3D).

These findings demonstrate that ARSO effectively ameliorated structural abnormalities in the islets and enhanced MAFA expression, a β-cell-specific marker. Furthermore, considering the improved antioxidant status of the pancreas in the ARSO-treated group (Table 1), the increase in MAFA gene and protein expression could be attributed to the antioxidant properties of ARSO.

4. DISCUSSION

Despite studies reporting the antidiabetic properties of *S. oblonga* through reduced glucose absorption, improvement in insulin secretion, and reduction in peripheral resistance [12], its action on β-cells remains elusive. In the present study, ARSO exhibited notable antidiabetic properties in rats with STZ-induced diabetes, as evidenced by a reduction in FBS levels, improvement in antioxidant status, restoration of pancreatic histoarchitecture, and upregulation of MAFA gene and protein expression. These findings indicate a novel molecular mechanism underlying the action of *S. oblonga* on β cells.

4.1. Glycemic control and lipid modulation

The hypoglycemic effect of ARSO administration was consistent with the established α-glucosidase and α-amylase inhibitory properties of *S. oblonga*, which delay carbohydrate digestion and mitigate postprandial hyperglycemia [15]. Comparative studies have indicated that hydroalcoholic root extracts of *S. oblonga* achieved glycemic control comparable to the hypoglycemic efficacy of standard medications, such as glibenclamide and acarbose, with the additional benefit of improving lipid profiles [10,15]. Our previous findings verified the positive effect of ARSO on insulin secretion and lipid profiles, establishing its beneficial role in normalizing lipid metabolism in addition to glucose metabolism [25].

4.2. Antioxidant effects

In diabetes, oxidative stress is involved in the progression of β-cell dysfunction [26]. In the present study, ARSO administration enhanced antioxidant status (SOD, CAT, and GSH) and attenuated lipid peroxidation (TBARS) in the pancreatic tissues of diabetic animals, suggesting potent antioxidant activity. Our results aligned with those reported in a prior study by Bhat *et al.* [9] and may be attributed to the phytoconstituents of ARSO, such as flavonoids, phlobatannins, terpenoids, phenols, and alkaloids [27]. Flavonoids, by enhancing antioxidant capacity, have been shown to protect β-cells from autophagy, apoptosis, and necroptosis [28]. These mechanisms are regulated by nuclear factor kappa B (NF-κB) and nuclear factor erythroid 2-related factor 2 [29], and the modulation of which by ARSO requires further investigation.

4.3. Pancreatic histopathology and ultrastructure

STZ-induced diabetes leads to pancreatic β-cell degeneration, which was ameliorated by *S. oblonga* treatment, as observed in the histopathological and ultrastructural analyses. Histopathological findings were in agreement with the results of a previous study conducted by Gladis Raja Malar *et al.* [30], which showed a reduction in inflammatory changes, β-cell degranulation, and cytoplasmic vacuolation following the administration of *S. oblonga*. Transmission electron microscopy results showed that the ARSO-treated group had well-defined cell organelles and prominent insulin granules compared to group III animals, reflecting the functional status of β-cells, which was consistent with previous studies [17]. The variable electron density observed in the insulin granules of the treated groups (IV and V) indicated the co-existence of both immature and mature granules within the β-cell mass. The presence of immature granules suggests the existence of proinsulin, which eventually matures into insulin-secreting granules [23]. These findings demonstrate that the administration of ARSO effectively preserved pancreatic architecture, with a particular emphasis on the mediation of β-cell preservation.

4.4. MAFA upregulation

The transcription factor MAFA specifically binds to the conserved region of the insulin gene promoter (RIPE3b1) and regulates insulin gene expression in mature β cells [3]. Its expression is highly sensitive to oxidative stress, which downregulates MAFA and other transcriptional regulators such as PDX1, Pax4, and Nkx6.1 [29]. In this study, untreated diabetic rats showed reduced MAFA gene and protein expression, which was significantly restored by ARSO treatment, likely due to its antioxidant effects. The upregulation of MAFA by *S. oblonga* is a significant finding because glucose-dependent expression of MAFA is essential for the normal production of insulin in mature β-cells [31]. Our research group previously reported that ARSO upregulates PDX-1 expression, further supporting its role in β-cell preservation and function [25].

4.5. Mechanistic insights from other plant extracts and phytochemicals

Various plant extracts influence MAFA expression through distinct mechanisms in experimental models of diabetes. Extracts of *Hibiscus rosa-sinensis* and *Centaurium erythraea* upregulate MAFA and PDX-1 by alleviating oxidative stress, inflammation, and apoptosis [32,33]. Furthermore, they modulated the expression of transcriptional regulators of cellular antioxidant defense mechanisms, NF-κB, and Nrf2. Extracts of *Gynura divaricate* and mulberry branch bark powder also elevated the expression of MAFA and PDX-1, along with anti-apoptotic effects (Bax and caspase-3), and improved insulin signaling [Phosphatidylinositol 3-Kinase/Protein Kinase B (PI3K/AKT), glycogen synthase, phosphorylated glycogen synthase kinase 3β (GSK3β), GLUT4, and PPARγ] [34,35].

Among the isolated phytochemicals, gymnemic acid, a triterpenoid saponin from *Gymnema sylvestre*, enhances *MAFA* expression via the activation of PI3K/AKT, E-cadherin, and β-catenin, while inhibiting forkhead box O1, GSK3β, and cyclin-dependent kinase inhibitor 1 [36]. Kotalagenin 16-acetate, a triterpenoid saponin, present in *S. oblonga* [12], may exert a similar influence on β-cell regeneration, although the exact mechanism remains to be elucidated. Other compounds such as quercetin, resveratrol, ebselen (a GSH peroxidase mimetic), and luteolin have also been shown to enhance MAFA expression by modulating oxidative stress, mitochondrial biogenesis [via sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)], or the inflammatory signaling pathway (NF-κB/inducible nitric oxide synthase/nitric oxide) [37–40].

In contrast, extracts of *Nigella sativa* and *Securigera securidaca* have been reported to downregulate MAFA and PDX-1 [41,42]. This downregulation was attributed to disrupted phosphorylation pathways in a hyperglycemic environment, which compromised MAFA transcriptional function despite apparent gene upregulation through altered extracellular signal-regulated kinase and p38 signaling.

In line with these findings, our study demonstrated that ARSO administration enhanced MAFA gene and protein expression, suggesting a potential role in improving β-cell function. Mechanisms observed with other plant extracts and phytochemicals, particularly in modulating oxidative stress, inflammation, and key regulators and signaling pathways such as PI3K/AKT, SIRT1, PGC-1α, and NF-κB, need to be evaluated further to establish ARSO-mediated MAFA regulation.

Herbal formulations containing *S. oblonga* in combination with agents such as *G. sylvestre, Tinospora*

cordifolia, Curcuma longa, and *Emblica officinalis* have demonstrated additive effects in preclinical and clinical studies [43]. These combinations target complementary pathways, including insulin secretion, glucose uptake, β-cell regeneration, and inflammation. Such multimodal combinations could enhance therapeutic efficacy and may reduce the need for higher doses of conventional drugs, potentially lowering side effects. However, standardization of the extraction processes and identification of the active compounds, particularly those influencing MAFA, are essential for reproducibility and clinical translation. Despite promising clinical data, large-scale prospective studies are required to ascertain the long-term efficacy, safety, and interactions of *S. oblonga* with other pharmacological agents.

4.6. Limitations

The limitations of this study include its short duration (8 weeks) and the lack of a dose-dependent response, β-cell-specific transcriptome profiling, detailed evaluation of inflammatory mediators, and intracellular mechanistic pathways are required, which would significantly enrich our understanding of the protective effects of ARSO on β cells. Moreover, extrapolating these findings from animal models to human populations, potential adverse effects following prolonged use and interactions with other pharmaceutical agents have not been examined and should be evaluated in the future.

5. CONCLUSION

This study demonstrated that ARSO exhibits significant antidiabetic effects through various mechanisms. It improved glycemic control and antioxidant status, restored pancreatic β-cell structure and function, and upregulated MAFA gene and protein expression in the pancreatic tissues of diabetic rats. The upregulation of MAFA, an important transcription factor for β-cell function and insulin production, represents a novel mechanism of action of *S. oblonga* in diabetes management. This effect was likely mediated by the antioxidant properties of the extract. The findings on MAFA expression indicate that *S. oblonga* is one of the few natural substances that directly affect this key regulator of β-cell function. This may pave the way for its potential use as a nutraceutical or adjunct therapy for the management of diabetes. Further research is required to identify the active compounds responsible for MAFA upregulation, elucidate the detailed molecular pathways involved, and clinical trials are to be conducted to validate its efficacy and safety in humans.

6. ACKNOWLEDGMENTS

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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9. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

The study protocol was approved by the Scientific Review Committee and Institutional Animal Ethics Committee of the Sri Venkateswara Medical College Hospital and Research Centre (SVMC/SRC/2022/24/CTR 744 and 1/IAEC/SVMCH/01/2023, respectively). This study was performed in accordance with the principles of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

12. PUBLISHER'S NOTE

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13. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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