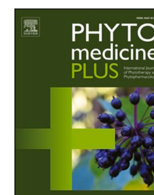
Phytomedicine Plus 2 (2022) 100284

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus



Synergistic hypoglycemic and hypolipidemic effects of ω-3 and ω-6 fatty acids from Indian flax and sesame seed oils in streptozotocin-induced diabetic rats

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ARTICLE INFO

Keywords:
Omega fatty acid
ω-3/ω-6 ratio
Synergism
Diabetes mellitus
Histopathology

ABSTRACT

Background: Omega-3 and 6 fatty acids (FAs) of flaxseed oil (FSO) and sesame seed oil (SSO) that are being used in traditional foods and medicine have been investigated for their various synergistic biological potencies. However, their synergistic antidiabetic and antilipidemic properties at the world health organization (WHO) recommended ratio have not been studied.
Purpose: To evaluate the synergistic antidiabetic and antipidemic potentials of bioactive-fatty acids from flaxseed (FS) and sesame seeds (SS) against streptozotocin (STZ)-induced diabetes mellitus Wistar albino rats.
Study design: The study was to determine the *in vitro* and *in vivo* synergistic antidiabetic and antipidemic potentials of ω-3 and ω-6 FAs at 1:5 ratio from FSO and SSO in STZ-induced diabetes mellitus rats.
Methods: Fifty-four Wistar albino rats were divided into 9 groups: normal control; diabetic control received streptozotocin 45 mg/kg b.w IP; diabetic groups received standard drug tolbutamide orally received 5mg/kg b.w; FSO and SSO orally administered with 250 and 350; 516 and 700 mg/kg b.w PO respectively, and FSO+SSO at 43+292 and 86+584 mg/kg b.w PO respectively. Blood glucose levels were observed on weekly basis. At the end of the experiment, biochemical factors such as the level of antioxidant enzymes, serum liver enzymes, malondialdehyde level, lipid profiles, blood parameters, and pancreas morphology were studied.
Results: Twenty-eight days of the treatment significantly increased the *in vivo* antioxidant enzyme activities such as catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH), whereas, malondialdehyde (MDA) level was markedly decreased in a dose-dependent manner in the pancreas and liver. A noteworthy reduction was observed in the lipid profiles of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and increase in high-density lipoprotein (HDL), and also significant reduction was observed in the hematological parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, urea, glycosylated hemoglobin (HbA1c) and decreased blood glucose levels, while, total protein (TP), hemoglobin (Hb) and insulin levels were markedly increased in the treated rats in a dose-dependent manner compared to the diabetic control. Additionally, the results were also confirmed by histopathological examinations.
Conclusion: The study suggested that the ω-3 and ω-6 FAs from FSO and SSO, respectively, showed potential synergistic antidiabetic and antilipidemic effects that were mainly mediated by ω-3 and ω-6 FAs present in the respective seed oils.

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https://doi.org/10.1016/j.phyplu.2022.100284

Available online 18 April 2022
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Abbreviations

ALA	α-linolenic acid
ALP	alkaline phosphate
ALT	alanine transaminase
AST	aspartate transaminase
CAT	catalase
CVDs	cardiovascular diseases
DHA	docosahexaenoic acid
DM	diabetes mellitus
DNS	3, 5-dinitrosalicylic acid
EPA	eicosapentaenoic acid
FAs	fatty acids
FS	flaxseed
FSO	flaxseed oil
GSH	glutathione
Hb	hemoglobin
HbA1c	glycosylated hemoglobin
HDL	high-density lipoprotein
I.P	intraperitoneal
LA	linoleic acid
LCPUFA	long-chain polyunsaturated fatty acids
LDL	low-density lipoprotein
MDA	malondialdehyde
MUFA	monounsaturated fatty acid
NIDDM	non-insulin-dependent diabetes mellitus
OA	oleic acid
PO	per orally
PUFA	polyunsaturated fatty acid
ROS	reactive oxygen species
SFA	saturated fatty acid
SOD	superoxide dismutase
SS	Sesame seed
SSO	sesame seed oil
STZ	streptozotocin
T2DM	type-2 diabetes
TC	total cholesterol
TG	triglycerides
TP	total protein
VLDL	very-lowdensity lipoprotein

Introduction

Diabetes mellitus (DM) is included in a group of chronic metabolic disorders, which is characterized by hyperglycemia, hyperuricemia, hypoinsulinemia, and impaired carbohydrates, proteins, and lipids metabolism (Nazir et al., 2018; JV et al., 2019). According to International Diabetes Federation, approximately 463 million people are suffering from diabetes around the globe, rising to 700 million by 2045, and projects that DM will be the seventh leading cause of death (Saeedi et al., 2019). The American Diabetes Association classified the DM cases into type-1 diabetes mellitus (T1DM) and type-2 diabetes mellitus (T2DM). Type-1diabetes (insulin-dependent DM; IDDM) is an immune-mediated disease characterized by the deficiency of insulin secretion *i.e.*, destruction of pancreatic β-cells, which leads to absolute insulin deficiency (Iwase et al., 2015). The high concentration of blood glucose level and other biochemical abnormalities, result in either insufficient amount of insulin secretion by pancreatic cells of islet of Langerhans or insensitivity of target organs to insulin, that is seen in non-insulin-dependent DM (NIDDM), which is also known as type-2 diabetes (T2DM) that is more prevalent in adult age (Gandhi and Sasikumar, 2012).

Although the mechanisms of DM complications remain unclear, preclinical and clinical trials have proved that there is an increase in the production of reactive oxygen species (ROS), including superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH•) or reduction in the antioxidant defense system (Kamalakkannan and Prince, 2004; Makni et al., 2011; Bouhrim et al., 2019). About 80% of diabetic patients are from developing and underdeveloped countries, of which China and India shared larger contributions (Ramachandran et al., 2010; JV et al., 2019). Thus, the increase in the prevalence of T2DM is considered a global problem and it will become an epidemic disease in the future if proper preventive measurements are not taken.

Several synthetic hypoglycemic agents such as sulfonylureas, biguanides, meglitinides, thiazolidinediones, glucosidase inhibitors, and peptide analogs are currently used either alone or in combination to treat diabetes but, some like biguanides and sulfonylureas cause serious side effects associated with drawbacks, such as hypoglycemia, gastrointestinal discomfort, weight gain, nausea, liver toxicity, and also have a high price (Ramachandran et al., 2012). Therefore, extracts from some medicinal plants have proved promising anti-diabetic effects due to the presence of antioxidant and free radical scavenging agents like polyphenols and omega fatty acids. These might also help in the regeneration of β-cells previously damaged due to the oxidation caused by high blood glucose levels. Omega fatty acids might also protect pancreatic islets against the cytotoxic effects of streptozotocin (STZ) and thus, can offer an alternative approach in treating diabetes (Nazir et al., 2018).

The dietary long-chain polyunsaturated fatty acids (LCPUFA) like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are present in some marine sources and natural seeds have been shown to prevent CVDs, hyperglycemia, and dyslipidemia (Makni et al., 2010; Kaithwas and Majumdar, 2012). Many investigations indicated that intake of unsaturated fatty acids from fish oils has improved glycemic controls, enhancement of high-density lipoprotein (HDL) cholesterol levels, and enhanced membrane lipid peroxidation induced by free radicals in T2DM patients (Sirtori and Galli, 2002; Makni et al., 2010).

Makni et al. (2011) have reported that the mixture of flax and pumpkin seeds showed antioxidant and hypolipidemic effects in alloxan-induced diabetes in rats. Combined treatment of ω−3 and metformin showed a significant elevation in estradiol, which was strongly associated with indices of bone mineralization in STZ-induced diabetic rats (Adeyemi et al., 2020). Khadke et al. (2020) have demonstrated that flax/fish oil and glibenclamide synergistically ameliorated the lipid abnormalities through modulation of expression of transcription factors (NF-kβ, SREBP1-c, and PPAR-γ) and their regulatory genes (FASN, ACACA, FABP, CPT1, and TNF-α) in diabetic dyslipidemic conditions.

Flaxseed oil (FSO) and sesame seed oil (SSO) are the oldest commercial oils that have been used for centuries as fungicidal lotion, insecticide, paints and varnishes, and also extensively used in traditional foods (Kaithwas and Majumdar, 2012). In our previous study, we have demonstrated that the ω−3 and ω−6 FA from FSO and SSO, respectively, have shown more synergistic antioxidant potential compared to their treatments against CCl_4-induced liver damage in rats (Sunil et al., 2021). Nevertheless, the balanced diet of ω−3 and ω−6 FA is important due to their competitive nature with different biological roles to ensure the conversion of ALA to EPA and DHA (Simopoulos, 2016).

An optimal ratio of ω−3:ω−6 FAs in the diet prevents the pathogenesis of many inflammatory diseases (Nehdi et al., 2019). Both ω−3 and ω−6 FA are beneficial in improving lipid profiles in T2DM patients and among healthy individuals (Jeppesen et al., 2013). Greenlandic Inuit, who eats the diet rich in marine foods enriched with ω−3 FA, have low rates of DM and coronary heart diseases compared with Danes, who eats a Western diet (Kromann and Green, 1980). The Dietary guidelines for Americans and American Heart Association, recommend 5–10% of energy be obtained from LA. But, French national guidelines recommend 4%. Because, based on some studies, researchers hypothesized that LA can have harmful effects, since it competes with ω−3 FA (Wu et al., 2017). The Global Burden of disease data suggests that both long chain ω−3 and ω−6 FA need to be increased worldwide, theories suggest that ω−3 and ω−6 FAs compete in some metabolic pathways. So that, the ω−3/ω−6 ratio is more important than their individual intake (Hartweg et al., 2008). According to the 2015–16, National Health and Nutrition

Examination Survey data that indicates ω−3 (ALA): ω−3 (LA) ratio of ~1:9 for United Sates (US) males and females of 20 years and above, suggesting a highly unbalanced intake of ω−3:ω−6 FAs in the Western diet as compared to the ω−3:ω−6 FAs ratio of ~1:2 for the outmoded hunter-gatherer diet intake by pre-agricultural humans (Zirnheld et al., 2019).

Based on the literature survey, and also as per recommendations of the World Health Organization (WHO), also based on preliminary findings of our previous study, the study was designed to obtain the recommended standard ω−3:ω−6 FAs ratio from the flaxseed per oil (FSO) and sesame seed oil (SSO) by blending in the proportion of 1:5 ratio (Makni et al., 2011; Khadke et al., 2020; Gurumallu et al., 2022).

Owing to various health beneficial and disease preventive synergistic properties of PUFAs such as hypoglycemic, dyslipidemic, antioxidant, anti-diabetic effects, the present study was undertaken to investigate the synergistic anti-diabetic and antilipidemic effects of FSO rich in ω−3 FA and SSO in ω−6 FA in streptozotocin-induced diabetic rats.

Materials and methods

Chemicals

The chemicals such as 3, 5-dinitrosalicylic acid (DNS), p-nitrophenyl-*α*-D-glucopyranoside (PNPG), nitoblue tetrazolium (NBT), 5, 5-dithio bis-2 nitro benzoic acid (DTNB), thiobarbituric acid (TBA), boron tri-fluoride methanol (BF_3), porcine pancreatic *α*-amyalse, *Saccharomyces cerevisiae* *α*-glucosidase, streptozotocin and standard fatty acid mixtures (Supelco-37) were purchased from Sigma-Aldrich Chemical Co., St. Louis Mo, USA. The diagnosis kits were purchased from Span Diagnostics Ltd., India. Tolbutamide tablets were procured from a local pharmaceutical company in Mysore, India. All other chemicals, reagents, and solvents were purchased from SD Fine-Chem Ltd., Mumbai, India., and the water used was double distilled.

Seed material

Both FS and SS were procured from the Department of Oil Science, University of Agricultural Sciences (UAS), Dharwad, India. The seeds were certified as Mugad and SRI-2 varieties of FS and SS, respectively, and stored at 4 °C for future analyses.

Extraction of oil and determination of fatty acid composition

The FS and SS were subjected to screw press oil extraction (German double-barrel screws-2005, Shakti Farms, Mysuru, India) at 27 °C. The fixed oil compositions of the oils were determined by gas chromatography-mass spectrometry (GC–MS) analysis (PerkinElmer, USA). The FA profile was assessed through GC–MS. Before analysis, the oils were dried under nitrogen and methylated according to the method of Morrison and Smith (1964) with boron tri-fluoride methanol complex solution (15% BF_3).

Preparation of oil mixture

The FSO and SSO were blended in the proportion of 1:5 ratio as per our previous study, and also as per recommendations of the WHO to obtain the recommended standard ω−3:ω−6 FAs ratio (Makni et al., 2011; Khadke et al., 2020).

Determination of in-vitro anti-diabetic activities

α-Amylase enzyme inhibition

The porcine pancreatic α-amylase inhibition potential of FSO and SSO was evaluated using the 3, 5-dinitrosalicylic acid (DNS) assay with minor modifications (Banerjee et al., 2017). In brief, 100 μL of oil in methanol at 1:1 ratio were added to 200 μL of an α-amylase enzyme (2 units/mL) and incubated at 30 °C for 10 min. Later on, 200 μL of 1% (w/v) starch was added and then the mixture was incubated at room temperature (RT) for 3 min. The reaction was stopped by adding 200 μL of DNS reagent (12 g of sodium potassium tartrate dissolved in 8 mL of 2 M NaOH and 20 mL of 96 mM DNS). The reaction mixture was incubated in a circulating water bath at 90 °C for 10 min followed by the addition of 5 mL distilled water. The absorbance was measured at 540 nm. The α-Amylase enzyme inhibition was calculated by using the equation:

$$\%\ \alpha\text{- amylase inhibition} = ((A_{control}\text{-}A_{sample})/A_{control}) \times 100 \qquad ( )$$

α-Glucosidase enzyme inhibitory assay

The *Saccharomyces cerevisiae* *α*-glucosidase inhibition potential of FSO and SSO was determined by the method of Ranilla et al. (2010) with minor modifications. In brief, 100 μL of oil in methanol at 1:1 ratio were added to 100 μL of the *α*-glucosidase enzyme (0.5 U/mL), in 600 μL of 0.1 M phosphate buffer at pH 6.9. The mixture was incubated at 37 °C in a circulating water bath for 15 min. The enzymatic reaction was initiated by adding 100 μL of 5 mM p-nitrophenyl-*α*-D-glucopyranoside, and again it was incubated at 37 °C for 15 min at RT. The reaction was stopped by adding 400 μL of 0.2 M sodium carbonate. The absorbance was measured at 405 nm. The *α*-glucosidase enzyme inhibition was calculated by using the equation,

$$\%\ \alpha\text{- glucosidase inhibition} = ((A_{control}\text{-}A_{sample})/A_{control}) \times 100 \qquad ( )$$

The IC_{50} values of *α*-amylase and α-glucosidase were determined from the plots of% inhibition *versus* log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. All the tests were performed in triplicates. Acarbose was used as the standard.

Experimental animals

Fifty-four, male Wistar albino rats weighting 175–200 g were obtained from the animal house, Department of Studies in Zoology, Manasagangotri, University of Mysore, Mysuru, India, after approval of the Institutional Animal Ethics Committee, University of Mysore [UOM/IAEC/15/2020; Dated: 10/3/2020]. The animals were maintained as per the principle and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. All the animals were maintained under standard laboratory conditions (24 ± 2 °C, 12/12 h light-dark cycle), fed with a standard pellet diet, and allowed free access to water *ad libitum* during 28 days of the experimental period. Before starting the experiment, all the animals were allowed to acclimatize for laboratory conditions for a week.

Induction of diabetes

The experiment was conducted in nine groups, each consisting of six animals. After overnight fasting, diabetes was induced by a single intraperitoneal injection (I.P.) of freshly prepared solution of streptozotocin (STZ; 45 mg/kg b.w.) in 0.1 M citrate buffer of pH 4.5, A glucosamine derivative of nitrosourea, which selectively destroys pancreatic islets of *β*-cells and results in the development of symptoms like hyperglycemia and glycosuria. Then, the diabetic rats were allowed to drink 5% (w/v) glucose solution overnight to overcome the early phase of drug-induced hypoglycaemic death. The blood glucose levels of rats were measured after 48 h, through tail tipping using a glucometer (Glucocard-01 Mini, Bengaluru). Those rats with fasting blood glucose levels >250 mg/dL were considered as diabetic and included in the study. At the time of induction, control rats were injected with 0.2 mL of vehicle (0.1 M citrate buffer of pH 4.5) alone and STZ-treated rats were given food and water *ad libitum*.

- Group-1: Control rats received an equal volume of vehicle orally (P.O.)
- Group-2: Diabetic group received STZ 45 mg/kg/ b.w. I.P.
- Group-3: Diabetic rats received the standard anti-diabetic drug-tolbutamide (20 mg/kg b.w. P.O; Positive group)
- Group-4: Diabetic rats received FSO [258 mg per kg b.w P.O, FSO contains 150 mg of ω−3 FA]
- Group-5: Diabetic rats received SSO [350 mg per kg b.w P.O, SSO contains 150 mg of ω−6 FA]
- Group-6: Diabetic rats received FSO [516 mg per kg b.w P.O, FSO contains 300 mg of ω−3 FA]
- Group-7: Diabetic rats received SSO [700 mg per kg b.w P.O, SSO contains 300 mg of ω−6 FA]
- Group-8: Diabetic rats received FSO+SSO [43+292 mg/kg b.w P.O, FSO+SSO contains 25+125 mg of ω−3 & ω−6 at 1:5 ratio]
- Group-9: Diabetic rats received FSO+SSO [86+584 mg/kg b.w P.O, FSO+SSO contains 50+250 mg of ω−3 & ω−6 at 1:5 ratio]

During the experimental period, the blood glucose level and body weights of the rats were measured on weekly basis *i.e.*, on days 0, 7, 14, 21, and 28. Glycosylated hemoglobin and lipid profile were estimated on the 28th day. The rats were sacrificed under ether anesthesia and euthanized to collect blood samples, and the organs *i.e.*, pancreas and liver, were separated and preserved for further analyses.

Determination of in vivo antioxidant activities

Pancreas/liver homogenates were prepared by using a phosphate buffer of 125 mM, pH 7.4, and used to measure enzymatic and non-enzymatic antioxidants.

Catalase

Catalase activity was determined according to the method of Baureder et al. (2014). In brief, 10 μL of tissue homogenates were put into 1.9 mL of 125 mM phosphate buffer, pH 7.4. The reaction was initiated by adding 1 mL of 30 mM H_2O_2. A decrease in the optical density due to the decomposition of H_2O_2 was measured at the end of 1 min at 240 nm. Catalase activity was expressed as U/mg protein.

Superoxide dismutase

Superoxide dismutase activity was based on the reduction of nitroblue tetrazolium (NBT) to water-insoluble blue formazan as described by the methods of Siddhuraju and Becker (2003). 5 μL of tissue homogenates were taken and 1 mL of 125 mM sodium carbonate, 0.4 mL of 24 μM NBT, and 0.2 mL of 0.1 mM ethylenediaminetetraacetic acid (EDTA) were added. The reaction was initiated by adding 0.4 mL of 1 mM hydroxylamine hydrochloride. Zero-time absorbance was measured at 560 nm followed by recording the absorbance after 5 min. A single unit of SOD activity was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was presented as U/mg protein.

Reduced glutathione

The glutathione (GSH) level was determined by the method of Moron et al., 1979 based on the development of a yellow color when 5, 5-dithio bis-2 nitro benzoic acid (DTNB) was added to compounds. In brief, 500 μL of tissue homogenate was added to 3 mL of 4% (w/v) sulfosalicylic acid. The mixture was centrifuged at 1100 g for 15 min and the supernatant was collected. 2 ml of 0.6 Mm Ellman's reagent (DTNB) was added to 500 μL of the supernatant and the absorbance was measured at 420 nm after 10 min.

Measurement of malondialdehyde

Lipid peroxidation activity was carried out according to the method described by the methods of Arora et al. (2008). Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen. 5 μL of tissue homogenate and 1 mL of 0.15 M KCl were taken, and peroxidation was initiated by adding 250 μL of 0.2 mM $FeCl_3$ and incubated at 37 °C for 30 min. The reaction was arrested by adding 2 mL of an ice-cold mixture of 0.25 N HCl containing 15% (v/v) trichloroacetic acid, 0.30% (w/v) TBA, and 0.05% (v/v) butylated hydroxytoluene, heated at 80 °C for 60 min. The samples were cooled, and the results were expressed as MDA an equivalent that was calculated by using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$. One unit of lipid peroxidation activity is defined as the amount of TBA that gets converted to TBA reactive substances. Thus, the specific activity was expressed as U/mg of protein.

Protein concentration in tissue homogenates was estimated by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Estimation of biochemical parameters

The blood samples were collected by direct cardiac puncture under mild ether anesthesia and were stored with and without disodium EDTA tubes depending upon the estimation of respective biochemical parameters. The hemoglobin (Hb), glycosylated hemoglobin (HbA1c), and blood glucose were determined using whole blood. The aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), total protein, creatinine, urea, and insulin in serum were measured by using commercially available biochemical diagnostic kits using semiautomatic Erba Chem 5x clinical chemistry analyzer (Kaithwas and Dipak, 2012; Azad and Sulaiman, 2020). The low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels were calculated by using the following equation (Ramachandran et al., 2012)

$$VLDL = Triglycerides/5$$

$$LDL = TC - (HDL + VLDL)$$

Histopathological evaluation

A small portion of the pancreas from the control and treatment groups was fixed in a 10% formalin solution. The tissues were processed as per the standard protocol of the paraffin section method and technique. Pancreas tissues were cut into 4 μm sections using a rotary microtome, stained with hematoxylin and eosin (Sy and Ang, 2019). Thereafter, the slides were observed under the microscope (Zeiss A2m) and photographed (Axion Vision LE64).

Statistical analysis

The data were analyzed by using the statistical package program SPSS version 16.0 by One Way Analysis of Variance (ANOVA); $P < 0.05$ was to determine the significant differences between treatments, and the values are expressed as mean ± SD.

Results

The FSO confirmed the presence of 5.34 ± 0.00% palmitic acid, 3.92 ± 0.01% stearic acid, and 0.09 ± 0.01% eicosanoid acid, whereas, those were in the range of 8.87 ± 0.09%, 4.90 ± 0.00%, and 0.47 ± 0.00%, respectively, in the case of SSO. Similarly, 12.19 ± 0.00% oleic acid, 18.05 ± 0.01% linoleic acid and 58.28 ± 0.01% alpha-linolenic acid were present in the FSO, while SSO exhibited their presence as 40.54 ± 0.00%, 42.86 ± 0.00%, and 0.28 ± 0.06%, respectively (Table 1). Thus, the analysis showed that the sum of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) was higher in SSO compared to FSO. However, the sum of MUFA and PUFAs was higher in FSO compared to SSO. The degrees of unsaturation of these oils were higher than the common vegetable oils. These levels of unsaturation could be

Table 1
Fatty acids composition of FSO and SSO.

Fatty acids*	FSO (%)	SSO (%)
Saturated fatty acids (SFAs)		
Lauric acid, C12:0	0.35 ± 0.00	ND
Palmitic acid, C16:0	5.34 ± 0.00	8.87 ± 0.09
Stearic acid, C18:0	3.92 ± 0.01	4.90 ± 0.00
Eicosanoic acid, C20:0	0.09 ± 0.01	0.47 ± 0.00
Total SFA	9.71 ± 0.03	14.25 ± 0.09
Monounsaturated fatty acids (MUFAs)		
Oleic acid, C18:1(ω−9)	12.19 ± 0.00	40.54 ± 0.00
Gondoic acid C20:1(ω−9)	0.08 ± 0.00	0.10 ± 0.01
Palmitoleic acid, C16:1 (ω−7)	ND	0.11 ± 0.00
Total MUFA	18.13 ± 0.00	43.08 ± 0.02
Polyunsaturated fatty acids (PUFAs)		
α-linolenic acid, C18:3 (ω−3)	58.28 ± 0.01	0.28 ± 0.06
Linoleic acid, C18:2 (ω−6)	18.05 ± 0.01	42.86 ± 0.00
Arachidonic acid, C20:4 (ω−6)	0.09 ± 0.00	0.10 ± 0.01
γ-linolenic acid, 18:3 (ω−6)	0.16 ± 0.00	ND
Total PUFA	70.72 ± 0.00	40.92 ± 0.08
Total fatty acids	98.59 ± 0.04	98.26 ± 0.19

* Data were expresses as mean±SD, $n = 3$; ND = Non detected FA.

an interesting potential in maintaining health (Makni et al., 2010; Figueiredo et al., 2017). The analyses confirmed the presence of the interested bioactives viz ALA and LA in FSO and SSO, respectively, and also met the required standards. Therefore, the oils reported in the present investigation were used in the study.

In vitro antidiabetic activities

The IC_{50} values of α-amylase were calculated by evaluating the plot of % α-amylase inhibition as a function of different concentrations of standard acarbose, FSO, SSO, and FSO+SSO from 20 to 100 μg/mL. The % of α-amylase inhibition potential of FSO, SSO, and FSO+SSO were 43.36 ± 1.63, 47.19 ± 0.45, and 62.20 ± 2.20, respectively with their IC_{50} values 96.68 ± 2.03, 91.02 ± 3.50, and 54.73 ± 2.44 μg/mL, respectively at the highest concentration (100 μg/mL). It was observed that the combination of FSO+SSO was more effective than their individual treatments and showed the highest % inhibition of α-amylase with the lowest IC_{50} value (Fig. 1). Acarbose was used as a standard, which showed 78.07 ± 1.07% inhibition with 34.03 ± 0.58 IC_{50} value. All the samples showed concentration-dependent activities.

The α-glucosidase activities of FSO, SSO, and FSO+SSO were compared with standard acarbose at the same concentration (20–100 μg/mL). The % of α-glucosidase enzyme inhibition potential of FSO, SSO, and FSO+SSO were 46.89 ± 0.53, 39.74 ± 0.51 and 57.95 ± 1.24, respectively with their IC_{50} values 92.11 ± 1.43, 109.07 ± 2.98, and 60.24 ± 1.57 μg/mL, respectively at the highest concentration (100 μg/mL). The combination of FSO+SSO exhibited more potential than their individual treatments and showed the greater% inhibition of α-glucosidase with the lowest IC_{50} value (Fig. 2). Acarbose was used as a standard, which showed 69.10 ± 0.45% inhibition with 40.41 ± 1.81 IC_{50} value. All the samples showed concentration-dependent activities.

In summary, it was clear that FSO+SSO inhibited α-amylase and α-glucosidase enzymes significantly, and their efficacies were comparable to the standard acarbose.

Bodyweight and blood glucose level in STZ-induced diabetic rats

The present studies showed no lethality reactions at any of the selected FSO and SSO dosages until the end of the study.

STZ-induced diabetic rats showed a significant reduction in body weight as presented in Table 2. Diabetic control rats exhibited decreased body weights (18.56%) and showed a significant difference ($p < 0.001$) when compared to the normal control group. The body weight was significantly increased (33.89%) in the tolbutamide treated group ($p < 0.001$) compared to the diabetic control group. The administration of FSO (258 and 516 mg/kg b.w) and SSO (350 and 700 mg/kg b.w) gradually increased body weight in dose-dependent manner. The combined treatment of FSO+SSO (43+292 and 86+584 mg/kg b.w) to diabetic rats resulted in the highest weight gain (33.84 and 34.87%, respectively) that was closer to the normal control group, in dose-dependent mode ($p < 0.001$) when compared to the diabetic control group.

Table 3 shows the levels of blood glucose in animals of normal control and treatment group rats on days 0, 7, 14, 21, and 28 after drug administration. The diabetic control group rats exhibited an increase in blood glucose levels ($p < 0.001$) until 28 days of the experimental study when compared to the normal control group. There was a significant reduction ($p < 0.001$) in the tolbutamide-treated animals compared to the diabetic control group. Administration of FSO (258 and 516 mg/kg b.w) and SSO (350 and 700 mg/kg b.w) gradually decreased the blood

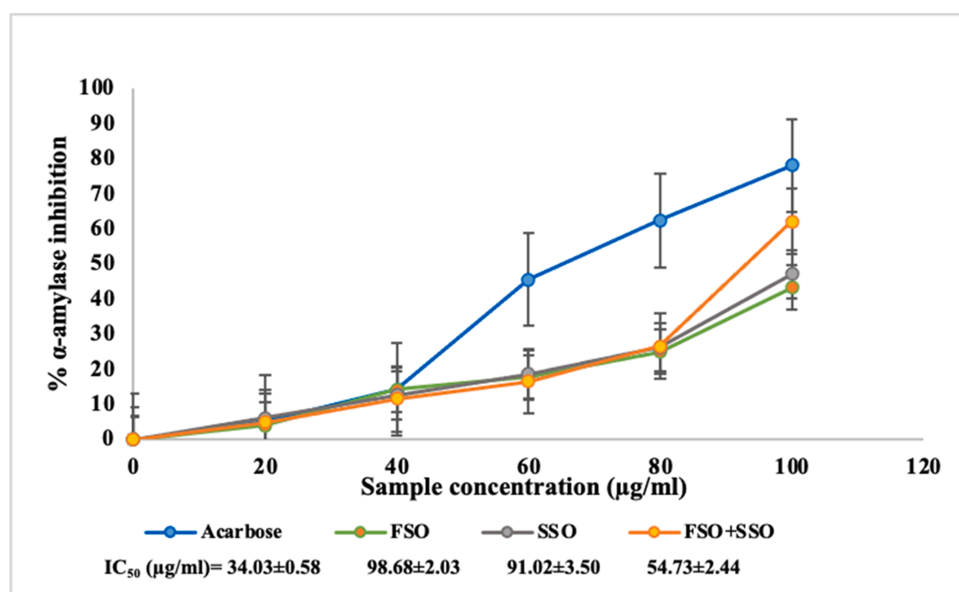

Fig. 1. Inhibitory effects of FSO, SSO, FSO+SSO and standard against porcine pancreatic α-amylase at different (20–100 μg/mL) concentrations.
*Data were expressed as mean ± SD, $n = 3$.

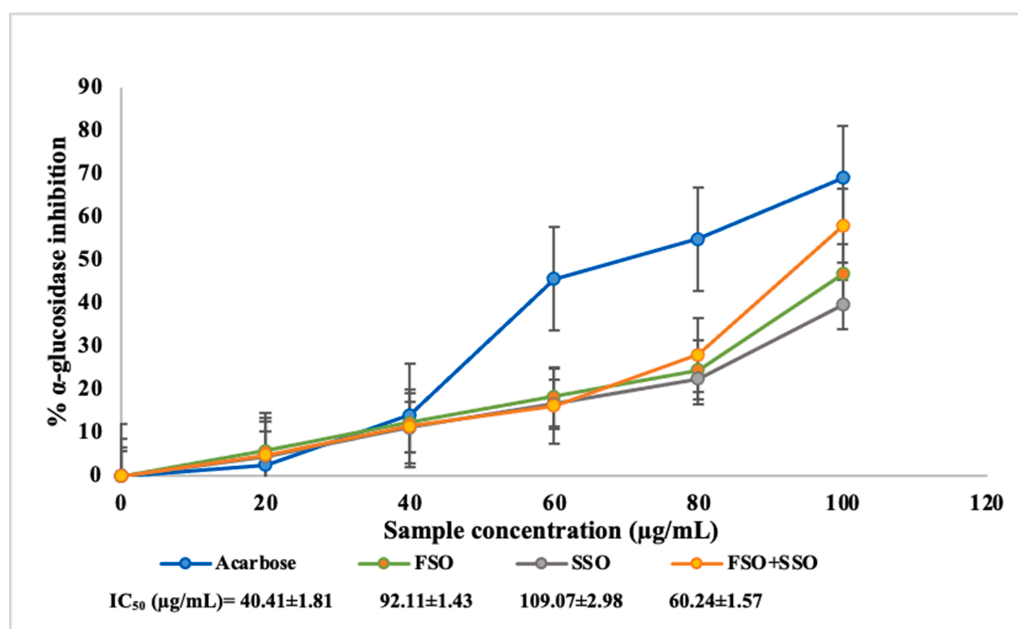

Fig. 2. Inhibitory effects of FSO, SSO, FSO+SSO and standard against *Saccharomyces cerevisiae* α-glucosidase at different (20–100 µg/mL) concentrations. *Data were expressed as mean ± SD, $n = 3$.

Table 2
Changes in body weight of STZ-induced diabetic rats.

Treatment*	0th day^	28th day^	Difference in body weight^	% body weightchanges
Normal control	188.00 ± 5.65	262.66 ± 6.21	74.66 ± 2.58	45.92⇧
Diabetic control (STZ)	186.66 ± 5.71	152.00 ± 6.29	34.66 ± 2.06[b]	18.56⇩
Diabetic+Tolbutamide (20 mg/kg b.w)	187.33 ± 5.57	250.83 ± 4.87	63.50 ± 2.16[a]	33.89⇧
Diabetic+FSO (258 mg/kg b.w)	186.66 ± 5.35	221.83 ± 6.73	35.16 ± 2.40[NS]	18.84⇧
Diabetic+SSO (350 mg/kg b.w)	186.66 ± 5.00	219.50 ± 6.50	32.83 ± 2.48[NS]	17.59⇧
Diabetic+FSO (516 mg/kg b.w)	185.66 ± 8.86	222.16 ± 7.88	36.50 ± 2.07[NS]	19.65⇧
Diabetic+SSO (700 mg/kg b.w)	185.16 ± 7.67	222.00 ± 5.83	36.83 ± 3.76[NS]	19.89⇧
Diabetic+FSO+SSO (43+292 mg/kg b.w)	186.66 ± 7.42	249.83 ± 7.96	63.16 ± 1.60[a]	33.84⇧
Diabetic+FSO+SSO (86+584 mg/kg b.w)	187.33 ± 8.57	252.66 ± 7.60	65.33 ± 4.17[a]	34.87⇧

[*] Data were expressed as mean ± SD, $n = 6$; ^in grams.
[a] $p < 0.001$ compared to respective diabetic control group.
[b] $p < 0.001$ compared to respective normal control group.
[NS] $p < 0.05$ compared to respective diabetic control group.

glucose levels ($p < 0.001$) in a dose-dependent manner and the combined treatment of FSO+SSO (43+292 and 86+584 mg/kg b.w) to diabetic rats showed the highest reduction in blood glucose levels ($p < 0.001$) in a dose-dependent manner when compared to the diabetic control group.

Taken together, the above findings suggest that the combined treatment of FSO+SSO to diabetic rats showed the highest weight gain that was closer to the normal control group, in dose-dependent mode, when compared to the diabetic control group. Similarly, the combined treatment resulted in a pronounced reduction in blood glucose levels in a dose-dependent manner when compared to the diabetic control group.

In vivo antioxidant activities

Antioxidant enzymes (CAT and SOD), GSH, and MDA levels in the pancreas and liver of the control and treatment groups are shown in Table 3. The CAT levels in both pancreas and liver were significantly reduced ($p < 0.001$) in STZ-treated rats when compared to normal control rats (Table 4). Oral gavage of FSO and SSO alone in a dose-dependent manner induced appreciable improvements ($p < 0.05$), whereas the FSO+SSO at 43+292 and 86+584 mg/kg b.w showed significant improvement ($p < 0.001$), in a dose-dependent manner, and showed the highest activity, which is greater than the tolbutamide treated group and equivalent to control group. Tolbutamide treated group at 5 mg/kg b.w exhibited a significant increase ($p < 0.001$) in the activity of CAT of pancreas and liver homogenates.

A significant decrease ($p < 0.001$) in SOD activity was observed in pancreas and liver homogenates of STZ-treated rats when compared to normal control group rats. SOD activity in both organs homogenates revealed that oral gavage of FSO and SSO alone activate this antioxidant enzyme in a dose-dependent manner, whereas combined treatment of FSO and SSO at 43+292 and 86+584 mg/kg b.w showed a significant elevated ($p < 0.001$) activity in a dose-dependent manner, which is higher than the tolbutamide treated group (Table 4). Tolbutamide treated group at 5 mg/kg b.w showed a significant increase ($p < 0.001$) in SOD activity.

The GSH levels were significantly decreased in the pancreas and liver of the diabetic control group when compared to normal control. The treatment of FSO (516 mg/kg b.w) and SSO (700 mg/kg b.w) alone were significantly increased the GSH levels in pancreas ($p < 0.01$) and liver ($p < 0.05$), respectively. Similarly, maximal activity ($p < 0.001$) was observed in FSO+SSO (86+584 mg/kg b.w) in both organs homogenates when compared to the tolbutamide treated group (Table 4).

A significant increase ($p < 0.001$) in MDA levels in the STZ-induced rats was on par with the lipid peroxidation levels of both pancreas and liver (Table 4). Oral administration of FSO and SSO alone significantly inhibited MDA levels in the organs. The combination of FSO and SSO (43+292 and 86+584 mg/kg b.w) decreased the formation of lipid peroxidation products in both homogenates when compared to the tolbutamide treated group.

In conclusion, the administration of FSO+SSO significantly elevated

Table 3
Blood glucose levels in different experimental groups.

Treatment[*]	Blood glucose (mg/dL) Before STZinduction	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	67.66 ± 3.44	70.83 ± 1.83	68.83 ± 2.04	69.66 ± 2.58	71.83 ± 1.72	72.83 ± 1.94
Diabetic control (STZ)	65.33 ± 3.93	339.33 ± 4.63	351.66 ± 4.50	344.16 ± 4.40	352.16 ± 3.18	350.33 ± 2.50[b]
Diabetic+Tolbutamide (20 mg/kg b.w)	67.33 ± 3.44	338.16 ± 4.16	325.16 ± 4.35	272.50 ± 3.01	190.33 ± 3.20	93.83 ± 2.71[a]
Diabetic+FSO (258 mg/kg b.w)	67.16 ± 4.35	335.16 ± 4.26	330.50 ± 4.08	314.16 ± 4.70	287.16 ± 4.28	251.83 ± 4.21[a]
Diabetic+SSO (350 mg/kg b.w)	68.50 ± 3.27	337.66 ± 3.93	333.83 ± 3.25	320.83 ± 3.31	296.83 ± 3.31	266.16 ± 4.83[a]
Diabetic+FSO (516 mg/kg b.w)	66.66 ± 4.58	333.83 ± 4.44	328.00 ± 4.09	305.8 ± 4.02	272.83 ± 4.07	228.66 ± 4.76[a]
Diabetic+SSO (700 mg/kg b.w)	63.66 ± 4.54	340.16 ± 4.70	334.50 ± 4.59	315.50 ± 5.16	289.33 ± 4.67	254.33 ± 5.95[a]
Diabetic+FSO+SSO (43+292 mg/kg b.w)	68.33 ± 4.22	341.16 ± 3.18	331.83 ± 3.18	293.16 ± 4.35	234.83 ± 5.19	168.66 ± 3.14[a]
Diabetic+FSO+SSO (86+584 mg/kg b.w)	66.50 ± 4.92	336.66 ± 2.94	325.83 ± 4.49	278.66 ± 3.14	202.66 ± 4.03	109.50 ± 2.88[a]

[*] Data were expressed as mean ± SD, $n = 6$.
[a] $p < 0.001$ compared to respective diabetic control group.
[b] $p < 0.001$ compared to respective normal control group.

Table 4
Effects of FSO and SSO on *in vivo* antioxidant enzyme activities of STZ-induced diabetic rats.

Treatment[*]	Liver CAT (μmol/mg protein)	SOD (U/mg protein)	GSH (μg/mg protein)	MDA (nmol/mg protein)	Pancreas CAT (μmol/mg protein)	SOD (U/mg protein)	GSH (μg/mg protein)	MDA (nmol/mg protein)
Normal control	1.26 ± 0.18	15.83 ± 1.00	31.57 ± 1.28	4.29 ± 0.41	1.12 ± 0.13	16.33 ± 0.97	29.53 ± 1.24	494 ± 0.37
Diabetic control (STZ)	0.67 ± 0.09[b]	6.49 ± 1.02[b]	23.89 ± 1.03[b]	9.38 ± 0.70[b]	0.65 ± 0.11[b]	5.91 ± 1.08[b]	21.81 ± 1.41[b]	10.61 ± 1.22[b]
Diabetic+Tolbutamide (20 mg/kg b.w)	1.06 ± 0.16[a]	14.60 ± 0.73[a]	29.81 ± 1.18[a]	5.03 ± 0.28[a]	1.11 ± 0.13[a]	13.28 ± 0.55[a]	27.84 ± 0.87[a]	5.85 ± 0.56[a]
Diabetic+FSO (258 mg/kg b.w)	0.76 ± 0.10[NS]	7.35 ± 0.55[NS]	24.80 ± 1.25[NS]	8.57 ± 0.43[d]	0.69 ± 0.13[NS]	5.93 ± 0.87[NS]	23.39 ± 1.13[d]	9.49 ± 0.66[NS]
Diabetic+SSO (350 mg/kg b.w)	0.82 ± 0.07[d]	8.00 ± 0.79[d]	24.41 ± 1.36[NS]	8.71 ± 1.08[NS]	0.80 ± 0.17[NS]	6.14 ± 0.95[NS]	23.04 ± 1.19[NS]	9.96 ± 1.35[NS]
Diabetic+FSO (516 mg/kg b.w)	0.77 ± 0.06[NS]	8.04 ± 0.71[c]	25.38 ± 1.08[d]	7.61 ± 0.97[c]	0.73 ± 0.04[NS]	6.49 ± 0.42[NS]	24.48 ± 1.45[c]	8.53 ± 0.77[c]
Diabetic+SSO (700 mg/kg b.w)	0.83 ± 0.14[NS]	8.30 ± 0.52[d]	25.62 ± 1.23[d]	7.16 ± 0.47[a]	0.82 ± 0.11[d]	7.80 ± 0.29[c]	24.17 ± 0.85[c]	7.67 ± 0.74[a]
Diabetic+FSO+SSO (43+292 mg/kg b.w)	1.05 ± 0.10[a]	13.61 ± 0.91[a]	26.15 ± 1.53[c]	5.02 ± 0.38[a]	1.13 ± 0.14[a]	12.60 ± 0.72[a]	26.30 ± 1.15[a]	5.33 ± 0.72[a]
Diabetic+FSO+SSO (86+584 mg/kg b.w)	1.55 ± 0.19[a]	14.89 ± 0.93[a]	28.71 ± 1.65[a]	4.88 ± 0.40[a]	1.20 ± 0.14[a]	14.67 ± 0.92[a]	27.71 ± 1.54[a]	5.15 ± 0.52[a]

[*] Data were expressed as mean ± SD, $n = 6$.
[a] $p < 0.001$ compared to respective diabetic control group.
[b] $p < 0.001$ compared to respective normal control group.
[c] $p < 0.01$ compared to respective diabetic control group.
[d] $p < 0.05$ compared to respective diabetic control group.
[NS] $p < 0.05$ compared to respective diabetic control group.

the activities of antioxidant enzymes viz CAT, SOD, and GSH of pancreas and liver homogenates, whereas, MDA levels of those organs were pronouncedly inhibited by them.

Lipid profile in STZ-induced diabetic rats

The levels of lipidic parameters *i.e.*, TG, TC, HDL, LDL, and VLDL in normal, diabetic, and FSO and SSO treatment groups are shown in Table 5. Diabetic control rats showed a significant increase ($p < 0.001$) in TG, TC, LDL, and VLDL while a significant decrease ($p < 0.001$) was observed in HDL when compared to normal control rats (Table 5). The FSO (258 and 516 mg/kg b.w) and SSO (350 and 700 mg/kg b.w) showed a significantly decreased level ($p < 0.001$) in TG, TC, LDL, and VLDL and increased levels of HDL in a dose-dependent manner, that showed appreciable results as compared to tolbutamide treated group, whereas co-treatment of FSO and SSO at 43+292 and 86+584 mg/kg b.w showed significantly elevated levels in TG, TC, LDL and VLDL ($p < 0.001$) and increased levels of HDL in dose-dependent mode, which is equivalent to normal and tolbutamide treated group.

The above results suggest that the treatment of FSO+ SSO significantly reduced the levels of TG, TC, LDL, and VLDL, while it pronouncedly increased the level of HDL.

Changes in the levels of biochemical parameters of STZ-induced diabetic rats

In the biochemical analysis performed in serum collected from rats, there were significant differences ($p < 0.05$) in all tested parameters when the STZ-induced group was compared to the normal control group (Table 6). The levels of parameters to test liver and renal function *i.e.*, AST, ALT, ALP and creatinine, and urea activities were significantly increased ($p < 0.001$) in diabetic control rats when compared to normal control rats. The FSO (258 and 516 mg/kg b.w) and SSO (350 and 700 mg/kg b.w) showed a significant depletion of AST, ALT, ALP and creatinine, and urea ($p < 0.05$) in dose-dependent mode, when compared to STZ-induced control rats, whereas co-treatment of FSO and SSO at 43+292 and 86+584 mg/kg b.w showed a strong decrease in levels ($p < 0.001$) of AST, SLT, ALP and creatinine, as well as urea, in a dose-dependent manner, and were similar to those determined to tolbutamide treated rats, when compared to STZ-induced control rats. Tolbutamide treatment group exhibited the highest decreased levels ($p < 0.001$) at 5 mg/kg b.w. (Table 5). Thus, the liver and renal function tests indicated that there was no dysfunction of both the organs by the above treatments.

The significantly decreased levels ($p < 0.001$) of total TP, Hb, insulin, and increased level ($p < 0.001$) of HbA1c were noticed in STZ-induced

Table 5
Effect of FSO and SSO on lipid profile levels in STZ-induced diabetic rats.

Treatment*	Lipid profiles (mg/dl) TG	TC	HDL	LDL	VLDL
Normal control	74.58 ± 2.22	68.21 ± 1.34	33.64 ± 1.50	19.64 ± 1.24	14.91 ± 0.44
Diabetic control (STZ)	161.94 ± 3.46[b]	131.96 ± 2.79[b]	18.10 ± 1.32[b]	81.47 ± 2.01[b]	32.38 ± 0.69[b]
Diabetic+Tolbutamide (20 mg/kg b.w)	81.17 ± 2.59[a]	74.78 ± 1.71[a]	31.21 ± 1.07[a]	27.33 ± 2.87[a]	16.23 ± 0.51[a]
Diabetic+FSO (258 mg/kg b.w)	131.54 ± 2.96[a]	105.47 ± 1.72[a]	21.26 ± 1.21[c]	57.90 ± 2.09[a]	26.30 ± 0.59[a]
Diabetic+SSO (350 mg/kg b.w)	134.18 ± 1.99[a]	108.24 ± 1.66[a]	20.34 ± 1.20[d]	61.06 ± 1.91[a]	26.83 ± 0.39[a]
Diabetic+FSO (516 mg/kg b.w)	119.78 ± 2.02[a]	96.71 ± 1.60[a]	22.05 ± 1.17[a]	50.69 ± 2.91[a]	23.95 ± 0.40[a]
Diabetic+SSSO (700 mg/kg b.w)	124.16 ± 2.63[a]	100.18 ± 2.17[a]	23.86 ± 0.98[a]	51.48 ± 1.95[a]	24.83 ± 0.52[a]
Diabetic+FO+SSO (43+292 mg/kg b.w)	88.63 ± 1.60[a]	84.10 ± 1.01[a]	28.04 ± 1.18[a]	38.32 ± 2.07[a]	17.72 ± 0.32[a]
Diabetic+FSO+SSO (86+584 mg/kg b.w)	83.73 ± 1.38[a]	76.96 ± 1.44[a]	31.83 ± 1.38[a]	28.38 ± 2.51[a]	16.74 ± 0.27[a]

[*] Data were expressed as mean ± SD, $n = 6$.
[a] $p < 0.001$ compared to respective diabetic control group.
[b] $p < 0.001$ compared to respective normal control group.
[c] $p < 0.01$ compared to respective diabetic control group.
[d] $p < 0.05$ compared to respective diabetic control group.

Table 6
Effects of FSO and SSO on the levels of SGOT, SGPT, ALP, creatinine and urea in STZ-induced diabetic rats.

Treatment*	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mg/dL)	Urea mg/dL
Normal control	141.11 ± 3.22	44.41 ± 2.71	74.12 ± 3.94	0.32 ± 0.02	35.35 ± 2.76
Diabetic control (STZ)	392.97 ± 4.32[b]	235.51 ± 5.49[b]	154.70 ± 3.97[b]	0.83 ± 0.04[b]	81.62 ± 3.35[b]
Diabetic+Tolbutamide (20 mg/kg b.w)	177.70 ± 3.58[a]	75.81 ± 4.95[a]	114.62 ± 5.32[a]	0.37 ± 0.01[a]	49.19 ± 2.53[a]
Diabetic+FSO (258 mg/kg b.w)	314.41 ± 4.66[a]	194.60 ± 4.78[a]	144.18 ± 3.39[a]	0.78 ± 0.01[d]	76.37 ± 2.80[d]
Diabetic+SSO (350 mg/kg b.w)	310.04 ± 5.99[a]	205.05 ± 4.70[a]	145.39 ± 4.17[c]	0.71 ± 0.01[a]	78.34 ± 2.00[NS]
Diabetic+FSO (516 mg/kg b.w)	284.84 ± 4.63[a]	177.72 ± 5.25[a]	137.69 ± 4.82[a]	0.77 ± 0.02[c]	72.13 ± 2.01[a]
Diabetic+SSO (700 mg/kg b.w)	290.54 ± 5.10[a]	183.65 ± 4.77[a]	132.03 ± 2.97[a]	0.72 ± 0.02[a]	65.80 ± 3.02[a]
Diabetic+FSO+SSO (43+292 mg/kg b.w)	200.14 ± 3.92[a]	99.70 ± 4.64[a]	110.99 ± 3.13[a]	0.43 ± 0.02[a]	59.80 ± 3.43[a]
Diabetic+FSO+SSO (86+584 mg/kg b.w)	186.55 ± 4.61[a]	91.85 ± 3.57[a]	104.75 ± 4.43[a]	0.41 ± 0.02[a]	61.14 ± 2.01[a]

[*] Data were expressed as mean ± SD, $n = 6$.
[a] $p < 0.001$ compared to respective diabetic control group.
[b] $p < 0.001$ compared to respective normal control group.
[c] $p < 0.01$ compared to respective diabetic control group.
[d] $p < 0.05$ compared to respective diabetic control group.
[NS] $p < 0.05$ compared to respective diabetic control group.

diabetic rats when compared to normal control rats. Oral administration of FSO (258 mg/kg b.w) and SSO (350 mg/kg b.w) alone showed non-significant decreased levels of Hb, insulin and significant increased level of HbA1c, respectively, and SSO (350 mg/kg b.w) increased ($p < 0.05$) TP. The second concentration of FSO (516 mg/kg b.w) and SSO (700 mg/kg b.w) alone was able to significantly increase the level ($p < 0.001$) of HbA1c and to decrease levels of ($p < 0.01$) insulin, respectively, and FSO (516 mg/kg b.w) increased ($p < 0.05$) TP while SSO (700 mg/kg b.w) increased ($p < 0.001$) total Hb, when compared to diabetic control group (Table 7). Furthermore, the combination of FSO and SSO at 43+292 and 86+584 mg/kg b.w promoted a significant increase ($p < 0.001$) and decrease ($p < 0.001$) of TP, Hb, insulin, and HbA1c, respectively, when compared to the diabetic control group, which is equivalent to the tolbutamide treatment group and normal control rats. Tolbutamide treatment group presented decreased levels of TP, Hb, insulin ($p < 0.01$), ($p < 0.001$), ($p < 0.001$), and increased level ($p < 0.001$) of HbA1c, when compared to the diabetic control group.

Histopathology of the pancreas of experimental rats

The pancreas is a tubule-acinar gland consisting of exocrine and endocrine parts. The endocrine part of the pancreas is the islets of Langerhans, which consist of three types of endocrine cells, alpha, beta, and delta. The pancreas of normal control group rats showed a normal histological structure of islet of Langerhans compared to STZ-induced diabetic control rats. The shrunken and distorted islets of Langerhans were observed in the diabetic control group. Oral administration of FSO (258 and 516 mg/kg b.w) and SSO (350 and 700 mg/kg b.w) groups exhibited signs of recovery towards normal cellular architecture. The tolbutamide and FSO+SSO treatments (43+292 and 86+584 mg/kg b.w) did not differ from their controls and exhibited normal histological architecture (Fig. 3).

Discussion

Type 2 diabetes accounts for more than 95% of all diabetes cases and causes economic problems in developing countries (Cheng, 2005). Patients with T2DM are characterized by both hyperglycemia and

Table 7
Levels of total protein, hemoglobin, glycosylated hemoglobin and insulin in STZ-induced diabetic rats.

Treatment*	Total protein (g/dL)	Hb(mg/dL)	HbA1c (%Hb)	Insulin (μmol/ml)
Normal control	8.36 ± 0.76	14.20 ± 0.78	5.78 ± 0.47	22.95 ± 1.76
Diabetic control (STZ)	5.25 ± 0.81[b]	8.41 ± 1.05[b]	11.42 ± 0.66[b]	10.92 ± 1.29[b]
Diabetic+Tolbutamide (20 mg/kg b.w)	7.05 ± 0.77[c]	13.38 ± 0.79[a]	6.62 ± 0.46[a]	20.42 ± 1.18[a]
Diabetic+FSO (258 mg/kg b.w)	6.11 ± 0.72[NS]	9.06 ± 0.74[NS]	9.55 ± 0.54[a]	12.15 ± 0.86[NS]
Diabetic+SSO (350 mg/kg b.w)	6.27 ± 0.43[d]	8.85 ± 0.82[NS]	9.36 ± 0.47[a]	12.12 ± 1.13[NS]
Diabetic+FSO (516 mg/kg b.w)	6.29 ± 0.40[d]	10.78 ± 1.03[c]	8.84 ± 0.64[a]	13.44 ± 0.61[c]
Diabetic+SSO (700 mg/kg b.w)	6.35 ± 0.35[c]	11.07 ± 1.07[a]	8.09 ± 0.40[a]	13.17 ± 1.12[c]
Diabetic+FSO+SSO (43+292 mg/kg b.w)	7.11 ± 0.37[a]	12.49 ± 1.11a	8.37 ± 0.45[a]	18.95 ± 1.06[a]
Diabetic+FSO+SSO (86+584 mg/kg b.w)	7.18 ± 0.20[a]	12.75 ± 0.96[a]	7.44 ± 0.36[a]	19.26 ± 1.10[a]

[*] Data were expressed as mean ± SD, $n = 6$.
[a] $p < 0.001$ compared to respective diabetic control group.
[b] $p < 0.001$ compared to respective normal control group.
[c] $p < 0.01$ compared to respective diabetic control group.
[d] $p < 0.05$ compared to respective diabetic control group.
[NS] $p < 0.05$ compared to respective diabetic control group.

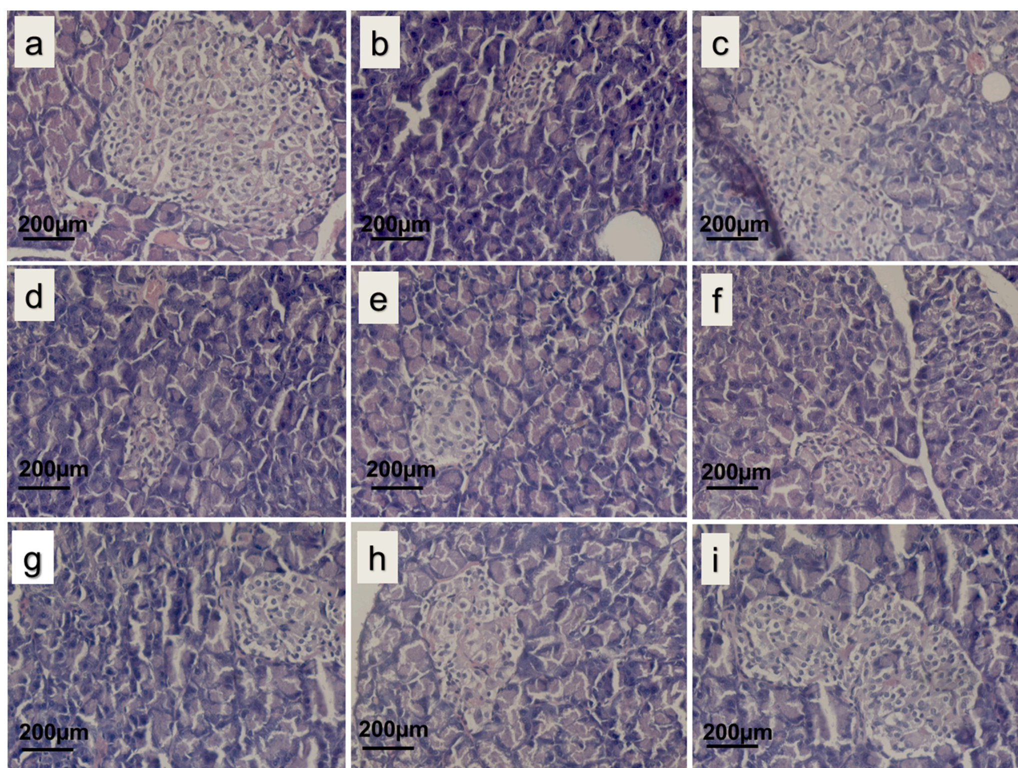

Fig. 3. Microphotographs of pancreas histological sections (40X). a- Normal control; b- Diabetic control (STZ); c- Diabetic+Tolbutamide (20 mg/kg b.w); D- Diabetic+FSO (258 mg/kg b.w); e- Diabetic+SSO (350 mg/kg b.w); f- Diabetic+FSO (516 mg/kg b.w); g- Diabetic+SSO (700 mg/kg b.w); h- Diabetic+FSO+SSO (43+292 mg/kg b.w); i- Diabetic+FSO+SSO (86+584 mg/kg b.w).

hyperinsulinemia. Most of the T2DM patients are obese due to excessive food intake and lack of physical activity. However, hyperglycemia, over the long term causes abnormal secretion of insulin, which exhausts the β-cells of the pancreas. Pancreatic β-cell dysfunction causes secretion of inflammatory cytokines and accumulation of M1 macrophages, which cause inflammation and potentiate insulin resistance (Iwase et al., 2015).

Streptozotocin is a commonly used chemical to induce T2DM in experimental animals, which is mediated by alkylation of pancreatic DNA and generation of $O_2^{\bullet-}$, H_2O_2, and OH• radicals. These are responsible for partial damage of pancreatic β-cells, bringing about inadequate insulin discharge, resulting in necrosis and creating T2DM (Ramachandran et al., 2012; Jayaprasad et al., 2016). Tolbutamide is a standard anti-diabetic drug and insulin stimulant in the STZ-induced rat model of diabetes used as a positive control when comparing the anti-diabetic effects of various hypoglycemic bioactive compounds (Azad and Sulaiman, 2020). Although there is progress in the management of diabetes by synthetic agents, they have side effects in the long term. Therefore, a search for improved and safe natural antidiabetic agents is going on while WHO has also recommended the development of bioactives from natural sources in this concern (El-Soud et al., 2011).

With understanding the bioavailability and bioaccessibility of bioactives to treat the pathogenic condition of many diseases, currently, drug discovery shifted from *'One drug, one target'* to *'Multidrug, multitarget"* model is a new phenomenon of pharmaceutical approach in the area of health sciences, which gives a complementary approach in Rational drug design (Long et al., 2015). By understanding the aim of the many researchers, there is an urgent need for a multi-therapeutic and systematic approach to identifying the pathways that are targeted by drugs (Zimmermann et al., 2007). Despite the extensive experience in the use of bioactives, scientific study and investigations for the identification of bioactives effect both synergistic and combinatorially can lead to the discovery of new therapeutic benefits and the production of combinatorially effective natural bioactives in the future.

During the past few decades, epidemiological shreds of evidence have suggested that dietary LCPUFAs, namely DHA and EPA, decrease the risk of metabolic diseases such as CVDs, DM, hypertension, stroke, and improve symptoms in mood disorders as well as cognitive function (Kaithwas and Majumdar, 2012). Jeppesen et al. (2013) have reported that both ω−6 and ω−3 FAs are beneficial in improving lipid profiles by lowering the TG and VLDL cholesterol in T2DM patients.

The presence of fatty acid composition in the oilseeds like flax and sesame vary due to various factors, including agronomic. In FSO, ALA is a major fatty acid and it constitutes 39.90±0.14% out of the total 52.24% PUFA. Similarly, in SSO, LA constitutes 28.35 ± 0.46 out of the total 28.69% PUFA (Guimarães et al., 2013). Apart from FAs, both oils also contain natural antioxidants like tocopherols and phenolic compounds, which exert different biological properties.

Several studies have investigated that PUFAs from many natural sources have the potential to counteract free radicals (Makni et al., 2011; Han et al., 2016). In our previous study, we have assessed the hepatoprotective effects of ω−3 and ω−6 FA from FSO and SSO at a 1:5 ratio on toxin-induced liver damage in rats (Sunil et al., 2021). Therefore, in the present investigation, we focused on elucidating the anti-diabetic effects of ω−3: ω−6 PUFA from FSO and SSO at 1:5 ratio on STZ-induced diabetic rats.

The α-amylase inhibitory activity is the most common to determine the antidiabetic activity. Some bioactives are not only capable of reducing oxidative stress but also significantly slow down or prevent the absorption of starch in the human body, mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltotriose, maltose, and other simple sugars. The fatty acids such as palmitic, linoleic, and oleic acids of *Clausena indica* fruits synergistically exerted the strongest α-amylase suppression activity that was higher than acrobose, which were demonstrated as being involved in antidiabetic effects (Su et al., 2013; La Anh et al., 2020). The results of

our study (Fig. 1) showed that FSO+SSO effectively inhibited carbohydrate hydrolyzing enzymes, which was near to acarbose. Thus, the findings of our current study are in accordance with previous reports (Shettar et al., 2017; La Anh et al., 2020).

Su et al. (2013) have reported that the LA and OA showed strong inhibitory effects on α-glucosidase. The avocado methanolic leaves extract had 3 times higher α-glucosidase inhibitory activity than acarbose (Uysal et al., 2015). The % inhibition of the activity of α-glucosidase by FSO, SSO, and FSO+SSO delayed the carbohydrate degradation, which in turn caused a decrease in the absorption of glucose, increase in insulin action, or insulin secretion and β-cell function due to the reduction in the elevation of postprandial blood glucose level. The ability of bioactives to modulate glucose liberation from starch and absorption is a therapeutic modality in the management of DM. The α-glucosidase and α-amylase inhibitors are the first-line clinical drugs, for example, acarbose, which helps to reduce postprandial glucose levels in diabetic patients (Mwakalukwa et al., 2020). The methanolic extract of *Tinospora sinensis*, and chloroform, and ethyl acetate extracts of *Elaeagnus umbellata* have exhibited inhibitory effects against α-amylase and α-glucosidase, respectively, which resulted in the management of postprandial hyperglycemia in the treatment of T2DM (Banerjee et al., 2017; Nazir et al., 2018). The results of our present investigation (Fig. 2) indicated that the FSO+SSO effectively delayed carbohydrate degradation and inhibited the activities of α-amylase and α-glucosidase. This could be due to the presence of ω−3 and ω−6 FA present in FSO and SSO, respectively, and thus, these are in coherent with previous studies (Banerjee et al., 2017; Nazir et al., 2018).

STZ-induced diabetes in rats induces multiple alterations including severe body weight loss, polydipsia, polyphagia, a significant increase in blood glucose levels, and decreased insulin section. This is due to the catabolism of proteins and fats and a decrease in the pancreatic secretion of insulin from existing β-cells of islets of Langerhans (Kamalakkannan and Prince, 2004; Ramachandran et al., 2012; Deepti et al., 2019). The administration of FSO, SSO, and FSO+SSO significantly increased body weight, insulin, and decreased blood glucose levels compared to diabetic control rats (Tables 2 and 3), which shows preventive effects of FSO and SSO on the degradation of structural proteins and reversal of insulin resistance or increased insulin secretion, which could be due to regeneration of damaged β-cells of the pancreas. Similarly, all other symptoms were also observed and recorded in our present study. Additionally, compromised motility and excessive urination (often at night) were also observed, when compared with normal control rats. Some authors have demonstrated that linoleate reduced the voltage-gated K^+ current in β-cells through GPR40 and cAMP-protein kinase-A system, leading to an increase in Ca^{2+} and insulin secretion in rats (Feng et al., 2006; Makini et al., 2011). When FSO and SSO were administered in a combined manner to glucose-loaded rats, the animals exhibited a significant reduction in their blood glucose levels after 60 min. This may be due to the prevention of damage to the β-cells of islets of Langerhans. Added to this, the closing of the K+ATP channel and release of insulin from the healthy β-cells might also be the reason for this decrease in glucose level, which resulted from the synergistic antioxidant effects of FSO and SSO. Therefore, the results of our present study are in agreement with other investigations (Ramachandran et al., 2012; Azad and Sulaiman, 2020).

Ramachandran et al. (2012) have reported that hyperglycemia is linked with the formation of ROS, which causes organ damage. The levels of ROS (H_2O_2, $O_2^{\bullet-}$ and OH•) and lipid peroxidation are common markers of oxidative stress in diabetic rats. Diminished levels of both endogenous enzymatic (CAT, SOD) and non-enzymatic (GSH) antioxidants in the pancreas and liver are registered in the present study. Reduced antioxidant level, which increased free radical accumulation in STZ-induced diabetes has been previously reported (El-Soud et al., 2011; Ali et al., 2020). These endogenous enzymatic antioxidants are a mutually supportive group of defenses against ROS. Catalase is a hemeprotein, that catalyzes the production of H_2O_2 and protects the tissue from OH• levels and SOD scavenges the $O_2^{\bullet-}$ by forming H_2O_2 and molecular oxygen (Kaithwas and Majumdar 2012). Some researchers have reported that SOD and CAT are partially inactivated by OH• and H_2O_2. Glutathione peroxidase catalyzes the reaction of H_2O_2 with reduced GSH to form glutathione disulfide and the reduced product of H_2O_2. In the present investigation, the decline in the activities of these enzymes in diabetic rats was due to oxidative stress elicited by STZ (Ramachandran et al., 2012; Gomathi et al., 2014), and significantly elevated activities of antioxidant enzymes were attributed to ω−3 and ω−6FA of FSO and SSO, respectively.

A significant increase in CAT, SOD, and GSH levels were noticed in FSO and SSO alone and combined therapy (Table 4). This is because ω−3 and ω−6 FA are present in respective oils and thus, antioxidants are proved to be involved in the regeneration of damaged extracellular matrix proteins and promotion of cell growth (Smith et al., 1992). Thus, the antioxidative role of omega fatty acids might have helped in the management of DM, and the results of our current study are in line with other reports (Gomathi et al., 2014; Sheweita et al., 2016; Ali et al., 2020).

Chronic diabetes is also characterized by lipid peroxidation (Maxwell et al., 1997). The development of both type I and type II diabetes mellitus show lipid peroxide mediated tissue damage. Malondialdehyde is a marker of oxidative stress and the end-product of lipid peroxidation (Krishnaraju et al., 2009). An increase in lipid peroxidation in diabetes is due to increased oxidative stress in cells resulting in depletion of the antioxidant scavenging system and leading to cytotoxicity and various pathological conditions. Several studies have reported that insulin secretion is closely associated with lipoxygenase-derived peroxides (Kamalakkannan and Prince, 2004; Ramachandran et al., 2012). Ramachandran et al. (2012) have reported that hypoinsulinemia activity of fatty acyl-coenzyme A oxidase, which initiates the β-oxidation of FA, results in lipid peroxidation. Oral administration of FSO and SSO alone and in combination showed a significantly lowered MDA level in diabetic rats (Table 4). Therefore, all the results of our present study are coherent with previous reports (Krishnaraju et al.,2009; Ramachandran et al., 2012).

The common abnormalities of lipids in diabetes are hypercholesterolemia and hypertriglyceridemia. This is due to increased mobilization of free FAs from peripheral fat depots, which result in significantly increased TG, TC, LDL, and VLDL and markedly reduced HDL levels in diabetic rats (Table 5). The increase in HDL is one of the major norms of anti-atherogenic agents. Besides, many studies have demonstrated that high levels of HDL are linked with a lower incidence of CVDs. Administration of FSO, and SSO alone and in combination reduced the levels of TG, TC, LDL, and VLDL, and increased the level of HDL in diabetic rats. This is attributed to ω−3 (ALA) and ω−6 (LA) FA present in FSO and SSO, respectively, which exhibited strong hypercholesterolemia and hypertriglyceridemia effects. The above conditions could be beneficial in preventing diabetic complications like atherosclerosis and coronary heart diseases (Makni et al., 2011; Ramachandran et al., 2012). Hence, the findings of our current study are in close agreement with other reports (Makni et al., 2011; Ramachandran et al., 2012; Azad and Sulaiman, 2020).

In STZ-diabetic rats, increased levels of serum creatinine and urea are due to renal damage caused by increased glucose and glycosylated protein tissue levels, suggesting compromised renal function (Azad and Sulaiman, 2020) and reduction in total protein. This could be due to hypoproteinemia and proteinuria or increased catabolism of protein, which are the clinical markers of diabetic nephropathy (Lal et al., 2009). Treatment of FSO and SSO alone and in combination normalized these levels (Table 6) and exhibited their beneficial effects on the kidney. Thus, the results of the present study are on par with other reports (Ramachandran et al., 2012; Azad and Sulaiman, 2020).

It has been investigated that the STZ causes hepatotoxicity by inducing CYP2E1dependent oxidative stress and leads to the release of liver microsomal enzymes (Nazir et al., 2018). An elevated level of AST, ALT and ALP in serum reflects active hepatic damage in T2DM disease

(Nazir et al., 2018; Azad and Sulaiman, 2020). In our current study, elevation in hepatic enzyme levels was noticed (Table 6). Therefore, increased levels of AST, ALT, and ALP might have been released from the liver to the blood. However, this has been corrected by administration FSO and SSO alone and in combination, and all these results are agreeing with our previous hepatoprotective study and other reports, and also our previous study (Khan et al., 2017; Bouhrim et al., 2019; Sunil et al., 2021).

The HbA1c is considered as a diagnostic marker as per International Diabetes Federation (Jayaprasad et al., 2016). During diabetes, excess glucose present in the blood reacts with Hb to form HbA1c (Gandhi and Sasikumar, 2012). Lanjhiyana et al. (2011) have reported that HbA1c concentration helps to know the long-term blood glucose levels, degree of protein glycation, and correlation of diabetic-associated complications. The increased HbA1c and reduced Hb levels were observed in our present study (Table 7). This could be due to hyperglycemia. The administration of FSO and SSO alone and in combination in diabetic rats showed a significant decrease in HbA1c and increase in Hb levels, which is attributed to ω−3 and ω−6 fatty acids and FSO and SSO, respectively. Therefore, the findings of our present investigation are in close agreement with previous reports.

In the current study, nuclear shrinkage, decrease in the number of secretory granules, and swelling of mitochondria have been observed in pancreas tissue of diabetic control rats. Treatment with FSO and SSO alone and in combination showed restoration of normal cellular population and hyperplasia (cell proliferation) or hypertrophy (increase in cell size) of pancreas β-cells (Fig. 3). This regeneration and hyperplasia of pancreatic β-cells could be due to the prevention of free radical formation by ω−3 and ω−6 FA present in FSO and SSO, respectively, and these results are in agreement with earlier reports (Khan et al., 2017; Azad and Sulaiman, 2020). The present study supported the cell numbers and structural integrity of pancreatic β-cells that were restored indicated that the β-cells were stimulated for insulin synthesis by ω−3 and ω−6 FAs.

Conclusion

In conclusion, this study confirmed that the combination of FSO+SSO possesses the highest antidiabetic and antilipidemic activities than their individuals. The co-treatment of FSO+SSO significantly increased the activities of antioxidant enzymes like CAT, SOD, GSH, and decreased MDA levels in the pancreas and liver. The treatment also lowered various biochemical parameters including AST, ALT, ALP, serum creatinine, urea, and HbA1c, as well as lipids including TG, TC, VDL, and VLDL, and increased HDL levels, and TP, Hb, insulin in diabetic rats. The restoration of blood glucose levels indicated a strong antidiabetic potential of FSO+SSO. The histopathological results of the pancreas also supported the anti-diabetic potential by recovering the normal cellular population and hyperplasia or hypertrophy of the pancreas β-cells. Therefore, it is concluded that the combination of FSO+SSO exhibited more synergistic antioxidant and antidiabetic potential compared to their individual treatments. This is attributed to ω−3 (ALA) and ω−6 (LA) fatty acids of FSO and SSO, respectively. However, further studies are essential to explore the mechanisms regarding the anti-diabetic effects of ω−3: ω−6 of FSO and SSO at molecular levels.

Authorship contribution statement

Conception and design of the study: SCG and RJ. Acquisition of data: SCG, TA, and RJ. Analysis and/or interpretation of data: SCG, TA, HNS, AB, and RJ. Drafting the manuscript: SCG and TA. Revising the manuscript critically for important intellectual content: SCG, CMFP, and RJ. Approval of the version of the manuscript to be published: SCG, TA, HNS, AB, CMFP, and RJ.

All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Author agreement statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the corresponding author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs

Funding

The authors gratefully acknowledge the University Grants Commission, New Delhi, India for funding this research work (Grant No. F. No.41–1280/2012(SR).

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors thank Yuvaraja's College, University of Mysore, Mysuru, India for supporting this research work by facilitating the requirements. Mr. SCG immensely thanks to the Special cell, University of Mysore, Mysuru, India for providing fellowship to carry out this research work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2022.100284.

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