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ORIGINAL ARTICLE

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Seed from olive cake prevents hyperglycemia, hyperlipidemia, and oxidative stress in dexamethasone-induced diabetes rats

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ABSTRACT

Background: Diabetes mellitus, a leading cause of global morbidity and mortality, can be managed with both pharmacological and non-pharmacological interventions. However, current medications often come with significant costs and side effects, including hypoglycemia, liver failure, and congestive heart failure. This highlights the need for exploring alternative strategies, such as dietary antioxidants and nutraceuticals, for diabetes prevention and complication management. **Aims:** This study investigated the potential of olive seed powder (OSP) supplementation to prevent diabetes, hyperlipidemia, and oxidative stress induced by dexamethasone in rat, utilizing both *in vivo* and in silico approaches. **Material and Methods:** Male rats were distributed into three groups: control (Ctl), preventive (D-OSP, receiving OSP supplementation), and dexamethasone-treated (Dx). Over 14 days, all groups received intraperitoneal dexamethasone injections, while the preventive group received a diet supplemented with 10% OSP. **Results:** Compared to the Dx group, supplementation significantly mitigated hyperglycemia, improved glucose tolerance, and reduced the triglyceride-glucose index, glycated hemoglobin levels, and increased hepatic glycogen content. OSP also ameliorated the lipid profile, decreasing triglycerides, total cholesterol, LDL-C, atherogenic index, and coronary risk index levels, while increasing HDL-C levels. Enzyme biomarker activities of liver function were elevated in the Dx compared to Ctl and OSP supplementation attenuated these changes. Furthermore, the Dx group displayed increased lipid peroxidation products and protein oxidation, alongside decreased activity of antioxidant enzymes in the liver and pancreas. PSO supplementation alleviated these markers of oxidative stress. Histopathological analysis revealed liver and pancreas damage in the Dx group, which was reduced by OSP. Additionally, the *in-silico* investigations indicated the potential biding of vanillic acid to the active sites of protein kinase 1, insulin receptor substrate 1, phosphatidylinositol 3-kinase, and glycogen synthase kinase-3 beta complex. **Conclusion:** This study demonstrates that OSP supplementation effectively prevented dexamethasone-induced hyperglycemia, dyslipidemia, and oxidative stress in rats. These beneficial effects are likely attributed to the bioactive compounds present in OSP suggesting its potential as a promising functional food for diabetes prevention and complication management.

Keywords: Dexamethasone, diabetes, in silico, olive seed powder, oxidative stress.

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1 Introduction

Over the last few decades, the incidence of diabetes mellitus (DM) has witnessed a significant increase across both developed and developing nations. DM has emerged as a leading cause of death and disability worldwide. According to estimates, 537 million individuals were living with diabetes in 2021. This number is projected to reach 643 million by 2030, and 783 million by 2045 [1]. Even more alarmingly, projections suggest diabetes will become the seventh leading cause of death globally by 2030 [2]. Notably, type 2 diabetes accounts for approximately 90% of all diabetes cases worldwide [3]. This chronic condition is characterized by hyperglycemia and frequently co-occurs with lipid metabolism abnormalities, resulting from either insulin resistance or inadequate insulin production [4].

Dexamethasone (Dex), a widely used synthetic glucocorticoid, plays a crucial role in treating inflammatory and immune-related disorders. However, its therapeutic application is not without drawbacks, as Dex administration has been linked to adverse events such as hypertension and diabetes mellitus [5,6]. In animal models, particularly rodents, Dex administration has been associated with disruptions in

glucose homeostasis, ultimately leading to diabetes [7] This diabetogenic effect is attributed to Dex-induced insulin resistance achieved through alterations in the insulin signaling pathway and the promotion of glycogen degradation [8]. These combined effects worsen hyperglycemia and contribute to the development of diabetes. Furthermore, Dex administration is known to induce dyslipidemia and an imbalance in redox status, both of which contribute to the development and progression of diabetic complications [9].

The contemporary management of diabetes relies on both pharmacological and non-pharmacological interventions. However, ~~the~~ current pharmacological agents, besides their exorbitant cost, are often associated with significant drawbacks, including high costs and a spectrum of adverse effects such as hypoglycemia, liver dysfunction, and congestive heart failure [10]. In addition, in developing countries, access to timely diagnosis and affordable medication remains a challenge for patients with DM [11]. Given these limitations and the chronic nature of diabetes, emphasis on preventative strategies with minimal side effects and lower costs is paramount. In this context, dietary antioxidants and nutraceuticals have emerged as promising candidates for DM prevention and management of its chronic complications [12]. Olive seeds (OS), a by-product generated during the olive oil industry and pitted table olive processing, constitute approximately 3 – 5% of the total fruit weight. Recent research suggests their potential application as functional foods or food supplements. OS are rich source of oil, containing significant levels of sterols and polyunsaturated fatty acids (PUFAs) [13]. They further exhibit appreciable amounts of potassium, calcium, magnesium, essential amino acids, and tocopherols (vitamins) [14]. Additionally, OS are rich in phenolic compounds, predominantly flavones and glycosidic compounds such as salidroside, nüzhenide-glucose, nüzhenide-oleoside, alongside other phytonutrients with potential health benefits [13]. These bioactive components contribute to the observed biological activities including radical scavenging potential and anti-neurodegenerative properties [15]. *In-vitro* study has demonstrated the antioxidant and inhibitory activities of OS protein hydrolysates against dipeptidyl peptidase-IV, an enzyme implicated in the pathogenesis of type 2 diabetes [16]. It has also been reported that vanillic acid, a phenolic acid isolated from olive pomace, has been reported to protects against diabetes, diabetic nephropathy [17], hyperinsulinemia, hyperglycemia, and hyperlipidemia [18]. Inspired by this existing evidence, the current study aimed to investigate the preventive effects of olive seed powder (OSP) derived from olive cake (OC) against hyperglycemia, dyslipidemia, and lipid peroxidation in dexamethasone-induced diabetes rats. Furthermore, to elucidate the potential mechanism action of OSP, *in silico* molecular docking simulations were performed, evaluating the interaction of vanillic acid with protein kinase 1, glycogen synthase kinase-3, insulin receptor substrate 1, and phosphatidylinositol 3-kinase.

2 Material and Methods

OC was procured following the olive oil extraction process from a reputable olive oil producer located in Sig, western Algeria. The OC was subsequently transported to the Clinical and Metabolic Nutrition Laboratory (LNCM) at the University Oran1 (Oran, Algeria) for further processing. At the LNCM, the OC underwent a drying process at a controlled temperature of 60°C. Subsequently, the olive seeds were meticulously separated from the remaining pulp using a manual sieving technique. The isolated OS fraction was then pulverized to obtain a homogenous powder. Finally, the OS powder was stored at 25°C in sealed containers to ensure optimal preservation.

2.1 Preparation of extract

Ten grams (10 g) of olive seed powder (OSP) were subjected to a defatting process using 100 mL of n-hexane. Following complete desiccation, a five-gram (5 g) portion of the defatted residue was homogenized in a pre-determined solvent mixture. This solvent mixture consisted of 35 mL of methanol and 15 mL of water (70:30 v/v). The homogenization process was carried out for 45 minutes at a controlled temperature of 55°C. Subsequently, the homogenate was filtered through filter paper to obtain the crude extract. This crude extract served as the test solution for the quantification of total phenolic content (TPC) and total flavonoids content (TFC) and the determination of antioxidant activity.

2.2 Phenolic compounds determination

The TPC was assessed using the Folin-Ciocalteu method while the TFC was determined using the aluminum chloride assay [19]. The phenolic profile of the OSP extract was characterized using a high-performance liquid chromatography system coupled to a diode array detector (HPLC-DAD, USA). The system employed a C18 column (4.6 mm × 250 mm internal diameter, 5 µm). Two mobile phases were utilized: mobile phase A, consisting of acidified water prepared with 0.1% formic acid and mobile phase B , composed of acetonitrile. A linear gradient elution program was implemented as follows: 0 min (87% A); 0 – 18 min (linear gradient to 45% A); 18 – 23 min (isocratic elution at 40% A); 23 – 25 min (linear gradient to 87% A); 25 – 28 min (isocratic elution at 87% A). The injection volume was set to 10 µL, and the flow rate of the mobile phase was maintained at 0.7 mL/min. Detection was performed at a wavelength of 320 nm at 25°C. The resultant HPLC profile of the OSP extract was then compared to the profile generated using standard reference compounds.

2.3 *In vitro* antioxidant activity of OSP

The antioxidant activities of OSP were assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity [20]; superoxide anion scavenging (SASA) [21] and hydroxyl radical scavenging (HRSA) [22].

2.4 Animals and diets

***In vivo* study:** Adult male Wistar rats (245 ± 15 g) were obtained from the animal house facility of the LNCM. The animals were housed under standard laboratory conditions, maintaining a controlled temperature of 25 ± 2°C, humidity of 55%, and a 12 – hour light-dark cycle. Standard laboratory chow and water were provided *ad libitum* (freely accessible). All experimental procedures adhered to established ethical principles for the care and use of laboratory animals [23]. The study protocol received prior approval from the Institutional Animal Care and Use Committee of the University of Oran 1 (approval number: D00L01UN310120190002).

Following a one-week acclimatization, period, the rats were randomly divided into three groups (n = 6 rats/group) as follows:

- ***Control group (Ctl):*** rats in this group received daily intraperitoneal injection of a sterile 9‰ saline solution (1 mL/100g body weight) and were fed with a standard laboratory diet (SD).
- ***Dexamethasone group (Dx):*** rats in this group received daily intraperitoneal injection of dexamethasone (1 mg/100g body weight) and were fed with a standard laboratory diet (SD).
- ***Preventive group (D-OSP):*** rats in this group received a daily intraperitoneal injection of dexamethasone (1 mg/100g body weight) and were fed with the SD supplemented with 10% olive seed powder from olive cake.

The experimental protocol lasted for 14 days. During this period, body weight and food consumption were monitored and recorded.

2.4.1 Fasting blood glucose (FBG) and oral glucose tolerance test (OGTT)

Fasting blood glucose (FBG) levels were assessed in all animals from each group on days 0, 7, and 14 of the experimental protocol. Following an 8-hour fasting period, blood samples were collected from the rat's tail vein. Blood glucose concentration was measured using a commercially available glucometer (Vital Check, USA). Animals exhibiting FBG levels exceeding 200 mg/dL were classified as diabetic, as previously described [24].

An OGTT was performed on the final day of the experiment. After a 12-hour fasting period, rats received a single oral gavage of glucose solution (0.2 g/100 g body weight). Blood glucose levels were subsequently measured at baseline (0 min) and at 30-, 60-, and 120-minutes post-gavage. The trapezoidal rule was employed to calculate the area under the curve (AUC) for blood glucose concentration over the specified time points.

2.4.2 Blood and organ collection

Following a 14 – day treatment period, rats were euthanized using an appropriate method of anesthesia by chloral 10% (w/v) (3 mL/kg BW). Blood samples were collected from the abdominal aorta via cardiac puncture into pre-chilled, dry tubes. The blood was centrifuged at 3000 x g for 20 minutes at 25°C to separate the serum. Subsequently, the liver and pancreas tissues were harvested promptly, rinsed with chilled saline solution, and dissected into two sections. One section was designated for histopathological examination, while the other section was homogenized (10%, w/v) in a suitable buffer for subsequent biochemical analysis.

2.4.3 Biochemical analyses

In this study, a comprehensive biochemical evaluation was performed to assess the rats metabolic status, lipid profile, and liver function. Glycosylated hemoglobin (HbA1c) was measured using a commercially available assay kit (Kit Biosystem, Spain). Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were measured using commercially available diagnostic kits (Kit Cypress diagnostic, Belgium). Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald formula: LDL-C= TC- (HDL-C + (TG/5) [25]. The atherogenic index (AI) and coronary risk index (CRI) were calculated as follows: AI=LDL-C/HDL-C; CRI=TC/HDL-C.

Serum activities of alanine aminotransferase (ALT), aspartic aminotransferase (AST), and alkaline phosphatase (AKP) were determined using commercially available diagnostic kits (Biolobo, France). Hepatic glycogen content was measured according to Carroll et al. method [26]. The triglyceride-glucose index (TyG index) was estimated as TyG index = Ln [TG (mg/dl) x glucose (mg/dl)/2] [27].

2.4.4 Lipid and protein oxidation and antioxidant enzymes analysis in tissues

The activities of superoxide dismutase (SOD) [28], catalase (CAT) [29] and glutathione peroxidase (GSH-Px) [30] were measured in liver and pancreas homogenates. Additionally, the levels of thiobarbituric acid reactive substance (TBARS) [31] and advanced protein oxidation products (AOPPs) [32] were determined in these tissues.

2.4.5 Histological examination

Tissues were fixed in a 4% formaldehyde solution to preserve their morphology. Subsequently, the tissues were dehydrated through a graded series of ethanol solutions, embedded in paraffin wax. Serial sections with a thickness of 5 µM were obtained using a microtome then stained with Hematoxylin and Eosin. The stained sections were examined under an optical microscope (DN-107T, Japan) equipped with a digital camera. Images were captured using the Scope Image 9.0 software.

2.4.6 *In silico* molecular docking

The potential toxicity of the compounds identified by high-performance liquid chromatography (HPLC) was evaluated using ProTox-II, an online computational tool. This platform incorporates various established toxicity endpoints, including hepatotoxicity, cytotoxicity, carcinogenicity, and mutagenicity. Among the compounds found in olive seeds, vanillic acid exhibited no predicted toxicity (data not shown) and has been selected for further molecular docking.

Molecular docking simulations were performed using the AutoDock Vina software The 3D crystal structures of protein kinase 1 (AKT1 PDB ID: 4EKL), insulin receptor substrate 1 (IRS-1 PDB ID: 1QQG), glycogen synthase kinase-3 beta (GSK3 PDB ID: 3F7Z) and phosphatidylinositol 3-kinase (PI3K PDB ID: 5XGJ) were retrieved from the Protein Data Bank (https://www.rcsb.org), while the vanillic acid chemical structures were obtained using the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The potential binding interactions between vanillic acid and target protein were examined (BIOVIA, Dassault Systèmes, Discovery Studio Visualizer, v21.1.0.20298).

2.4.7 Statistical analysis

Data for phytochemical and *in vitro* antioxidant activity are presented as the means ± SE of three independent assays. For the *in vivo* study, results are expressed as the mean ± SE of six rats per group. One-way ANOVA was used for statistical analysis followed by Tukey's post-hoc test using a statistical package for the Social Science (SPSS) software version 25 (SPSS Inc., an IBM Company). A p-value was considered statistically significant when $p < 0.05$. [a] $p < 0.05$ when compared with the Ctl group and [b] $p < 0.05$ when comparing the D-OSP group with the Dx group.

3 Results

3.1 Antioxidant activity, content, and HPLC-analysis of OSP phenolic compound

TPC and TFC values of OPS represented respectively, 0.29 ± 0.02 mg gallic acid eq/g of OSP and 0.14 ± 0.05 mg catechin eq/g of OSP. Analysis of the phenolic profile of OSP by HPLC-DAD showed the presence of a number of compounds, including oleuropein, gallic acid, caffeic acid, vanillic acid, ferulic acid, and rutin (Fig. 1). The *in vitro* antioxidant activity of OSP extract showed its capacity to inhibit DPPH, hydroxyl anion and superoxide anion by 56.28 ± 5.13 %, 43.29 ± 7.79 and 37.62 ± 3.00 %, respectively.

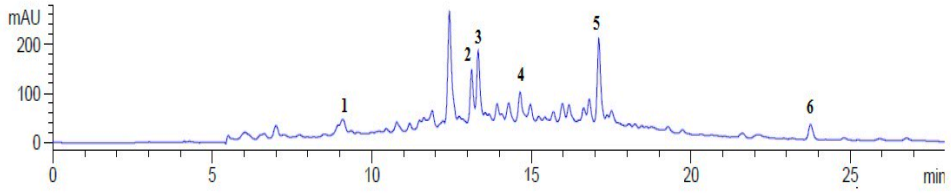

Figure 1. High-performance liquid chromatography (HPLC) chromatogram at 280 nm of OSP extract.
Peak identification (RT time retention; percentage) 1: Gallic acid (RT=13.335; 4%), 2: Cafeic acid (RT=13.130; 6%), 3: Vanilic acid (RT=12.435; 17%), 4: Ferulic acid (RT=14.647; 4%), 5: Oleuropein (RT=17.116; 9%), 6: Rutin (RT=23.753; 2.5%).

3.2 *In-vivo* study

3.2.1 Body weight (BW), FBG level and food intake

At day 14, the Dx group exhibited significant ($p < 0.05$) body weight loss and decreased food intake compared to the Ctl group throughout the study period. However, OSP supplementation partially prevented the drastic weight loss, without showing an effect on food intake compared to the Dx group. Although OSP resulted in an increase in BW than the Dx group, this difference was not statistically significant.

Fasting blood glucose (FBG) levels were measured in rats of different groups on days 0, 7, and 14. Our finding demonstrated a significant increase in FBG in the Dx group on day 7 and day 14 than in the Ctl group ($p < 0.05$). OSP

supplementation reduced FBG levels from day 7 onwards compared to the Dx group. This reduction reached a significant level on day 14 in the D-OSP group with a reduction of 20% *vs* the Dx group (Table 1).

Conversely, OSP supplementation led to reductions of 36% and 56% in Hb1Ac and TyG index, respectively, compared to the Dx group. Dexamethasone exposure resulted in an increase in liver glycogen content within the Dx group, as depicted in Figure 2e. This increase was attenuated by OSP

Table 1. Effect of OSP on body weight and food intake

	Days	Ctl	Dx	D-OSP
Body weight (g)	0	197.45 ± 13.56	200.33± 15.14	199 ± 10.23
	7	215.45 ± 9.7	169.00 ± 7.55[a]	173.75 ±14.98
	14	229.34 ± 8.53	151.20 ± 12.06[a]	164.33 ± 7.57[b]
Food intake (g/day/rat)	14	15.71 ± 1.21	6.28 ± 0.48[a]	8.02 ± 0.12
FBG (mg/dL)	0	83 ± 6.4	86 ± 8.4	85 ± 5.6
	7	85 ± 5.3	185 ± 10,01[a]	168 ± 7.39
	14	84 ± 3.6	241.5 ± 9.19[a]	192 ± 10.04[b]

Ctl: Control group; Dx: Dexamethasone group; D-OSP: Dexamethasone group treated with olive seed powder. Results are presented as mean ± SD. One-way ANOVA was used for statistical analysis followed by Tukey's post-hoc test. a $p < 0.05$, Dx vs Ctl group and b $p < 0.05$, D-OSP group vs Dx group.

Table 2. Serum lipid profiles and liver function markers

	Ctl	Dx	D-OSP
TG (mg/dL)	110.52 ± 5.27	181.60 ± 2.02[a]	129.82 ± 8.04[b]
CT (mg/dL)	80.40 ± 6.18	156.25 ± 3.25[a]	126.57 ± 4.98[b]
LDL-C (mg/dL)	22.72 ± 4.76	89.51 ± 5.15[a]	38.10 ± 5.09[b]
HDL-C (mg/dL)	46.70 ± 3.34	30.95± 2.08[a]	38.10 ± 2.82
AI	0.48 ± 0.08	2.91 ± 0.37[a]	1.42 ± 0.03[b]
CRI	1.96 ± 0.10	5.07 ± 0.48[a]	3.05 ± 0.05[b]
AST (U/L)	74.50 ± 5.33	220.00 ± 6.90[a]	173.38 ± 2.06[b]
ALT (U/L)	68.09 ± 2.47	154.814 ± 8.82[a]	100.31 ± 6.10[b]
AKP (U/L)	84.40 ± 4.44	154.66 ± 3.89[a]	129.53 ± 6.45[b]

Ctl: Control group; Dx: Dexamethasone group; D-OSP: Dexamethasone group treated with olive seed powder. Results are presented as mean ± SD. One-way ANOVA was used for statistical analysis followed by Tukey's post-hoc test. [a] $p < 0.05$, Dx vs Ctl group and [b] $p < 0.05$, D-OSP group vs Dx group. AI: Atherogenic index; CRI: Coronary risk index; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TC: Total cholesterol; TG: Triglyceride; AST: Aspartic aminotransferase ALT: Alanine aminotransferase; AKP: Alkaline phosphatase.

3.2.2 Effect of OSP on OGTT, AUC, Hb1Ac, TyG index and liver glycogen content

Figure 2a depicts the OGTT results for the various rat groups. Thirty (30) minutes after glucose administration, the Dx group exhibited a significantly greater increase in blood glucose levels compared to the Ctl group ($p < 0.05$). Furthermore, as shown in Figure 2b, the area under the curve (AUC) for blood glucose levels was significantly elevated in the Dx group compared to the Ctl group. Olive seed powder (OSP) supplementation in the D-OSP group significantly reduced the AUC compared to the Dx group ($p < 0.05$). The Hb1Ac level, as well as the TyG index, were both increased in the Dx group compared to the Ctl group (Fig. 2c-d). supplementation in the D-OSP group.

3.2.3 Effect of OSP on serum lipid profiles and liver function markers

As shown in Table 2, the Dx group exhibited a significant ($p < 0.05$) increase in serum TG, TC, and LDL-C values accompanied by a decrease in HDL-C levels compared with the Ctl group (Table 2). Conversely, OSP supplementation in the D-OSP group significantly reduced ($p < 0.05$) the concentrations of TG, TC, and LDL-C to levels comparable to the Ctl group. Additionally, HDL-C levels were significantly increased ($p < 0.05$) by 23% compared to the Dx group. Furthermore, the Dx group displayed a significant elevation ($p < 0.05$) in both the atherogenic index and

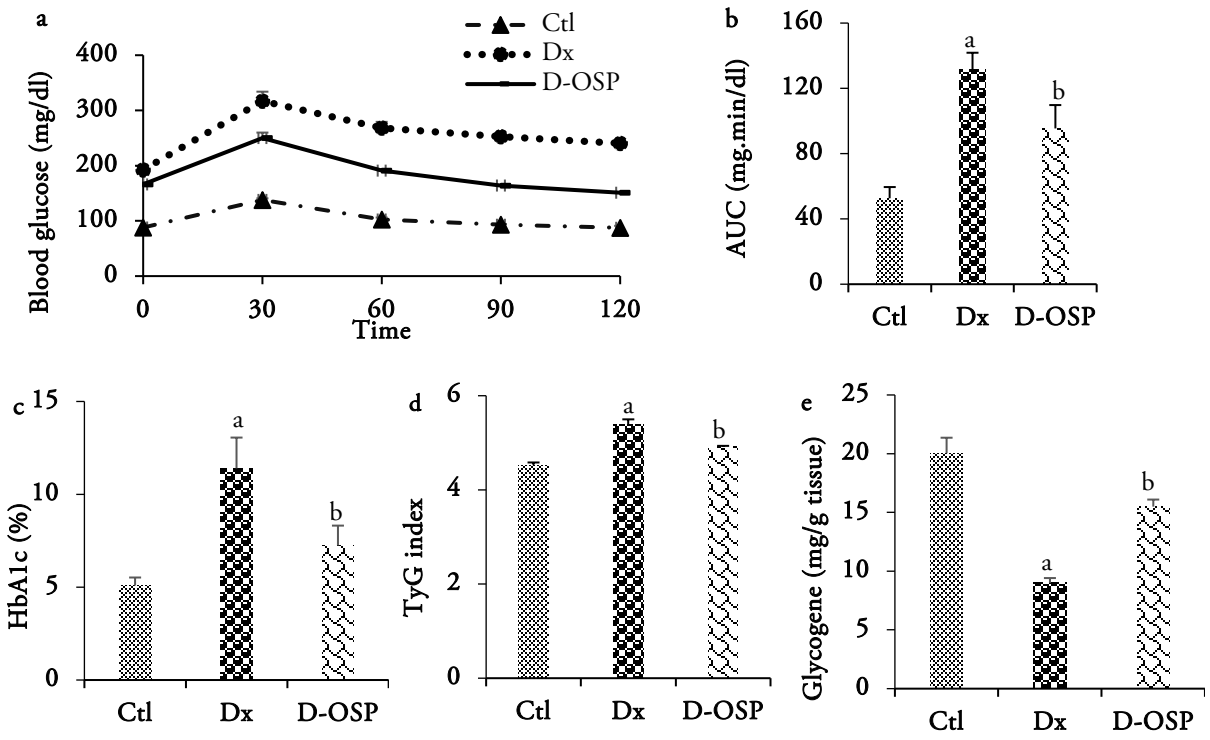

Figure 2. Effect of OSP on blood glucose and AUC, during OGTT, Hb1Ac, TyG index and liver glycogen content.
C: Control group; D: Dexamethasone group; D-OSP: Dexamethasone group treated with olive seed powder. Results are expressed as mean ± SD. Data among groups were analyzed by two-way ANOVA followed by Tukey post-hoc test. [a] $p < 0.05$ when compared to control group; [b] $p < 0.05$ when compared D-OSP group to D group.

coronary risk index compared to the Ctl group. Olive seed powder (OSP) supplementation in the D-OSP group significantly reduced ($p < 0.05$) both of these risk indices.

Aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AKP) were significantly elevated ($p < 0.05$) in the Dx group compared to the Ctl group. In contrast the D-OSP group displayed significantly reduced activities of these enzymes compared to the Dx group.

3.2.4 OSP effect on oxidative stress biomarkers of the liver and pancreas

Dexamethasone injection in the Dx group resulted in a significant decrease in antioxidant enzyme activity and an increase in the levels of TBARS and AOPPs within the liver and pancreas compared to the Ctl group. These findings suggest enhanced oxidative stress in the Dx group. Conversely, co-administration of OSP with dexamethasone in the D-OSP group significantly increased the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in the liver compared to the Dx group (SOD: 43% increase, CAT: 28% increase and GSH-Px: 57% increase, respectively). The pancreas, of the D-OSP group also displayed a similar pattern, with significant elevations ($p < 0.05$) in the activities of SOD (59%), GSH-Px (57%), and catalase (16%) compared to the Dx group. Furthermore, OSP significantly reduced ($p < 0.05$) the level of TBARS and AOPPs in both the liver and pancreas than the Dx group, as shown in Table 3.

3.2.5 Effect of OSP on histological changes in the liver and pancreas

As displayed in Figure 3a, histological examination of liver sections from the Ctl group revealed normal architecture with intact central veins and hepatocytes. In contrast, the livers from the Dx group exhibited severe dilatation and congestion (CG) of the central vein, accompanied by fat accumulation within the hepatocytes. OSP treatment in the D-OSP group significantly ameliorated lipid accumulation, dilation, and congestion of hepatic vessels compared to the Dx group.

Examination of pancreatic tissue sections from the Ctl group showed normal islets morphology, characterized by well-defined islets of Langerhans surrounded by acinar cells. (Fig. 3a). Pancreas from the Dx group rats presented with several histopathological alterations including hypertrophy of the

Table 3. Hepatic and pancreatic antioxidant enzyme activities and proxidative markers

	Ctl	Dx	D-OSP
Liver			
Thiobarbituric acid reactive substance (nM/mg protein)	5.55 ± 0.36	18.25 ± 1.17[a]	10.57 ± 1.05[b]
Advanced oxidation protein products (nM/mg protein)	13.60 ± 0.62	56.01 ± 6.07[a]	31.81 ± 6.02[b]
Superoxide dismutase (U/mg)	13.10 ± 1.5	5.70 ± 0.5[a]	10.20 ± 1.05[b]
Catalase (umol/min/mg)	110.82 ± 9.04	60.61 ± 7.42[a]	84.24 ± 11.11[b]
Glutathione peroxidase (µM GSH /min/mg)	1.03 ± 0.01	0.24 ± 0.02[a]	0.56 ± 0.04[b]
Pancreas			
Thiobarbituric acid reactive substance (nM/mL)	2.26 ± 0.25	6.69 ± 0.34[a]	4.15 ± 0.42[b]
Advanced oxidation protein products (nM/mg protein)	9.12 ± 1.06	43.39 ± 1.49[a]	16.45 ± 0.66[b]
Superoxide dismutase (U/mg)	5.90 ± 0.50	1.55 ±0.19[a]	3.77 ± 0.42[b]
Catalase (uM H_2O_2 /min/mg)	45.40 ± 4.19	24.12 ± 1.5[a]	28.42 ± 3.0[b]
Glutathione peroxidase (µm GSH /min/mg)	0.71 ± 0.07	0.18 ± 0.01[a]	0.42 ± 0.03[b]

Ctl: Control group; Dx: Dexamethasone group; D-OSP: Dexamethasone group treated with olive seed powder. Results are presented as mean ± SD. One-way ANOVA was used for statistical analysis followed by Tukey's post-hoc test. [a]$p < 0.05$, Dx vs Ctl group and [b]$p < 0.05$, D-OSP group vs Dx group.

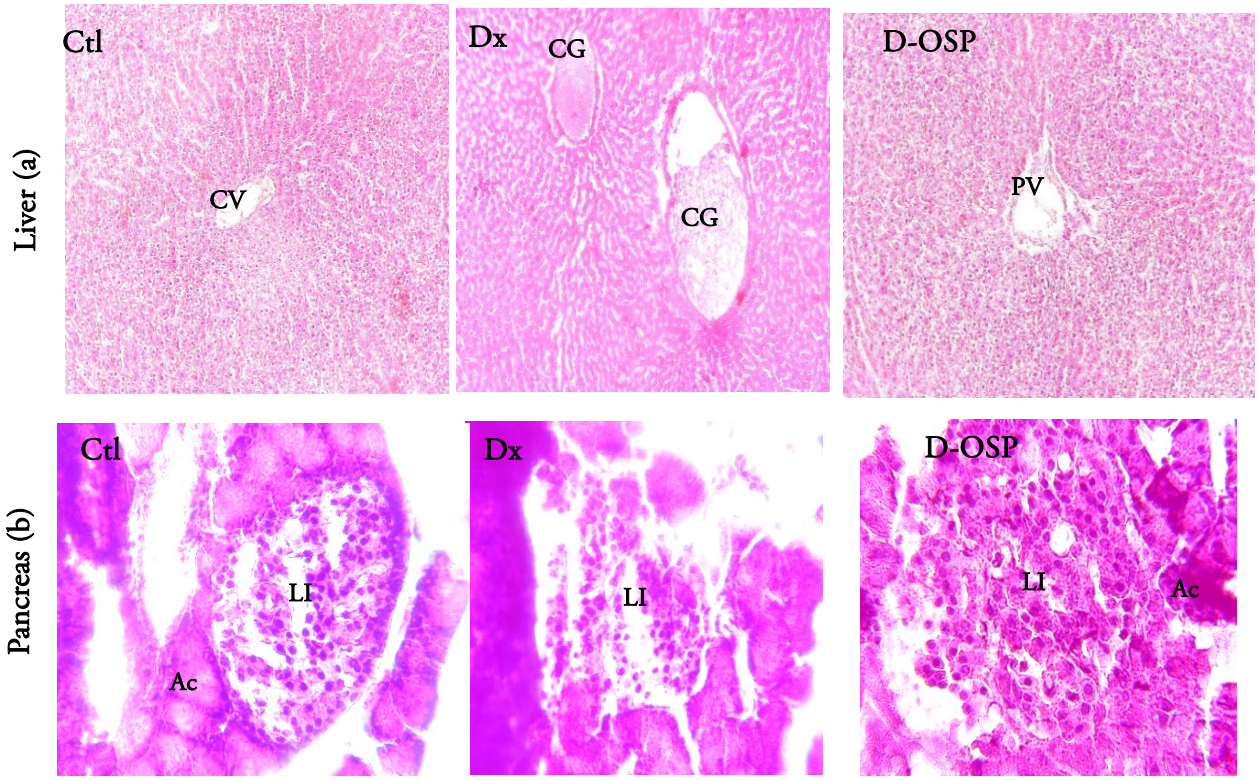

Figure 3. Representative histological changes in the liver and pancreas of each group of rats

(a) Section of rat liver of Ctl group showing normal architecture; liver of Dx group showing severe dilatation and congestion of the central hepatic vein; liver of D-OSP group, showing decreased lipid accumulation, dilatation and congestion of the hepatic vessels (HE X 100). (b) Representative histological changes in the pancreas of each group of rats. The pancreas of group Ctl showed normal islets. The pancreas of group Dx showed severe hypertrophy of the pancreatic islets with congested blood vessels. In the OSP group pancreas, a protective action of the pancreatic islets was demonstrated (X 400). Ctl: Control group; Dx: group receiving dexamethasone only; D-OSP: group receiving dexamethasone and fed concomitantly with 10% olive seed powder. PV= Portal vein; CV= Central vein; LI = Langerhans islets; AC = Acini cells.

islets Langerhans and congested blood vessels. Additionally, the acinar cells showed marked atrophy compared to the Ctl group, (Fig. 3b). In contrast, the pancreas of rats fed a diet containing OSP demonstrated partial restoration and improvement of islet architecture (Fig. 3C).

3.2.6 *In silico* study

Molecular docking analysis revealed binding affinities of vanillic acid with AKT1, IRS-1, PI3K, and GSK3 to be -6.2 kcal/mol, -6.1 kcal/mol, -5.5 kcal/mol, and -5.4 kcal/mol, respectively (Table 4). These values suggest favorable interactions between vanillic and the active sites of these target proteins Further analysis of the docking simulations (Fig. 4a-d) revealed that the interactions between vanillic acid and the active sites of these proteins involve various types of non-covalent bonds. These interactions include hydrogen bonds, π-alkyl interactions, π-different interactions (such as π-cation and π-sigma interactions), alkyl interactions, and van der Waals forces. Notably, vanillic acid forms distinct hydrogen bonds with specific amino acid residues within each target protein. For instance, in the interaction with AKT1 (Fig. 4a), vanillic acid establishes hydrogen bonds with residues ASP292, THR195, and GLU191. Similarly, in the complex with IRS-1 (Fig. 4b), vanillic acid forms hydrogen bonds with GLU200, SER199, and ARG184 residues. Additionally, with PI3K (Fig. 4c), vanillic acid forms two hydrogen bonds with residues ASP91 and ARG87. Regarding the interaction with GSK3 (Fig. 4d), the hydrogen bonds involve the amino acid residues LEU227, SER215, and ILE228.

Table 4. Affinities bending and residue interactions between vanilic acid with AKT1, IRS-1, PI3K and GSK3 complex

	PDB ID	Affinity (kcal/mol)	Number of H bond	Binding site interacting amino acid residues
AKT1	4EKL	-6,2	3	ASP292, THR195, GLU191, LEU295, LYS179, PHE 161 and HIS194,
IRS-1	1QQG	-6,1	3	GLU200, SER199, ARG (184, 89), PHE (70, 160) and VAL195,
PI3K	5XGJ	-6.0	2	ASP91, ARG87, PHE 119, ARG91
GSK3	3F7Z	-5,4	3	LEU227, SER215, ILE 228, VAL263 and ARG223

AKT1: Protein kinase 1; IRS-1: Insulin receptor subtrate-1; GSK-3: Glycogen synthase kinase-3 beta; PI3K: Phosphatidylinositol 3-kinase.

4 Discussion

Diabetes mellitus is a chronic metabolic disorder with a growing global prevalence. Despite advances in therapeutic strategies, the number of diagnosed cases continues to rise. The development of more effective management strategies for diabetes, particularly focusing on preventative measures is highly required. One of the main approaches to reducing the burden of diabetes, especially in developing countries where a large proportion of patients are undiagnosed or diagnosed at a late stage is preventing its development and associated complications. Recent research suggests a promising role for antioxidant-rich foods, polyphenols, and functional foods in reducing the incidence of metabolic diseases [33]. Numerous studies have demonstrated the beneficial effects of these dietary components in mitigating the risk of developing diabetes [34]. The present investigation tends to examine the preventive effects of olive seeds on hyperglycemia, hyperlipidemia, and oxidative stress in dexamethasone-induced diabetes rats, employing both *in vivo* and *in silico* approaches.

Dexamethasone (Dex) is a synthetic glucocorticoid widely used clinically as an immunosuppressive agent [35]. However, the administration of Dex at high doses for short duration induces acute hyperglycemia, hyperlipidemia, diabetes, hypertension, and other diseases [6,7].

It is used to induce insulin resistance [36] or diabetes in experimental animals [8]. In the current study, at the end, glucose levels in the DX group were around 240 mg/dL, which exceeds the 200 mg/dL threshold generally used to diagnose diabetes [25]. In contrast, the rats in the D-OSP group had glucose levels below this threshold. This observation suggests that OSP could potentially prevent dexamethasone-induced diabetes. The beneficial effect of OSP could be attributed to the presence of different components, which may act individually or in synergy via various mechanisms.

A high-performance liquid chromatography study of OSP revealed the presence of oleuropein, gallic acid, caffeic acid, vanillic acid, ferulic acid, and rutin in OSP, which are known for their antihyperglycemic effects. Literature has shown that oleuropein reduces blood sugar levels through modulation of insulin secretion, activation of hepatic AMP-activated protein kinase signaling, and improved glucose tolerance [37]. Moreover, vanillic acid attenuates hyperinsulinemia and hyperglycemia by reducing hepatic inflammation and insulin resistance (IR) [18]. In addition, the hypoglycemic effect of OSP may also be due to the presence of other compounds present in olive seeds, such as dietary fiber and peptides.

In the present study, the Dx group presented decreases in food consumption and BW gain than the Ctl group. This decrease is probably the result of increased muscle protein destruction, fat catabolism, and insulin resistance [38]. Furthermore, OSP was unable to prevent the reduction in dietary consumption and BW loss despite the blood glucose improvement, suggesting that dexamethasone-induced weight down is independent of its diabetogenic effect. In addition, the lack of effect of OSP on these two parameters may be due to the short duration of this study.

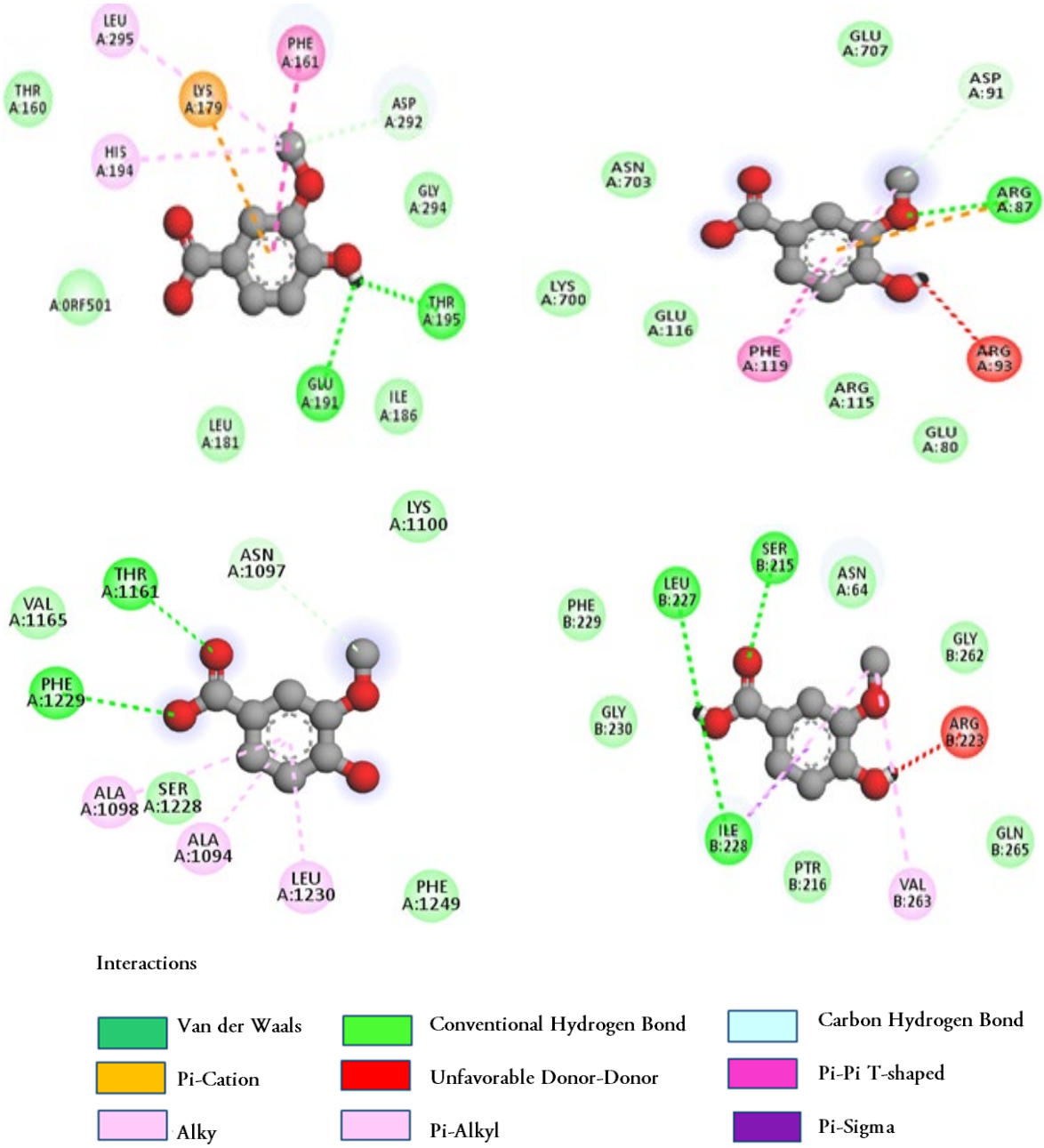

Figure 4. 2D interaction complexes of vanillic acid, with AKT1 (a) PI3K (b), IRS-1 (c) and GSK3 (d)

The estimation of glycosylated hemoglobin (HbA1c) is particularly used as a good indicator of glycemic control and is highly predictive of the development of complications related to diabetes [39]. During diabetes, excess glucose in the blood reacts with hemoglobin to form HbA1c. In the current study, the lower HbA1c values observed in the D-OSP group compared with the Dx group highlight the effectiveness of OSP in the control of blood glucose levels. PSO effect may be due to the decrease in blood glucose, which in turn induces a reduction in the glycosylation of blood proteins.

One of the characteristics of Dex-induced diabetes is insulin resistance [36,40]. There is evidence to suggest that dexamethasone induces IR by blocking hepatic hexokinase activity, inhibiting hepatic glucose oxidation, and increasing hepatic glucose synthesis. Dex can also inhibit insulin

signaling, preventing GLUT4 translocation in muscle cells, leading to hyperglycemia [41].

In the present study, insulin resistance was assessed by OGTT and TyG index [27]. A high TyG index reflects more severe IR while a low TyG index indicates insulin sensitivity. Our data showed an increase, in OGTT, AUC levels, and TyG index in the Dx group compared with the Ctl group, which indicated insulin-resistant status in this animal model. Interestingly, the D-OSP group had lower levels of these parameters than the Dx group. In addition, PSO favored an increase in hepatic glycogen compared with the Dx group. These results suggest that PSO may prevent or delay the onset of insulin resistance, probably by improving insulin sensitivity in the tissues, thereby enhancing hepatic glycogen metabolism and preventing hyperglycemia. This effect can be attributed to gallic acids present in OSP since it has been shown to attenuate insulin resistance, improve glucose absorption, and activate GLUT4 in adipose tissue [41].

The β-cells of the pancreas are highly specialized cells that secrete insulin to maintain glucose balance. Deficiency in β-cell development or dysfunction can lead to impaired glucose metabolism and diabetes [42]. In the present study, the histopathological section of the Dx group reveals an increase in islet mass, which leads to β-cell dysfunction and consequently diabetes. This increase in islet mass may be due to peripheral insulin resistance, which causes beta-cell proliferation [43]. In agreement with our results, it has reported beta cell hypertrophy and hyperinsulinemia in dexamethasone-induced hyperglycemic rats [44]. A decrease in islet mass after OSP supplementation suggests that OSP improves pancreatic islet function possibly due to an improvement in insulin sensitivity.

Dyslipidemia is one of the significant risk factors for cardiovascular disease (CVD) in DM. DM is highly associated with disturbances in lipid profiles such as hypertriglyceridemia and hypercholesteremia [45]. In this study, the Dx group exhibited dyslipidemia characterized by elevated levels of triglycerides, total cholesterol, LDL-C, CRI, and IA associated with a decrease in serum HDL-C amount. This dyslipidemia was prevented by olive seed powder supplementation, suggesting that PSO may exert anti-hyperglyceridemic and anti-hypercholesterolemic effects. OSP supplementation also reduced CRI and IA showing that the OSP would be effective against CVD and/or atherosclerosis. This effect was thought to be a consequence of the beneficial effects of OSP on markers of lipid profile. The lipid-lowering properties of OSP could be attributed to a synergistic effect of these constituents such as poly and monounsaturated fatty acids, vitamins, minerals, polyphenols, flavonoids, and dietary fiber. The fiber in OSP could reduce plasma lipid levels by increasing the excretion of cholesterol and bile acids in the feces [46]. In addition, olive seed peptides are able to stimulate lipoprotein lipase and inhibit HMG-CoA reductase, which are key enzymes involved in triglyceride metabolism and cholesterol synthesis, respectively [47]. Moreover, the increase in insulin sensitivity induced by OSP may decrease lipolysis in adipocytes and thus reduce serum lipids in rats.

Oxidative stress (OS) plays a central role in the pathogenesis of DM and complications associated with diabetes. In diabetic conditions, hyperglycemia generates free radicals, like superoxide anion, hydrogen peroxide, and hydroxyl radicals [48]. These radicals lead to lipid peroxidation, protein oxidation, and cellular damage. The present finding indicates a significant increase in OS in the liver and pancreas of rats in the Dx group, as shown by high levels of TBARS and AOPPs in these tissues. OSP supplementation significantly reduced OS in these organs. This suggests the protective role of OSP, which could be due to the antioxidant effect of phenolic compounds present in the seeds, which act as potent inhibitors of superoxide radicals and singlet oxygen, as shown in the *in vitro* study.

SOD, CAT, and GSH-Px are major antioxidant enzymes implicated in free radical scavenging [49]. A decrease in the activity of these enzymes has been reported in experimental models of diabetes [50]. In the present study, we noted a significant reduction of these enzymes' activities in both the liver and pancreas of the Dx group, which may be due to increased production of reactive oxygen radicals. Supplementation of rats with OSP significantly increased these activities in liver and pancreatic tissues. Diabetes is responsible for damage to many organs, including the liver, kidney, and pancreas. ALT, AST, and AKP are markers of liver function, and an increase in these enzymes is observed more consistently in experimental diabetic rats [51]. In the present study, these activities are increased in the Dx group compared to the Ctl group. The increased activity of these enzymes indicates liver injury [52]. In agreement with the biochemical results, histopathological analysis of the liver showed lipid accumulation and hepatocyte damage in the Dx group. Consistent with these findings, earlier reports have observed alterations in hepatocytes characterized by vacuolization and lipids accumulation in rats after dexamethasone administration [38]. Our study showed that OSP supplementation significantly reduced ALT, AST, and AKP activity as well as lipid accumulation in hepatocytes and liver damage, suggesting that OSP may prevent dexamethasone-induced diabetes-related liver damage. This effect could be attributed to OSP's hypoglycemic, lipolipidemic, and antioxidant effects.

The insulin signaling pathway plays a crucial role in regulating carbohydrate homeostasis. IRS-1, PI3K, AKT, and GSK3 are

considered essential components of this pathway [53]. Insulin action is initiated by its binding to its receptor, leading to the phosphorylation of IRS-1, which in turn activates phosphoinositide 3-kinase and protein kinase B phosphorylation [54]. AKT activation has various functions, such as facilitating GLUT4 transporter translocation and glucose uptake [55], as well as glycogen synthase kinase 3 (GSK3) phosphorylation [56].

Molecular docking was used to explore the mechanism action of olive seed against key target proteins involved in the pathogenesis of diabetes. The online toxicity test reveals that only vanillic acid exhibits no toxicity among the molecules identified in olive seeds. Therefore, it was employed to investigate the mechanism action of olive seeds by determining their affinity with the target proteins IRS-1, PI3K, AKT, as well as GSK3. These pathways are key components of the regulation of metabolism and insulin response in cells. Bioactive molecules capable of modulating these pathways have been considered promising therapeutic targets for diabetes and other metabolic disorders [56]. Our molecular docking results have revealed a strong affinity between vanillic acid and the proteins IRS-1, PI3K, AKT, and GSK3. Furthermore, these findings indicate that several amino acid residues are involved in the interactions of vanillic acid with the target proteins, including hydrogen bonds, van der Waals, alkyl, pi-alkyl, and pi-sigma interactions, as well as other types of interactions. These findings suggest that vanillic acid may have the ability to directly phosphorylate the IRS-1, PI3K, and AKT proteins, thereby regulating insulin sensitivity and reducing blood glucose levels. There is strong evidence that GSK3 was involved in the pathogenesis of various diseases, including diabetes and insulin resistance [57]. Phosphorylation of glycogen synthase by GSK-3β leads to its deactivation, resulting in reduced glycogen storage in hepatocytes and an increase in blood glucose levels. Several animal models of diabetes have shown improved insulin sensitivity and glucose metabolism following inhibition of GSK-3 activity [58]. The results of molecular docking have demonstrated that vanillic acid binds to the GSK active site through hydrogen bonding and hydrophobic interactions, suggesting that vanillic acid could inhibit GSK3, thereby contributing to the improvement of insulin sensitivity and glucose metabolism. These findings provide valuable insights for the development of new therapeutic interventions against diabetes and other complications associated with the use of glucocorticoids.

5 Conclusion

This study revealed that OSP is rich in bioactive compounds and exhibits significant anti-radical properties. Co-administration of OSP with dexamethasone effectively prevented hyperglycemia, the onset of dyslipidemia, and oxidative stress in Dex-induced diabetic rats. These protective effects may be mediated by OSP's ability to enhance insulin sensitivity, stimulate endogenous antioxidant activity, and reduce markers of lipid peroxidation and protein oxidation. Moreover, *in silico* investigations using molecular docking study were conducted to elucidate the potential mechanism of action of OSP. These stimulations indicated a strong interaction between vanillic acid and amino acids within the active sites of target proteins, leading to the inhibition of glycogen synthase kinase 3(GSK3) and modulation of the IRS-1/PI3K/AKT signaling pathway. These findings suggest that vanillic acid likely contributes to the underlying mechanism of the antidiabetic action of olive seeds. Isolation of vanillic acid from olive seeds and subsequent in vivo studies are necessary to validate its effects on the identified pathways within a relevant animal model.

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