Industrial Crops & Products 217 (2024) 118861

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

In vitro and in vivo exploitation of cell stress pathways using methanolic extracts of Phlomis stewartii in diabetic rat's model

Mamoon Ur Rasheed [a], Syed Ali Raza Naqvi [a,*], Sadaf Ul Hassan [b], Atta Ul Haq [a], Muhammad Ramazan Saeed Ashraf Janjua [a], Mohamed H. Mahmoud [c], Gaber El-Saber Batiha [d], Haroon Rashid [e], Muhammad Abdul Rahim [f], João Miguel Rocha [g,h,i,**]

[a] Department of Chemistry, Government College University, Faisalabad 38040, Pakistan
[b] Department of Chemistry, COMSATS university Islamabad, Lahore Campus, Lahore, Pakistan
[c] Department of Biochemistry, College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia
[d] Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, AlBeheira 22511, Egypt
[e] Department of Physiology, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
[f] Department of Food Science & Nutrition, Faculty of Medicine and Allied Health Sciences, Times Institute, Multan, Pakistan
[g] Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, Porto 4169-005, Portugal
[h] LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, Porto 4200-465, Portugal
[i] ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, Porto 4200-465, Portugal

ARTICLE INFO

Keywords:
Phlomis stewartii
phytochemicals
biological activities
antioxidants
phenolic acids
diabetes

ABSTRACT

Various bioactive constituents present in plants are used to treat metabolic disorders linked to oxidative stress and obesity. Low-grade to chronic inflammation and IR are considered as main causes of obesity, which is strongly correlated with the prevalence and incidence of Type-2 Diabetes Mellitus (T2DM). In this study, the biological influence of *Phlomis stewartii* extracts was evaluated *in vitro* and *in vivo* using rat diabetic models. The biological effects included anti-inflammatory, anti-proliferative, anti-apoptotic, metabolic hormonal via molecular signaling pathways, and antioxidant potential. The extracts from *P. stewartii* exhibited the strongest DPPH and FRAP potential for LM (38.92 μg/mL) and (45.28 μg/mL) respectively. Moreover, the high absorbance for reducing power was observed in leaves methanol (LM) 1.36 which is greater than flower methanol (FM) 1.12, and whole plant methanol (WPM) 1.21 at a concentration of 500 μg/mL when comparing treated groups to the negative control (NC), and positive control (PC) groups. The results of an Enzyme-linked Immunosorbent Assay (ELISA) study measuring a metabolic hormonal profile that includes Triiodothyronine (T3), Thyroxine (T4), Thyroid Stimulating Hormone (TSH), insulin, leptin, and glucokinase showed that LM has been greatly

Abbreviations: LM, Leaves Methanol; FM, Flower Methanol; WPM, Whole Plant Methanol; NC, Negative Control; PC, Positive Control; T2DM, Type-2 Diabetes Mellitus; T3, Triiodothyro-nine; T4, Thyroxine; TSH, Thyroid Stimulating Hormone; ELISA, Enzyme-linked Immunosorbent Assay; JAK, Janus kinase; STATS, Signal Transducer and Activator of Transcription; GRK-2, G protein-Coupled Receptor Kinase 2; FOXO-1, FOXO-3, FOXO-4, Forkhead Box 01, 03, 04; CALM-2, Calmodulin 2; OS, Oxidative Stress; ROS, Reactive Oxygen Species; PIAS-1, Proteins Inhibitor of Activated STAT 1; BCL-2, B-cell Lymphoma 2; BAX, Bcl-2 Associated X-protein; BID, BH3 Interacting Domain Death; IL-6, lL-1β, Interleukins; TNF-α, Tumor Necrosis Factor-α; IR, Insulin Resistance; MAPK3, Mitogen-Activated Protein Kinase 3; IFN, Inflammatory Cytokines Interferon; PI3K, Phosphoinositide 3-Kinase; ERK1/2, Extracellular Signal-Regulated Kinase 1–2; Ca^{+2}, Calcium ions; CaM, Calcium Calmodulin; MPs, Medicinal Plants; DPPH, 2,2-Diphenyl 1-Picrylhydrazyl; ABTS, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; FRAP, Ferric Reducing Antioxidant Power; CS, Cigarette Smoke; LPs, Lipids Peroxidation; AMPK, AMP-activated Protein Kinase; PDK4, Pyruvate Dehydrogenase Kinase Isoform 4; MCAD, Medium Chain acyl CoA dehydrogenase; UCP2, Mitochondrial Uncoupling Protein; DNAm, Deoxyribonucleic Acid Methylation; 5mC, 5-Methylcytosine; TFs, Transcription Factors; MTP, Microsomal Triglyceride Transfer Protein; ApoB, Apolipoprotein B; VLDL, Very Low-Density Lipoproteins; TGF-β, Transformation Growth Factors-β.

* Corresponding author.
** Corresponding author at: Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, Porto 4169-005, Portugal.
E-mail addresses: mamoon-sandhu68@gmail.com (M.U. Rasheed), draliraza@gcuf.edu.pk (S.A.R. Naqvi), sadafulhassan@cuilahore.edu.pk (S.U. Hassan), attaulhaq@gcuf.edu.pk (A.U. Haq), janjua@gcuf.edu.pk (M.R. Saeed Ashraf Janjua), mmahmoud2@ksu.edu.sa (M.H. Mahmoud), dr_gaber_batiha@vetmed.dmu.edu.eg (G.E.-S. Batiha), haroonrashid@iub.edu.pk (H. Rashid), mabdulrahim@t.edu.pk (M.A. Rahim), jmfrocha@fc.up.pt (J.M. Rocha).

https://doi.org/10.1016/j.indcrop.2024.118861
Received 29 January 2024; Received in revised form 6 May 2024; Accepted 27 May 2024
Available online 3 June 2024

recovered. The study examined the gene expression analysis of cell stress pathways, and it found that the PC groups had higher levels of Janus kinase (JAK), Signal Transducer and Activator of Transcription (STATS) pathways through JAK (JAK-2 and STAT-1), G protein-Coupled Receptor Kinase 2 (GRK-2), Calmodulin 2 (CALM-2), Proteins Inhibitor of Activated STAT 1 (PIAS-1), Forkhead Box 01, 03, 04, (FOXO-1, FOXO-3, FOXO-4), Bcl-2 Associated X-protein (BAX), B-cell Lymphoma 2 (BCL-2), Interleukins (IL-6, IL-1β), and Tumour Necrosis Factor-α (TNF-α). On the other hand, downregulation was observed in NC groups and *P. stewartii* extract-treated groups. The outcome of this study suggests a strong relationship towards ameliorative effects.

1. Introduction

Chronic inflammation which is initiated by oxidative stress and the autoxidation of lipoproteins and lipids, can lead to conditions including cancer, diabetes mellitus (DM), obesity, metabolic syndromes, cardiovascular illnesses, and obesity (Masenga et al., 2023). Oxidative stress (OS), imbalanced diets, and exercise intolerance are the primary causes of multifactorial and chronic inflammation, and they are all linked to illnesses related to obesity. Obesity is associated with T2DM to induce IR. OS and insulin resistance cause a significant upregulation of pro-inflammatory mediators, cytokines, and chemokines, which exacerbate the pathogenesis of T2DM (Tangvarasittichai, 2015). Reactive oxygen species (ROS) which are produced in a hyperglycemic environment and cause an imbalance in the oxidant/antioxidant ratio, are investigated using JAK-STAT pathways. These pathways are essential biological events that regulate many diverse processes, including apoptosis, differentiation, and cell division (Bhatti et al., 2022). It has been discovered that the JAK/STAT system is involved in several different liver activities, such as hepatoprotection, hepatic proliferation, and gluconeogenesis, among other metabolic processes. Furthermore, this signaling system plays a critical role in metastasis and the epithelial-mesenchymal transition, two phases of carcinogenesis (Park et al., 2023). Interestingly, it is associated with the production of cancer cells, which are important for the spread and resistance to therapy. Activation of FOXO-1, FOXO-3, and FOXO-4 is involved in gluconeogenesis and possesses extensive and exclusive interaction signals towards oncogenic partners such as mitogen-activated protein kinase 3 (MAPK3) (Ponugoti et al., 2012). The carcinoma and hematopoietic cancer signaling, JAK/STAT interacts with different regulators of apoptosis and necrosis which include BAX, BID, BCL-2, interleukins, and TNF (Tzifi et al., 2014).

The main pro-inflammatory cytokines interferon (IFN) and TNF are expressed by mast cells, fibroblasts, endothelial cells, neutrophils, and macrophages (Turner et al., 2014). They function via the endocrine, paracrine, and autocrine systems. Macrophages that are engaged in mediating the inflammatory responses are activated by the IL and IFN-γ. The diet-induced inflammation in adipose tissue, obesity, and glucose intolerance affect the secretion of IL and IFN-γ, which mediates IR (Zatterale et al., 2020). The intracellular signaling pathways, particularly the JAK/STAT pathway, are activated by IL-6. Similar to metabolic syndromes, several additional proteins, such as phosphoinositide 3-kinase (PI3K), and extracellular signal-regulated kinase 1–2 (ERK1/2), may also be activated concurrently with JAK/STAT. These cytokines are in charge of tissue repair, infection management, phagocytic activity and chemotaxis stimulation, fibroblast proliferation, and extracellular matrix protein breakdown following the healing process (Hu et al., 2023). One special, fundamental, and effective element for modulating biological reactions is calcium ions (Ca^{+2}). The 149 amino acid protein calcium calmodulin (CaM), which has a molecular weight of 17 kDa, is essential for conveying information that alters the concentration of Ca^{+2} in target tissues. Three genes (CALM1, CALM2 and CALM3) that encode distinct protein products but differ in nucleotide sequences are responsible for encoding the human CaM (Forest et al., 2008).

Plant-based dietary antioxidants are considered to be important in maintaining human health because these antioxidants give inadequate defense against constant ROS. Medicinal plants (MPs) are rich in antioxidants that protect cells from OS (Liu et al., 2018). Numerous techniques, including 2,2-diphenyl 1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), have been developed to determine the antioxidant characteristics of plants (Ozgen et al., 2006). Within the Lamiaceae family, *Phlomis* is a genus that has over 100 species. *Phlomis stewartii* is one of these species, and it can be found in Pakistan's Baluchistan desert from June through August. The most effective medicinal plant for treating oxidative stress-mediated metabolic syndrome, diabetes, cancer, Parkinson's disease, and cardiovascular illnesses is *P. stewartii* (Rasheed et al., 2022). Because of their biological potential, plants are the best source of natural antioxidants, including phenolic, alkaloids, ascorbic acids, amides, saponins, and diverse bioactive components from different parts of the plant. MPs also play a large role in human health. The previous studies mentioned that flavonoids such as sulforaphane, quercetin, curcumin, resveratrol, and genistein – reduce inflammation, induce apoptosis, and enhance antioxidation potential by modulating different signaling pathways involved in apoptosis such as Wnt, PI3K/Akt, MAPK and NF- kB (Ullah et al., 2020; Rahim et al., 2023). The flavonoid's role in apoptosis has been studied on cancer cell lines, which were mediated either by intrinsic (mitochondrial pathways) or by extrinsic (death receptor) pathways. The death receptor pathways are arbitrated by TNF-α receptors, whereas apoptotic markers – such as BID, BAX, BCl-2, and pro-survival BCL-XL – are involved in the intrinsic pathway (Guicciardi and Gores, 2009). The objective of this work was to demonstrate the capability of *P. stewartii* extracts to modulate the anti-carcinogenic and antidiabetic effects by in vitro and in vivo analyses.

2. Materials and methods

2.1. Identification of plant materials

In June–August of 2018, the fresh plant was harvested from Baluchistan's arid region. Additionally, it was verified by the University of Balochistan's Botany Department in Quetta, Pakistan. The plant was transported to the Government College University Faisalabad research laboratory using polythene bags.

2.2. Preparation of plant extract

The leaves, flowers, and whole plant (leaves, flower, stem, bark) were all cleaned with distilled water and dried at room temperature for 2 weeks. The components were then ground into a powder using a mechanical blender. Ten gram of plant powder was immersed in methanol (100 mL, 150 mL, and 200 mL) to facilitate percolation. For a month, the mechanical shaking procedure was carried out. The whole mixture (plant powder and solvent) was filtered through Whatman No. 1 filter sheets and then heated the mixture to 32 °C in a rotary evaporator to yield semi-solid extracts. These final extracts were refrigerated at 4 °C in preparation for further investigation.

2.3. Antioxidant potential

2.3.1. DPPH free radical scavenging potential assay

The DPPH radical scavenging action of *P. Strewatrii* plant extract was determined through a spectrophotometer (Thermo Scientific Multiskan

GO™ with SkanIt software 4.1) at 517 nm using the given protocol outlined by slight modification (Langley-Evans, 2000). The extracted sample was added into a 3 mL solution of 1 mM DPPH to determine the scavenging activity. The whole mixture was shaken thoroughly and allowed to stand for 30 minutes in the dark at room temperature. Ascorbic acid with concentrations 1, 2, 4, and 8 mg/mL was used as a positive reference. This formula (Eq. 1) was used to estimate the free radical inhibition activity.

$$\text{DPPH scavenging ability (\%)} = [(Ao - Ae) / Ao] \times 100 \quad (1)$$

Ae is the absorbance of the sample after 30 minutes of storage in the dark, and Ao is the absorbance of the DPPH control solution at 0 minutes. IC_{50} values were measured from the plot as the antioxidant concentration needed for providing fifty percent free radical scavenging potential.

2.3.2. Reducing power assay

The reducing power assay was carried out by following the method given by (Benzie and Strain, 1996). The sample was mixed with 2.5 mL of phosphate buffer of 0.2 M (pH 6.6) and 2.5 mL of one percent potassium ferricyanide in a test tube of 10 mL. A water bath was used for incubation at 50°C for 20 minutes. A volume of 2.5 mL of 10 % trichloroacetic acid was added to the whole mixture after incubation and centrifuge (Sigma model 1–14 14800 rpm/min Germany) at 5000 rpm for ten minutes. After that mixed the 2.5 mL supernatant in 2.5 mL of de-ionized water and 0.5 mL of 0.1 % ferric chloride. The reaction mixture's absorbance was measured at 700 nm.

2.3.3. Ferric reducing antioxidant power assay

The FRAP assay was carried out following certain changes (Oke et al., 2009). FRAP reagent having sodium acetate buffer (300 mmol/L, pH 3.6), 20 mmol/L $FeCl_3$ solution, and TPTZ solution in 40 mmol/L HCl in proportion of 10:1:1 (V/V/V), respectively. The freshly prepared 1.5 mL FRAP reagent was mixed with a 50 µl sample. The reaction's absorbance was noted at 593 nm, after four minutes.

2.4. Antibacterial test

Different methanolic extracts of *P. stewartii* plant were evaluated for antibacterial potential against *Staphylococcus aureus* and *Escherichia coli* by using the disc diffusion method (Samtiya et al., 2021). The Mueller Hinton agar (MHA) was used to grow the colonies and then the zone of inhibition was calculated through the vernier caliper.

2.5. Experimental design and animals used for in vivo studies

Following earlier research, rats were placed in a cigarette smoke (CS) chamber with an attached suction pump (dimensions: 1.000 × 850 × 750 mm^3). By employing the suction pump, CS was drawn out from the cigarette (composition per unit: 14 mg tar, 1.1 mg of nicotine, and 15 mg carbon monoxide) and filled the enclosed chamber. During the first week, the rats were exposed to 20 cigarettes twice a day, with the number progressively increasing from 8 to 20 for one hour. Consequently, twenty cigarettes were used in each smoking chamber (1 hour in the morning and 1 hour in the afternoon session) in 120–130 g rats for 8 weeks. Rats exhibiting cherry red to purple plantar skin, mouth breathing, agitation, and mouth wheezing were given to all clear, and the chamber was opened. Before administering a single intraperitoneal dose of 140 mg/kg of alloxan monohydrate by dissolving the drug in normal saline, the blood sugar levels of rats were assessed. The administration of alloxan resulted in acute hypoglycemia then 10 % dextrose solution was provided to rats to avoid mortality rate. A commercially available glucometer (OnCall® Ez II; SN 303S0014E09) was used to measure the fasting sugar level of rats' blood on the 3rd, 7th and 10th day post administration of alloxan injection. Rat's displaying fasting blood glucose level ≥ 250 mg/dL while kept on a normal diet. Thirty rats weighing 160–170 g were separated into 5 groups (n=6) as in Table 1. The dosage of *P. stewartii* LM extract was given at a concentration of 300 mg/kg body weight following the guidelines of basic, and clinical pharmacology and toxicology policies for clinical experimental policies. The Animal Ethical Committee (Study number:019306) of Government College University, Faisalabad, Punjab, Pakistan, authorized the experimental procedure.

2.6. Metabolic hormonal profile

In the experiment's 12th week, the blood sample was collected. The rats were anaesthetized with chloroform prior to their sacrifice, and their blood was collected in individual vacutainers for serum collection in platelet activator gel following 10-minute centrifugation (Sigma model 1–14, 14800 rpm/min, Germany). The separated serum was then evaluated through a multiscan plate reader (Thermo Scientific Multiskan GO™ with SkanIt software 4.1) for T3, T4, TSH, insulin, leptin, and glucokinase while following the guidelines mentioned in Thermo Fisher Scientific ELISA kits.

2.7. Gene expression

The gene expression analysis for cell stress pathways which includes JAK-STAT pathways PIAS-1, CLAM-2, GRK-2 gluconeogenesis pathways through FOXO-1, FOXO-3, FOXO-4 cell death pathways through BAX, BCL-2, BID, IL-6, IL-1β, and TNF-α were studied through Real-Time Quantitative Reverse Transcription (qRT-PCR) while following the guidelines with slight modifications mentioned in thermo-scientific SYBR green qPCR kit. The list of primers is mentioned in Table 2**.**

2.8. Statistical analysis

The one-way ANOVA followed by the least significant difference (LSD) of statistical analysis with post hoc was applied while taking the average from three replicates. The data were shown as mean ± SD with a 5 % level of significance and superscripts of different alphabets through international business machines (IBM), statistical package for social sciences (SPSS) software (21.0).

3. Results

3.1. DPPH radical scavenging activity

All tested extracts showed antioxidant potential. The lowest IC_{50} indicates the strongest capability of extract to act as a DPPH free radical scavenger. Methanolic extract LM, FM, WPM, and standard ascorbic acid had the lowest IC_{50} values 38.92, 55.29, 44.41, and 28 µg/mL, respectively. These results imply that LM possesses the most promising free radicals scavenging activity among all extracts as shown in Fig. 1. Different alphabets are used to calculate the significance level ($P<0.05$).

3.2. Free radical scavenging antioxidant ability

The methanolic extracts of *P. stewartii* plant revealed IC_{50} values of LM, FM, WPM and standard ascorbic acid 45.28, 54.43, 50.76, and 34.33 µg/mL, respectively. The results showed the existence of a concentration-dependent radical scavenging assay, with 150 µg/mL showing the highest activity as shown in Fig. 2. Different alphabets are used to calculate the significance level ($P<0.05$).

3.3. Reducing power assay

Table 3 shows the results of the antioxidant assay of *P. stewartii* plant extracts. All tested samples showed prominent antioxidant potential. The highest absorbance 1.36 was demonstrated by LM at a

Table 1
Experimental grouping to study the influence of *P. stewartii* extracts.

NCControl + vehicle
PCCS + alloxan-induced diabetes
LMCS + alloxan-induced diabetic rats were treated with LM extract
FMCS + alloxan-induced diabetes rats were treated with FM extract
WPMCS + alloxan-induced diabetes rats were treated with WPM extract

Negative control (NC), Positive control (PC), Leave methanol (LM), Flower methanol (FM), Whole plant methanol (WPM), and Cigarette smoke (CS).

Table 2
Detail list of reverse and forward primers used for qRT-PCR along with their amino acid sequences.

	Gene	Forward Primer	Reverse Primer
1.	Jak-2	CCATCCCCTACACCCCTAGT	CCAGAGAAGCCGGGAATCAG
2.	Stat-1	AAGCACCAGAACCGATGGAG	AGCCTCGAGACAGTGCAATC
3.	Grk-2	TGTACGAGATGATCGCAGCC	ACAGGTTCCATCCCGGAAAC
4.	Calm-2	CTCATGCATGCAGCTTGGTG	ACCCGTTTCCTGCACATCAT
5.	Pias-1	CCAGGTGGCAGGAGAGACTA	CCAGGACGCTTCTGTTCGAT
6.	Foxo-1	TTTGCTAAGAGCCGAGGACG	GAGCTGGTTCGAGGACGAAA
7.	Foxo-3	TACGAGTGGATGGTGCGCTG	AGGTTGTGCCGGATGGAGTTC
8.	Foxo4	GCCTGGCCTACCCAGATTGT	CTTCGACTTTCCGCCTCTCTC
9.	Bid	GGTAGAGCTCGGGTCCAAGT	TTCGGAGAAAGCCGAACACC
10.	Bax	AAGAGATGGTGCTTTTGGGGT	AAGCATCAGTTTCGTGTGGC
11.	Bcl-2	TTCTTCCTGACTCGGCTCCAC	TCCACAGACATAACCCTTCTGC
12.	IL-6	GCCCTTCAGGAACAGCTATGA	TGTCAACAACATCAGTCCCAA
13.	IL-1β	GACTTCACCATGGAACCCGT	CAGGGAGGGAAACACACGTT
14.	Tnf-α	CCTCTGCGGGACACTCATTT	CCACCTCCTTTGAAGCCACT
15.	Gapdh	ATGACTCTCCCACGGCAAG	TACTCAGCACCAGCATCACC

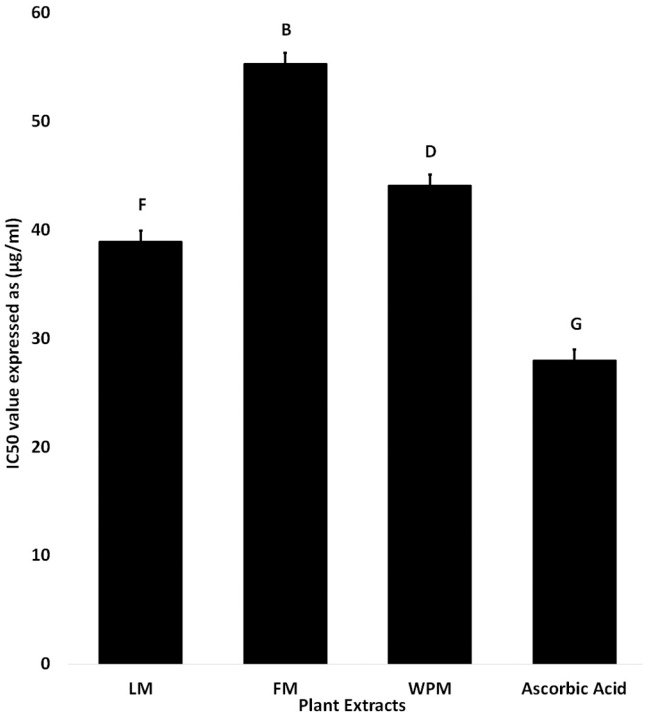

Fig. 1. : IC_{50} value of *P. stewartii* plant extracts for DPPH assay.

concentration of 500 µg/mL. The absorbance measured by FM and WPM at the highest concentration 500 µg/mL was 1.12 and 1.21, respectively. The outcomes of the present study indicate that the reducing power of the methanolic extracts of different parts of *P. stewartii* increased consistently with an increase in the volume of extracts from 200 to 500 µg. These findings show that *P. stewartii* extracts possess antioxidant potential. Reducing power assay revealed that methanolic leave extracts had the highest antioxidant potential compared with all other extracts. Different alphabets are used to calculate the significance level ($P>0.05$).

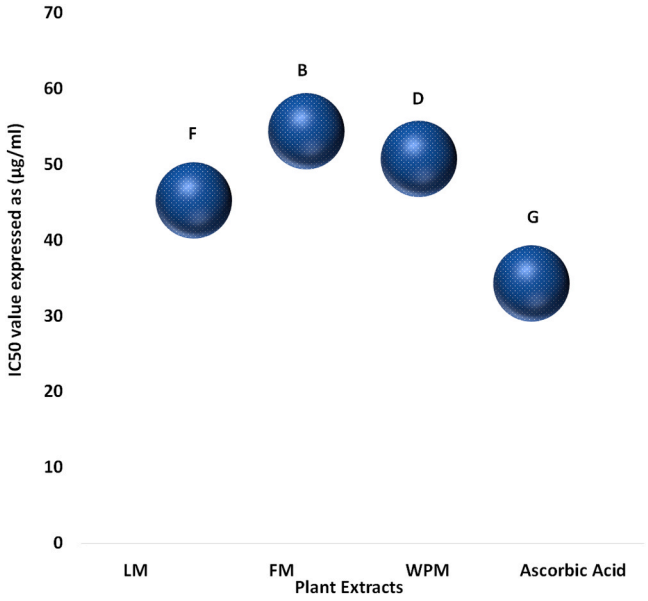

Fig. 2. : IC_{50} value of *P. stewartii* plant extracts for FRAP assay.

3.4. Anti-bacterial potential of P. stewartii extract

The result revealed that all methanolic extracts are potentially effective in suppressing bacterial growth with variable potency. LM was the most effective extract regarding the microbial growth of all tested pathogenic bacteria at a concentration of 10 mg/mL as shown in Fig. 3**a**. Other extracts FM and WPM also showed variable potential against bacterial strain. The evaluation of the antibacterial potential of different extracts is illustrated in Figs. 3**b**-3**c.** Different alphabets are used to calculate the significance level ($P<0.05$).

Table 3
Reducing power activity of different parts of *P. stewartii* methanolic extracts and standard ascorbic acid.

Sample Name	Sample Concentration (μg/mL)	Absorbance at 700 nm
LM	200	1.21 ± 0.02^{A}
	300	1.26 ± 0.12^{A}
	400	1.31 ± 0.30^{A}
	500	1.36 ± 0.09^{A}
FM	200	0.99 ± 0.10^{A}
	300	1.02 ± 0.13^{A}
	400	1.09 ± 0.05^{A}
	500	1.12 ± 0.07^{A}
WPM	200	1.11 ± 0.14^{A}
	300	1.15 ± 0.20^{A}
	400	1.18 ± 0.19^{A}
	500	1.21 ± 0.25^{A}
Ascorbic Acid	200	1.58 ± 0.18^{A}
	300	1.69 ± 0.31^{A}
	400	1.81 ± 0.54^{A}
	500	1.98 ± 0.38^{A}

Leave methanol (LM), Flower methanol (FM), Whole plant methanol (WPM). Means with different superscripts, and capital letters indicate significant differences ($p \leq 0.05$).

3.5. Metabolic hormonal profile

Our study's findings demonstrated a decline in T3, T4, and TSH levels in the PC group as compared to the LM-treated group, whereas insulin, leptin, and GCK levels increased as compared to the LM-treated groups (Fig. 4**a-f**). Fig. 4**a** shows that the T3 level in all treated groups increased significantly as compared to the PC group. Fig. 4**b** showed that the T4 level significantly increased in the LM and FM groups as compared to the PC group. Fig. 4**c** presented a significant increase in the level of TSH, whereas levels of insulin and leptin in Figs. 4**d**-4**e** showed a significant decrease as compared to PC groups. Fig. 4**f** indicated that the GCK level in the FM group was much lower than that of the PC group. Different alphabets are used to calculate the significance level ($P<0.05$).

3.6. Gene expression

3.6.1. Oxidative stress pathways

The findings of our examination showed that the expression level of JAK, STAT-1, GRK-2, PIAS-2, and CALM-2 increased in the PC group, while the LM, FM, and WPM-treated group downregulated it. Moreover, the expression of JAK-2 in the PC group was downregulated as compared to all treated groups shown in Figs. 5**a**-5**e.** Different alphabets are used to calculate the significance level ($P<0.05$).

3.6.2. Gluconeogenesis pathway

The Foxo-1, Foxo-4 and Foxo-3 in the PC group showed increased expression while all treated groups downregulated it to produce ameliorative effects (Fig. 6). Different alphabets are used to calculate the significance level ($P<0.05$).

3.6.3. Cell death markers

The TNF-α, IL-6, IL-1β, BAX, BCL-2, and BID in the PC group showed increased expression while all treated groups downregulated it to produce ameliorative effects (Fig. 7). Different alphabets are used to calculate the significance level ($P<0.05$).

4. Discussion

Over the past few years, numerous bioactive components found in MPs have been revealed to have promising effects and antioxidant potential against human diseases by lowering oxidative damage (Samtiya et al., 2021). Parallel to this, earlier research has also shown that extracts from the *Phlomis* plant have high concentrations of flavonoids and total phenolic content, both of which greatly enhance their antioxidant and antibacterial activity (Rasheed et al., 2022). Interestingly, the results of the DPPH free radical scavenging actions showed that all extracts had a notable antioxidant profile in Fig. 1. It was discovered that the antioxidant activity of LM extract was promising. For comparison purposes, we also checked some previous investigations of Lamiaceae plants, such as *P. bruguieri* Desf, *P. persica* Boiss, and *M. vulgare* L, which possess good antioxidant activities (Firuzi et al., 2010). Research by (Farooq et al., 2019) reported that methanolic extracts of *P. stewartii* had an IC_{50} of 28.42 μg/mL, whereas the current study reported 39.34 μg/mL in the LM extracts of *P. stewartii*. Our findings corroborate a previous finding that *Phlomis* leaves methanolic extracts had higher antioxidant activity. *P. biloba* Desf leaves extract in methanol has an IC_{50} of 47.78 ± 1.12 μg/mL, according to previously published results (Merouane et al., 2019). Another investigation has reported the IC_{50} 20.2 ± 66b μg/mL, 15.7 ± 0.28c μg/mL of methanolic extracts *P. umbrosa* and *P. megalantha*, respectively (Zhang and Wang, 2009). The current findings are consistent with published reports when comparing the data with those from earlier investigations.

Instead of relying on the transfer of hydrogen atoms, the FRAP test depends on the transport of electrons. The basis for this activity is PH's capacity to convert Fe^{+3} to Fe^{+2} (Chaves et al., 2020). This test was carried out at pH 3.6 to preserve iron stability. At low pH, the reaction increases the reduction potential, which is important in the reaction mechanism, and decreases the ionization potential, which promotes hydrogen atom movement in Fig. 2. In the presence of 2,4,6-trypyridyl--s-triazine, Fe^{+3} is reduced to Fe^{+2}, which is facilitated by the formation

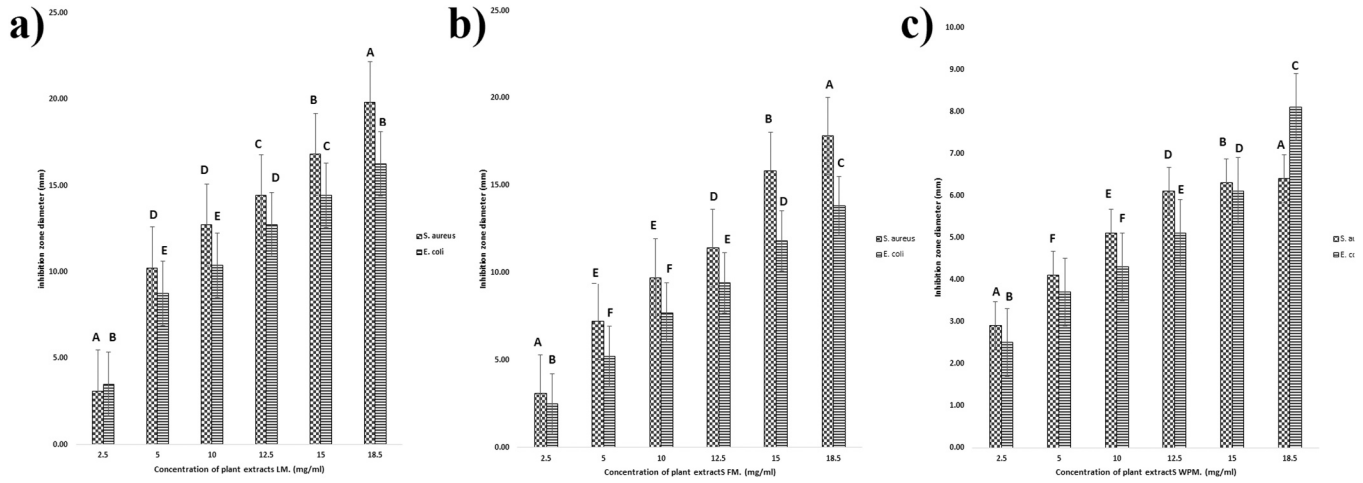

Fig. 3. : Zone of inhibition of *P. stewartii* methanolic extracts a) LM, b) FM, c) WPM at different concentrations for *Staphylococcus aureus* and *Escherichia coli.*

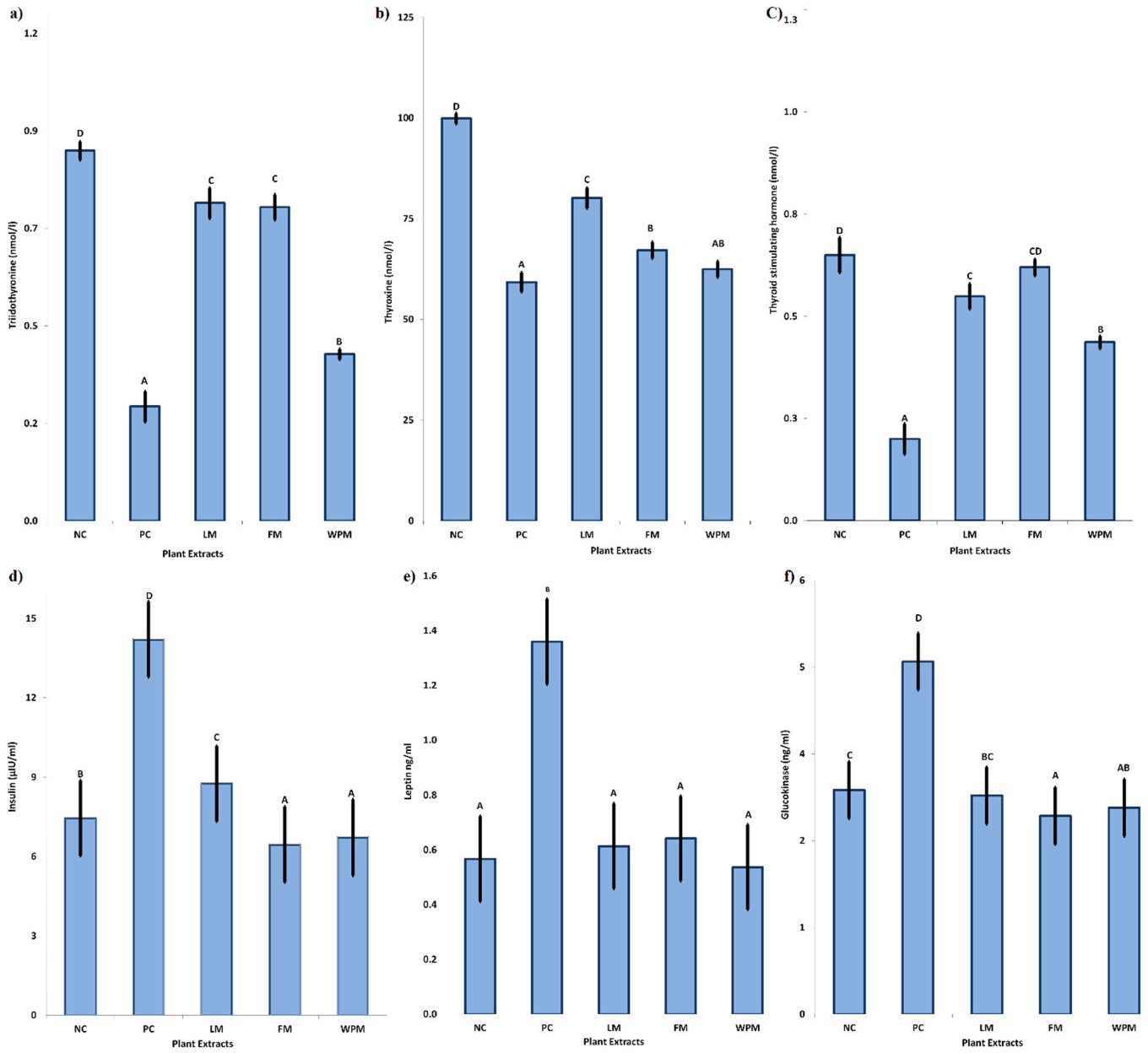

Fig. 4. : Effects of *P. stewartii* plant extracts on metabolic hormonal profile through serum analysis in diabetic rat's model.

of a colored complex with Fe^{+2} at absorption 593 nm (Hartati et al., 2020). Because this assay aids in the identification of ingredients with a redox potential of less than 700 mV, the capacity to reduce power appears to be associated with the degree of hydroxylation and amount of conjugation in pH. Nevertheless, the FRAP assay is not useful for identifying substances that function by hydrogen transfer, or radical quenching, especially proteins (Youn et al., 2019). According to reports, there is a direct relationship between antioxidant concentrations and a decrease in maximal absorption. There was a strong association between the DPPH assay and FRAP (Abate et al., 2022). The outcomes validate the conclusions of an additional research team that methanol extracts exhibited noticeably greater FRAP values in comparison to the other extracts.

Reducing power is a significant indicator of antioxidant potential and is associated with it. Various bioactive components found in plants with reducing power show that they can oxidize lipid peroxidation process intermediates and signify electrons, hence suppressing OS and serving as primary and secondary metabolites (Erb and Kliebenstein, 2020). Among ROS, the hydroxyl free radicals are the most active and cause serious damage to adjacent biomolecular activities of living cells, which can be inhibited by antioxidants. Therefore, the scavenging potential of free radicals is important for antioxidant protection in living cells (Altemimi et al., 2017). Depending on the decreasing power of each component, the test extracts yellow color changes to various hues of green and blue in the reducing power ability. The Fe^{+3}/ferricyanide complex used in this procedure shifts to ferrous in response to the presence of distinct reducers (Gulcin, 2015). The concentration of Fe^{+3} ions can be seen and the production of Pearl's Prussian was observed at 700 nm.

However, other authors have argued that all MPs methanol extracts showed better reducing power (Gonzalez-Palma et al., 2016). This indicates that the polarity of the solvent has a substantial impact on the assessment of the chelating capacity of different extracts because of variations in MPs sections, growth seasons, or the kinds of bioactive elements that are present in plant material. According to earlier research, the methanol extracts of the *Phlomis* plant showed more

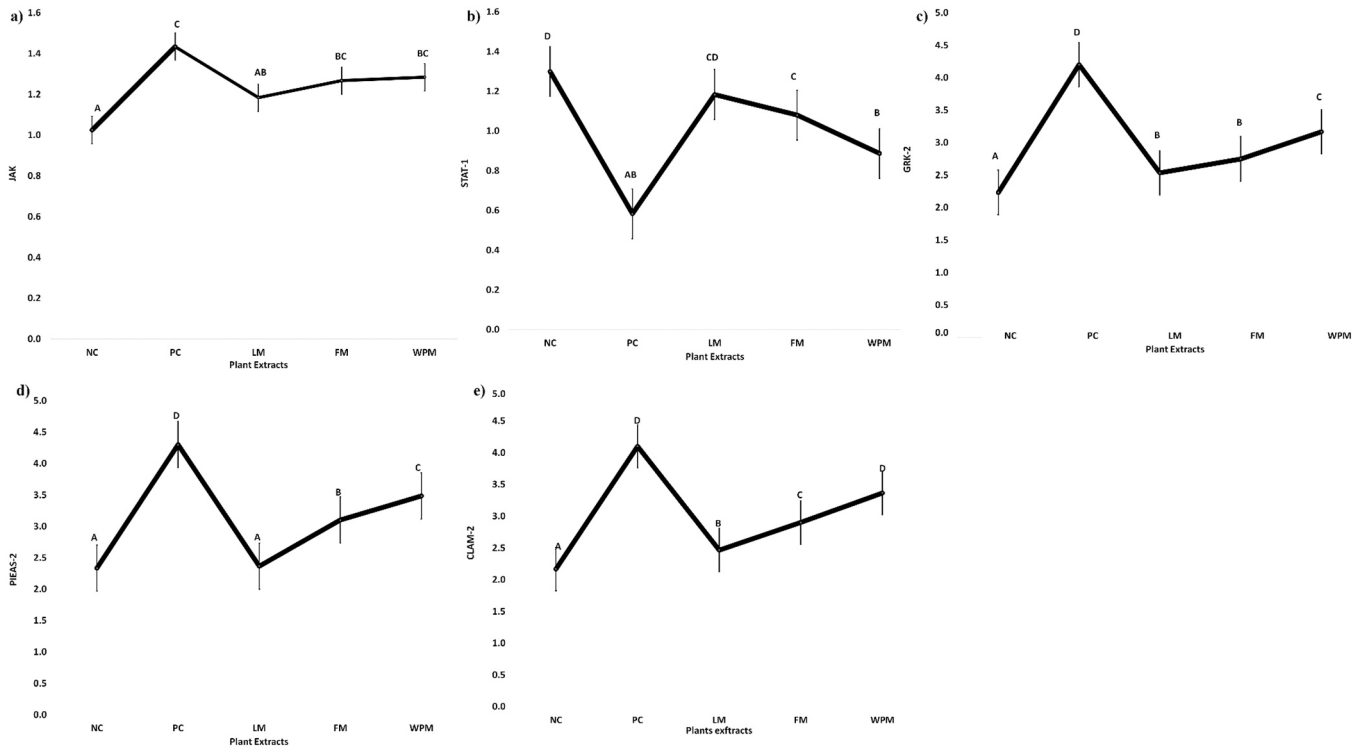

Fig. 5. : Effects of *P. stewartii* plant extracts on oxidative Stress Pathways in diabetic rat's model.

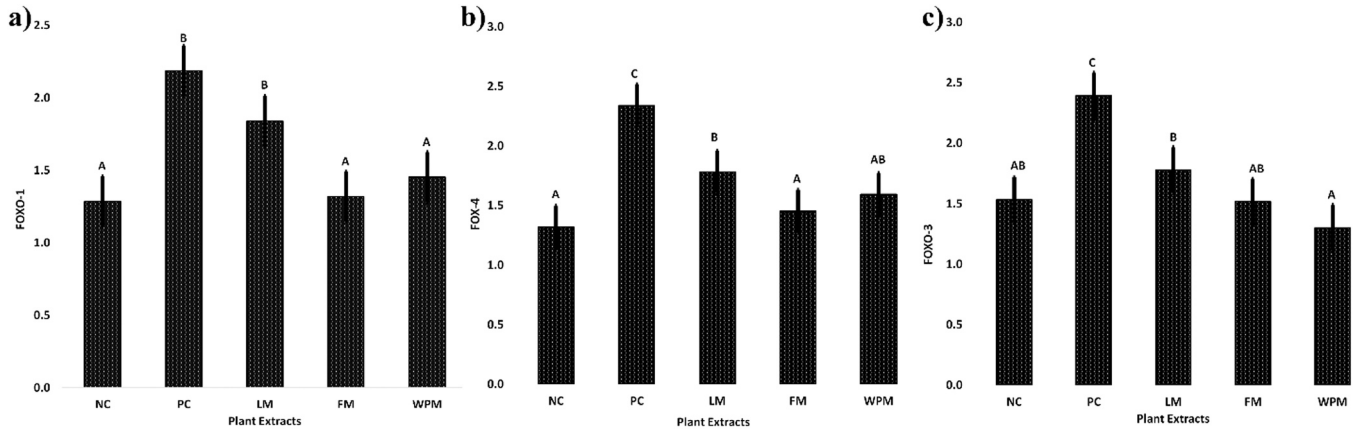

Fig. 6. : Effects of *P. stewartii* plant extracts to study gluconeogenesis at the cellular level in diabetic rat's model.

reduction capacities than other extracts, much like the DPPH test (Taskın et al., 2018). Our results are consistent with earlier research showing that *P. megalantha* and *P. umbrosa* methanolic extracts had absorbances of 2.2 ± 0.01 and 1.3 ± 0.01, respectively (Zhang and Wang, 2009). The findings showed that LM contains a variety of bioactive components.

MPs have various bioactive constituents, which are a significant class of antioxidants because they directly impact bacterial development and suppress pathogenic activity. It has been reported that three main processes involved in the supposed antibacterial activity are cytoplasmic leakage, suppression of nucleic acid synthesis, and outer membrane permeability (Delcour, 2009). The interaction of bioactive ingredients with non-specific forces (hydrogen bonding, hydrophobic impact lipophilic forces, and covalent bond formation) has been linked to microbial adhesion and transport proteins in the cell envelope. These bioactive components can chelate iron, which is necessary for bacterial growth and survival, thus they have antibacterial potential. These polyphenols break down the wall, increase the cytoplasm membrane's permeability, and ultimately, cause lipids peroxidation (LPs) to be released. The current results are supported by earlier research on this activity, which showed that antibacterial capability depends on the synergy of substances in a mixture rather than a single isolated component, however, for some bacteria, a single particular component is sufficient rather than a portion (Vaou et al., 2021).

Results of the antimicrobial activity of all extracts can suggest that *E. coli* was the most resistant strain to plant extract followed by *K. pneumonia* while *S. aureus* and *B. cereus* were the most susceptible strains to the extracted plant material, respectively in Figs. 3a-3c. The current study's findings corroborate previously published information indicating that methanol extracts had strong potential against every tested type of bacteria. In terms of antibacterial assay, comparison with other *Phlomis* species demonstrates its superior capability over *P. pungens, P. armeniaca,* and *P. nissolii*; additionally, it validates the use of methanol extracts against both gram-positive and gram-negative organisms (Sarikurkcu et al., 2016). According to earlier research,

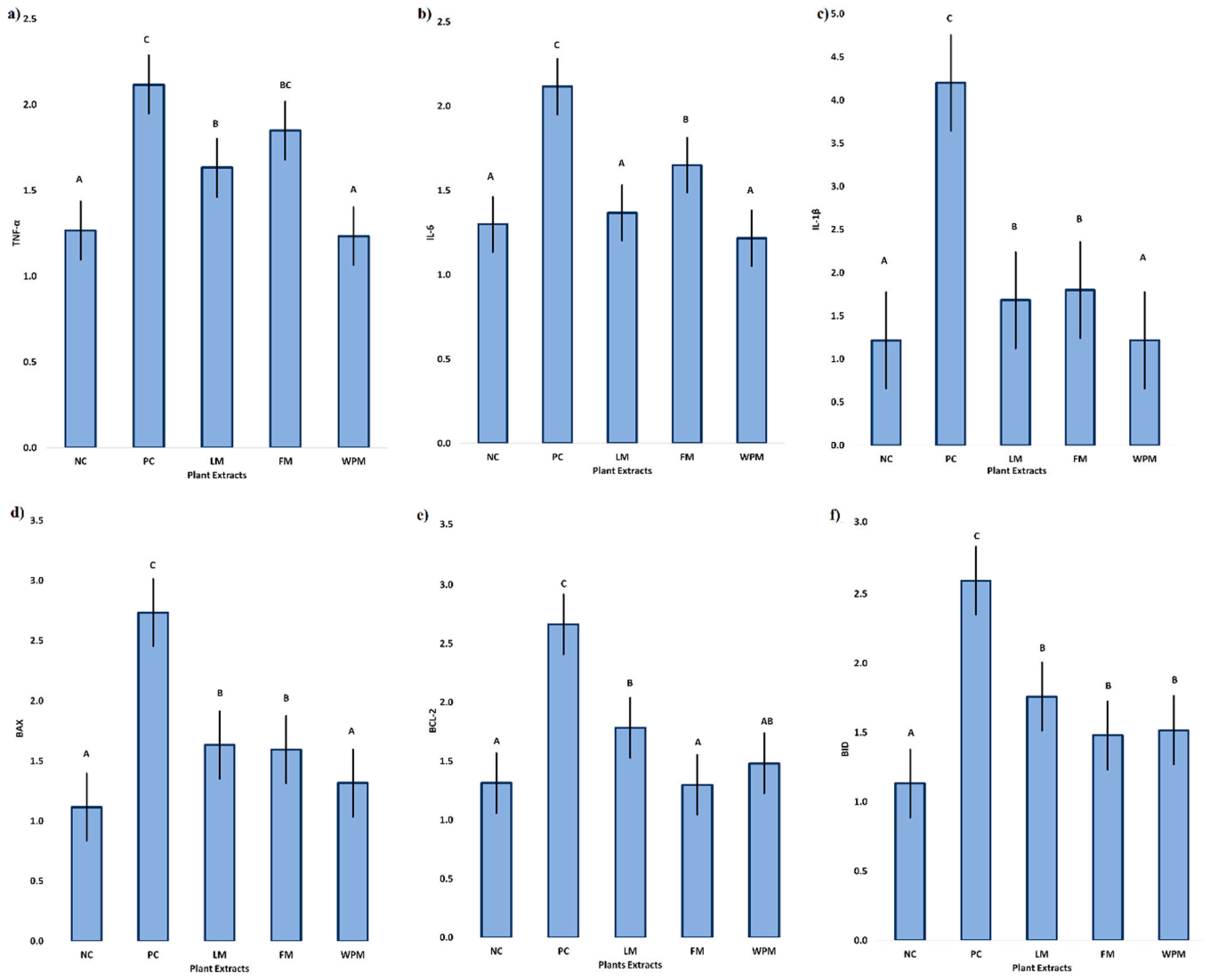

Fig. 7. : Influence of *P. stewartii* plant extracts to study anti-inflammatory, anti-proliferative, and anti-apoptotic at the cellular level in diabetic rat's model.

methanol can be used to extract various bioactive substances with antibacterial potential (Al-Farraj et al., 2020). Additionally, they discovered that the strong polarity of methanolic extracts facilitates the extraction of a variety of phytochemicals that are linked to their notable antibacterial activity. The antibacterial activity of *P. stewartii* plant is significantly enhanced by the presence of various phenolic components, according to the results of several invested antioxidants. Our results are consistent with earlier studies (Borges et al., 2020; Bashmil et al., 2021).

Metabolic hormones are concerned with the metabolism of carbohydrates, proteins, and lipids hence involved in the utilization and storage of energy. The expression of peroxisome prolifera-tor-activated receptor α (PPAR-α) activates the transcription and oxidation of fatty acids genes like cptla and Aox (Christofides et al., 2021). The PI3K-Akt pathways mediate the gluconeogenesis and insulin signaling pathways. Hepatocytes AMP-activated protein kinase (AMPK) guards against hepatic steatosis and atherosclerosis (Zhao and Saltiel, 2020). The fatty acid metabolism occurs in the mitochondria, which is also the site for regulating the thyroid hormone. The thyroid hormone increases the expression of cholesterol 7α- hydroxylase (CYP7A1), so increases the bile acid from the liver to emulsify the fats. The mitochondrial enzymes important in fatty acid oxidation which include pyruvate dehydrogenase kinase isoform 4 (PDK4), medium chain acyl CoA dehydrogenase (MCAD), and mitochondrial uncoupling protein (UCP2) are upregulated by thyroid hormone (Sinha et al., 2018). The results from our study showed that T3, T4, and TSH levels decreased in the PC group as compared to the LM extract-treated group, whereas the levels of insulin, leptin, and GCK increased as compared to the LM extract-treated group as shown in Figs. 4a-4f.

Deoxyribonucleic acid methylation (DNAm) is one of the important epigenetic modifications. Without changing the sequence of DNA, it modifies the gene expression, and tissue growth and function. Demethylation is reactivated to raise the expression level, whereas DNAm can block a gene's transcriptional potential and aid in lowering expression levels. The enzyme DNA methyltransferase (DNMT) produces 5-methylcytosine (5mC) by adding methyl to cytosine (Moore et al., 2013). The 5mC scavenges the binding of transcription aspects, which results in the inhibition of genes, the influence on metabolism and genome, how the genome is based on various metabolites, as well as how metabolic networks control the different changes at the genome level (Song et al., 2022).

The guardians of the biological processes are transcription factors (TFs). They regulate the expression of genes that code for essential proteins across the whole proteome, ranging from those involved in cell communication and metabolism to those governing the immune system and cell cycle (Liu et al., 2022). The JAK/STAT family of transcription factors is different from secondary messengers in that it controls gene expression that governs important cellular functions (Hu et al., 2023). There are seven members in this family. According to recent research,

they not only transduce signals that govern transcription but they also regulate mitochondrial anabolism and catabolism, as well as nuclear compartmentalization and genome integrity. While JAK/STAT members all contribute to cell signaling and biogenesis, member-specific protein interactions highlight the distinct roles that STAT members play in different biological processes. The upregulation of JAK in PC groups and down-regulation of STAT-1 in our studies results showed that *Phlomis* showed ameliorative effects in Figs. 5**a**-5**b**. The gluconeogenic proteins FOXO-1, FOXO-4, and FOXO-3 have unique and substantial interaction evidence with oncogenic partners such as Mitogen-activated protein kinase 3 (MAPK3) (Essaghir et al., 2009). The findings of our research indicated that the expression of FOXO-1, FOXO-4, and FOXO-3 was downregulated in all treated groups with *P. stewartii* as shown in Figs. 6**a**-6**c**.

The CALM-1, CALM-2, and CALM-3 are relatively abundant in the human heart, moreover the expression of all three transcripts increases due to TSDM. Multiple downstream pathways including mTORC1, calcium pathways through GRK-2, and CALM-2 were signaled to regulate glucose and lipid metabolism (Mao and Zhang et al., 2018). The results from our studies suggested that *P. stewartii* extracts downregulated GRK-2, CALM-2, and PIAS-2, as shown in Figs. 5**c**-5**e.** By raising glucose-6-phosphate levels and stimulating the hepatic production of microsomal triglyceride transfer protein (MTP), which catalyzes the transfer of lipid to apolipoprotein B (apoB) for the assembly and secretion of very low-density lipoproteins (VLDL), FOXOs regulate the expression of the glucokinase gene, which may promote the lipogenic response (Zhang et al., 2022). The PIAS are principally documented as inhibitors for STAT proteins. STAT families initiate the cellular mechanisms coupled with acquired and innate immunity to facilitate the release of cytokines involved in cellular transformation. The PIAS proteins regulate transcription factors through the involvement of nuclear hormone receptors and p53 in various signaling pathways like steroid hormones and transformation growth factors (TGF-β). The PIAS protein regulates its expression through inhibition of DNA-binding activity of transcription factors, recruiting transcriptional co-factors for repression, and activation of protein sumoylation (Shuai and Liu, 2005).

TNF-α, IL-6, and IL-1β are proinflammatory cytokines released from smooth muscle, and activated endothelial cells are involved in inflammation, cell proliferation, and smooth muscle migration and they are enhanced in patients with T2DM (Zhang, 2008). The results from our studies suggested that *P. stewartii* extracts have ameliorative effects in downregulating the expression of TNF-α, IL-6, and IL-1β, as shown in Figs. 7**a**-7**c.** The pro-inflammatory cytokines released from stellate macrophages and kupffer cells protect the hepatic tissue from alcoholic toxication, LPS, ischemia, and T cell-mediated injuries as well as bacterial and viral infections. In all the carcinoma and hematopoietic cancer networks, JAK-STAT interacts with different regulators of apoptosis and necrosis which include BID, BAX, BCL-2, and interleukins. The crosstalk between these pathways suggests that the formation of heterodimers is important in diseased states (Langley-Evans, 2000). Our research findings demonstrated that *P. strewartii* extracts decreased the expression of BID, BAX, and BCL-2 as shown in Figs. 7**d**-7**f.**

5. Conclusions

In conclusion, low-grade to chronic inflammation and IR are the root causes of obesity, which is strongly correlated with the prevalence and incidence of type T2DM. This research has clarified the immune-mediated processes underlying the genesis and progression of DM, a complex and complicated metabolic syndrome. Using diabetic rat models, we investigated the anti-inflammatory, anti-microbial, anti-apoptotic, anti-inflammatory, and anti-cancer properties of *P. Stewartii* extracts, both *in vitro* and *in vivo*, via molecular signaling pathways. Our investigation findings demonstrated that *P. stewartii* extracts' eco-friendly and time-saving techniques for pre-paring natural antioxidants make them suitable for use in industrial flowering. Targeting key cytoplasmic proteins would therefore reduce the amount of space available for oncogenic signaling to be transferred to other proteins in therapeutic efforts. To evaluate the impact of *P. stewartii* on systemic, beta-cell function, islet inflammation, glucose intolerance, islet inflammation, and IR, we advise doing more clinical trials.

Funding

No funding.

CRediT authorship contribution statement

Muhammad Saeed Ashraf Janjua: Software, Formal analysis. **Haroon Rashid:** Software, Formal analysis. **Muhammad Rahim:** Writing – original draft. **Mohamed Mahmoud:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Gaber Batiha:** Writing – original draft, Funding acquisition, Conceptualization. **Mamoon Rasheed:** Investigation. **Syed Naqvi:** Investigation. **João Miguel Rocha:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Sadaf Hassan:** Software, Formal analysis. **Atta Haq:** Software, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors acknowledge support from the Department of Chemistry, GCUF, Pakistan where all research activities were carried out. The authors would like to extend their gratitude to King Saud University (Riyadh, Saudi Arabia) for funding this research through Researchers supporting Project number (RSP-2024-R406). The work of the author J. M.R. was supported by national funds through FCT/MCTES (PIDDAC): LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020) and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020). Author J.M.R. also acknowledges the Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal.

Institutional review board statement

Not applicable.

Informed consent statement

Not applicable.

References

Abate, L., Bachheti, R.K., Tadesse, M.G., Bachheti, A., 2022. Ethnobotanical uses, chemical constituents, and application of plantago lanceolata L. J. Chem. 2022.

Al Farraj, D.A., Gawwad, M.R.A., Mehmood, A., Alsalme, A., Darwish, N.M., Al-Zaqri, N., Warad, I., 2020. In-vitro antimicrobial activities of organic solvent extracts obtained from Dipcadi viride (L.) Moench. J. King Saud. Univ. -Sci. 32 (3), 1965–1968.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., Lightfoot, D.A., 2017. Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6 (4), 42–65.

Bashmil, Y.M., Ali, A., Bk, A., Dunshea, F.R., Suleria, H.A., 2021. Screening and characterization of phenolic compounds from Australian grown bananas and their antioxidant capacity. Antioxidants 10 (10), 1521–1542.

Benzie, I.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239 (1), 70–76.
Bhatti, J.S., Sehrawat, A., Mishra, J., Sidhu, I.S., Navik, U., Khullar, N., Reddy, P.H., 2022. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: current therapeutics strategies and future perspectives. Free Radic. Biol. Med. 184, 114–134.
Borges, A., Jose, H., Homem, V., Simoes, M., 2020. Comparison of techniques and solvents on the antimicrobial and antioxidant potential of extracts from Acacia dealbata and Olea europaea. Antibiotics 9 (2), 48–67.
Chaves, N., Santiago, A., Alias, J.C., 2020. Quantification of the antioxidant activity of plant extracts: analysis of sensitivity and hierarchization based on the method used. Antioxidants 9 (1), 76–91.
Christofides, A., Konstantinidou, E., Jani, C., Boussiotis, V.A., 2021. The role of peroxisome proliferator-activated receptors (PPAR) in immune responses. Metabolism 114, 154338.
Delcour, A.H., 2009. Outer membrane permeability and antibiotic resistance. Biochim. Et. Biophys. Acta (BBA)-Proteins Proteom. 1794 (5), 808–816.
Erb, M., Kliebenstein, D.J., 2020. Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. Plant Physiol. 184 (1), 39–52.
Essaghir, A., Dif, N., Marbehant, C.Y., Coffer, P.J., Demoulin, J.B., 2009. The transcription of FOXO genes is stimulated by FOXO3 and repressed by growth factors. J. Biol. Chem. 284 (16), 10334–10342.
Farooq, A., Ali, S., Ullah, H., Khan, A., Jahan, N., Agha, I., Tareen, R.B., 2019. Evaluation of antioxidant, antimicrobial activity and GC-MS analysis of Phlomis stewartii. Pure Appl. Biol. (PAB) 8, 2420–2433.
Firuzi, O., Javidnia, K., Gholami, M., Soltani, M., Miri, R., 2010. Antioxidant activity and total phenolic content of 24 Lamiaceae species growing in Iran. Nat. Prod. Commun. 5, 193–207.
Forest, A., Swulius, M.T., Tse, J.K., Bradshaw, J.M., Gaertner, T., Waxham, M.N., 2008. Role of the N-and C-lobes of calmodulin in the activation of Ca2+/calmodulin-dependent protein kinase II. Biochemistry 47 (40), 10587–10599.
Gonzalez-Palma, I., Escalona-Buendia, H.B., Ponce-Alquicira, E., Tellez-Tellez, M., Gupta, V.K., Diaz-Godinez, G., Soriano-Santos, J., 2016. Evaluation of the antioxidant activity of aqueous and methanol extracts of Pleurotus ostreatus in different growth stages. Front. Microbiol. 7 (15), 1099–1108.
Guicciardi, M.E., Gores, G.J., 2009. Life and death by death receptors. FASEB J. 23 (6), 1625.
Hartati, R., Nadifan, H.I., Fidrianny, I., 2020. Crystal Guava (Psidium guajava L."Crystal"): Evaluation of in vitro antioxidant capacities and phytochemical content. Sci. World J. 17 (3), 1–7.
Hu, Q., Bian, Q., Rong, D., Wang, L., Song, J., Huang, H.S., Wang, P.Y., 2023. JAK/STAT pathway: extracellular signals, diseases, immunity, and therapeutic regimens. Front. Bioeng. Biotechnol. 11, 1110765.
Langley-Evans, S.C., 2000. Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. Int. J. Food Sci. Nutr. 51 (3), 181–188.
Liu, C., Omilusik, K., Toma, C., Kurd, N.S., Chang, J.T., Goldrath, A.W., Wang, W., 2022. Systems-level identification of key transcription factors in immune cell specification. PLoS Comput. Biol. 18 (9), 1010116.
Liu, Z., Ren, Z., Zhang, J., Chuang, C.C., Kandaswamy, E., Zhou, T., Zuo, L., 2018. Role of ROS and nutritional antioxidants in human diseases. Front. Physiol. 9, 477.
Mao, Z., Zhang, W., 2018. Role of mTOR in glucose and lipid metabolism. Int. J. Mol. Sci. 19 (7), 2043.
Masenga, S.K., Kabwe, L.S., Chakulya, M., Kirabo, A., 2023. Mechanisms of oxidative stress in metabolic syndrome. Int. J. Mol. Sci. 24.
Merouane, A., Saadi, A., Noui, A., Bader, A., 2019. Evaluation of phenolic contents and antioxidant properties of the leaves and flowers of Phlomis biloba Desf. Int. Food Res. J. 26, 167–173.
Moore, L.D., Le, T., Fan, G., 2013. DNA methylation and its basic function. Neuropsychopharmacology 38 (1), 23–38.
Oke, F., Aslim, B., Ozturk, S., Altundag, S., 2009. Essential oil composition, antimicrobial and antioxidant activities of Satureja cuneifolia Ten. Food Chem. 112 (4), 874–879.
Ozgen, M., Reese, R.N., Tulio, A.Z., Scheerens, J.C., Miller, A.R., 2006. Modified 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2, 2 '-diphenyl-1-picrylhydrazyl (DPPH) methods. J. Agric. Food Chem. 54 (4), 1151–1157.
Park, H., Lee, S., Lee, J., Moon, H., Ro, S.W., 2023. Exploring the JAK/STAT signaling pathway in hepatocellular carcinoma: unraveling signaling complexity and therapeutic implications. Int. J. Mol. Sci. 24.
Ponugoti, B., Dong, G., Graves, D.T., 2012. Role of forkhead transcription factors in diabetes-induced oxidative stress. Exp. Diabetes Res. *2012*.
Rahim, M.A., Ayub, H., Sehrish, A., Khan, F.A., Ambreen, S., Hussain, I., Rocha, J.M., 2023. Functional polyphenols: an overview, classification and health benefits. plant and food phenolics–chemistry, functionality and practical applications. SciKnowPub 2023 1–20.
Rasheed, M.U., Naqvi, S.A.R., Rasool, N., Shah, S.A.A., Zakaria, Z.A., 2022. Anti-diabetic and cytotoxic evaluation of phlomis stewartii plant phytochemicals on cigarette smoke inhalation and alloxan-induced diabetes in wistar rats. Metabolites 12 (11), 1133.
Samtiya, M., Aluko, R.E., Dhewa, T., Moreno-Rojas, J.M., 2021. Potential health benefits of plant food-derived bioactive components: an overview. Foods 10 (4), 839.
Sarikurkcu, C., Uren, M.C., Kocak, M.S., Cengiz, M., Tepe, B., 2016. Chemical composition, antioxidant, and enzyme inhibitory activities of the essential oils of three Phlomis species as well as their fatty acid compositions. Food Sci. Biotechnol. 25 (3), 687–693.
Shuai, K., Liu, B., 2005. Regulation of gene-activation pathways by PIAS proteins in the immune system. Nat. Rev. Immunol. 5 (8), 593–605.
Sinha, R.A., Singh, B.K., Yen, P.M., 2018. Direct effects of thyroid hormones on hepatic lipid metabolism. Nat. Rev. Endocrinol. 14 (5), 259–269.
Song, F., Akonyani, Z.P., Li, Y., Su, D., Wu, L., Pang, Y., Wu, J., 2022. The impact of different feeds on DNA methylation, glycolysis/gluconeogenesis signaling pathway, and gene expression of sheep muscle. PeerJ 10, 13455.
Tangvarasittichai, S., 2015. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J. Diabetes 6.
Taskın, T., Cam, M.E., Bulut, G., Hazar-Yavuz, A.N., Kabasakal, L., Bitis, L., 2018. Antioxidant and anti-inflammatory activities of Phlomis pungens and coridothymus capitatus. Marmara Pharm. J. 22 (1), 80–85.
Turner, M.D., Nedjai, B., Hurst, T., Pennington, D.J., 2014. Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease. Biochim. Et. Biophys. Acta (BBA)-Mol. Cell Res. 1843 (11), 2563–2582.
Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., Jaremko, M., 2020. Important flavonoids and their role as a therapeutic agent. Molecules 25 (22), 5243.
Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., Bezirtzoglou, E., 2021. Towards advances in medicinal plant antimicrobial activity: a review study on challenges and future perspectives. Microorganisms 9 (10), 2041–2052.
Youn, J.S., Kim, Y.J., Na, H.J., Jung, H.R., Song, C.K., Kang, S.Y., Kim, J.Y., 2019. Antioxidant activity and contents of leaves extracts obtained from Dendropanax morbifera LEV are dependent on the collecting season and extraction conditions. Food Sci. Biotechnol. 28 (6), 201–207.
Zatterale, F., Longo, M., Naderi, J., Raciti, G.A., Desiderio, A., Miele, C., Beguinot, F., 2020. Chronic adipose tissue inflammation linking obesity to insulin resistance and type 2 diabetes. Front. Physiol. 1, 1607.
Zhang, C., 2008. The role of inflammatory cytokines in endothelial dysfunction. Basic Res. Cardiol. 103, 398–406.
Zhang, Y., Wang, Z.Z., 2009. Phenolic composition and antioxidant activities of two Phlomis species: a correlation study. Comptes Rendus Biol. 332, 816–826.
Zhang, D., Wei, Y., Huang, Q., Chen, Y., Zeng, K., Yang, W., Chen, J., 2022. Important hormones regulating lipid metabolism. Molecules 27 (20), 7052.
Zhao, P., Saltiel, A.R., 2020. From overnutrition to liver injury: AMP-activated protein kinase in nonalcoholic fatty liver diseases. J. Biol. Chem. 295 (34), 12279–12289.