© 2025 Journal of Pharmacy & Pharmacognosy Research, 13 (2), 513-526, 2025
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres24.2043_13.2.513

Original Article

Administration of oleuropein or hydroxytyrosol improves diabetic markers in obese mice

[Administración de oleuropeína o hidroxitirosol mejora los marcadores diabéticos en ratones obesos]

Nurhidayati Harun[1,2], I Ketut Adnyana[1], Sophi Damayanti[3,4], Neng Fisheri Kurniati[1*]

[1]Department of Pharmacology-Clinical Pharmacy, School of Pharmacy, Institut Teknologi Bandung, Indonesia.
[2]STIKes Muhammadiyah Ciamis, Indonesia.
[3]Department of Pharmacochemistry, School of Pharmacy, Institut Teknologi Bandung, Indonesia.
[4]University Center of Excellence on Artificial Intelligence for Vision, Natural Language Processing & Big Data Analytics (U-CoE AI-VLB), Indonesia.
*E-mail: nfkurniati@itb.ac.id

Abstract

Context: Olive leaves are renowned for their medicinal properties in managing diabetes, primarily due to their high concentrations of hydroxytyrosol and oleuropein.

Aims: To evaluate the anti-diabetic effects of hydroxytyrosol and oleuropein isolates in a mice model of obesity-induced diabetes.

Methods: Male Swiss Webster mice were subjected to a high-fat diet and streptozotocin to induce obesity and diabetes, respectively. Following induction, the mice were treated with either oleuropein or hydroxytyrosol at a dosage of 20 mg/kg body weight over a period of 14 days. Fasting glucose levels, insulin sensitivity, and glucose tolerance were monitored throughout the treatment period. Additionally, histological examinations of the liver and pancreas were conducted. Furthermore, *in silico* docking studies were performed to assess the interactions of oleuropein and hydroxytyrosol with key metabolic receptors, including peroxisome proliferator-activated receptor gamma (PPARγ), hydroxysteroid 11-β dehydrogenase 1 (HSD11B1), and fructose-bisphosphatase 1 (FBP1).

Results: Treatment with oleuropein and hydroxytyrosol led to significant reductions in fasting blood glucose levels and marked improvements in insulin sensitivity in obese and diabetic mice. These compounds also provided protective effects against pancreatic and hepatic cellular damage. *In silico* analyses corroborated these findings, demonstrating strong binding affinities of oleuropein and hydroxytyrosol to the targeted receptors.

Conclusions: Oleuropein and hydroxytyrosol exhibit potent anti-diabetic effects *in vivo*, comparable to those of metformin, including glucose regulation, improved insulin sensitivity, and cellular protection. These findings suggest their potential as therapeutic agents for managing type 2 diabetes and its associated complications.

Keywords: high-fat diet; hydroxytyrosol; obesity; oleuropein; type 2 diabetes mellitus.

Resumen

Contexto: Las hojas de olivo son reconocidas por sus propiedades medicinales en el manejo de la diabetes, principalmente debido a sus altas concentraciones de hidroxitirosol y oleuropeína.

Objetivos: Evaluar los efectos antidiabéticos de los aislados de hidroxitirosol y oleuropeína en un modelo de ratones con diabetes inducida por obesidad.

Métodos: Se sometió a ratones macho Swiss Webster a una dieta rica en grasas y estreptozotocina para inducir la obesidad y la diabetes, respectivamente. Después de la inducción, los ratones fueron tratados con oleuropeína o hidroxitirosol a una dosis de 20 mg/kg de peso corporal durante un período de 14 días. Se monitorearon los niveles de glucosa en ayunas, la sensibilidad a la insulina y la tolerancia a la glucosa durante todo el período de tratamiento. Además, se realizaron exámenes histológicos del hígado y el páncreas. Además, se realizaron estudios de acoplamiento *in silico* para evaluar las interacciones de la oleuropeína y el hidroxitirosol con receptores metabólicos clave, como el receptor gamma activado por el proliferador de peroxisomas (PPARγ), la hidroxiesteroide 11-β deshidrogenasa 1 (HSD11B1) y la fructosa-bifosfatasa 1 (FBP1).

Resultados: El tratamiento con oleuropeína e hidroxitirosol produjo reducciones significativas en los niveles de glucosa en sangre en ayunas y mejoras notables en la sensibilidad a la insulina en ratones obesos y diabéticos. Estos compuestos también proporcionaron efectos protectores contra el daño celular pancreático y hepático. Los análisis in silico corroboraron estos hallazgos, demostrando fuertes afinidades de unión de la oleuropeína y el hidroxitirosol con los receptores objetivo.

Conclusiones: La oleuropeína y el hidroxitirosol exhiben potentes efectos antidiabéticos *in vivo*, comparables a los de la metformina, incluida la regulación de la glucosa, la sensibilidad a la insulina mejorada y la protección celular. Estos hallazgos sugieren su potencial como agentes terapéuticos para el manejo de la diabetes tipo 2 y sus complicaciones asociadas.

Palabras Clave: dieta rica en grasas; hidroxitirosol; obesidad; oleuropeína; diabetes mellitus tipo 2.

ARTICLE INFO
Received: April 6, 2024.
Accepted: September 23, 2024.
Available Online: October 27, 2024.

AUTHOR INFO
ORCID: 0000-0003-3454-9459 (NH) 0000-0002-8855-685X (SD)
0000-0001-5217-2312 (IKA) 0000-0002-3313-6612 (NFK)

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterised by the pancreas's inability to produce sufficient insulin or by insulin resistance, leading to elevated blood glucose levels (Fischer et al., 2019). Weight loss is a well-established preventive measure for diabetes (Al-Goblan et al., 2014). An increase in body weight is closely associated with increased body fat, which is metabolised into fatty acids released into the plasma, thereby impairing glucose transport to muscle cells (Den Hartogh et al., 2020). Concurrently, glucose production by the liver continues to rise (Camporez et al., 2015). If left unmanaged, elevated plasma glucose levels can cause extensive damage to various organs, including the kidneys (Tang et al., 2021), eyes (Kovacova and Shotliff, 2022), liver (Kim et al., 2016b), and nerves (Yorek et al., 2015).

The combination of diabetes and obesity often leads to multifactorial dysregulation of genes such as peroxisome proliferator-activated receptor gamma (PPARG), hydroxysteroid 11-β dehydrogenase 1 (HSD11B1), and fructose-bisphosphatase 1 (FBP1). PPARG plays a crucial role in adipogenesis, where inflammation can drive cells towards adipocyte differentiation, thereby modulating inflammatory gene expression in adipose tissue during obesity (Darwish et al., 2022; Gao et al., 2024). Obesity-induced alterations in glucocorticoid metabolism increase HSD11B1 expression, and inhibiting this gene is a novel therapeutic strategy under investigation. Additionally, targeting hepatic FBP1 to halt gluconeogenesis represents another approach for managing obesity-associated diabetes, potentially reducing blood glucose levels.

Olea europaea L. (family *Oleaceae*), commonly known as the olive tree, produces oleuropein and hydroxytyrosol, compounds abundant in its extracts and oils. These compounds have been extensively studied for their efficacy in various contexts. Oleuropein and hydroxytyrosol are known for their beneficial effects in managing cancer and neurological disorders, as well as significantly lowering blood pressure in individuals with metabolic syndrome (Casamenti and Stefani, 2017; Reboredo-Rodriguez et al., 2018). Despite the wealth of research on these compounds, assessing the efficacy of pure isolates remains challenging due to high production costs and the unavailability of isolation procedures.

The global rise in diabetes and obesity underscores the urgent need for novel therapeutic agents that offer efficacy with minimal adverse effects. Although hydroxytyrosol and oleuropein have been extensively studied for their anti-diabetic, antioxidant, and anti-inflammatory properties, significant gaps remain in understanding their precise mechanisms of action, particularly in the context of obesity-induced diabetes. The existing literature provides substantial evidence of their benefits; however, comprehensive elucidation of their molecular interactions and comparative efficacy in multifaceted biological models is still required (Harun et al., 2023).

The use of olive leaves in traditional Mediterranean medicine for managing diabetes highlights their ethnomedical significance. Hydroxytyrosol and oleuropein have been widely investigated for their antioxidant, anti-inflammatory, and anti-diabetic properties (Reboredo-Rodriguez et al., 2018). Nevertheless, this study's novelty lies in providing a comparative analysis of these compounds using both in vivo and *in silico* approaches. It explores their interactions with key metabolic receptors, including PPARG, HSD11B1, and FBP1, which are pivotal in obesity-related diabetes. This approach broadens the understanding of these compounds' anti-diabetic mechanisms, contributing novel insights beyond previous research that focused on isolated pathways such as modulation of gut microbiota, PI3K/Akt signalling, and GLUT4 translocation.

This study hypothesises that oleuropein and hydroxytyrosol can improve diabetic markers in obese, high-fat diet (HFD)-induced diabetic mice by lowering blood glucose levels, improving insulin sensitivity, and stabilising body weight. The specific objectives are to investigate the efficacy of these compounds in reducing glucose levels and insulin resistance in vivo, and to further explore their molecular interactions with PPARG, HSD11B1, and FBP1 via docking simulations, thereby providing insights into their therapeutic potential.

Oleuropein and hydroxytyrosol are prominent bioactive compounds found in extracts and oils derived from the fruit and leaves of *O. europaea*. Previous studies have demonstrated the effectiveness of these compounds in both *in vivo* and *in vitro* models (Vogel et al., 2014). In this study, we explore the capacity of oleuropein and hydroxytyrosol to interact with the PPARG, HSD11B1, and FBP1 receptors through molecular docking simulations and assess their effects on blood glucose levels, insulin resistance, and body weight in diabetic mice subjected to a high-fat diet (HFD). Therefore, this study contributes new data by highlighting the protective effects of hydroxytyrosol and oleuropein against cellular damage associated with diabetes, emphasising their roles in blood glucose regulation and insulin sensitivity. The findings, supported by both *in vivo* and *in silico* analyses, underscore the potential of these compounds as natural

therapeutic agents for diabetes management. This work strengthens the rationale for further investigation into olive leaf extracts as alternatives to synthetic drugs like metformin, particularly for patients seeking plant-based treatments. By building on existing knowledge and integrating new molecular insights, this study supports the continued exploration of olive leaf compounds in modern medicine.

MATERIAL AND METHODS

Chemicals and reagents

The following chemicals and reagents were utilised in the study: oleuropein isolate, streptozotocin, metformin, glucose, insulin, haematoxylin, formaldehyde, hydroxytyrosol isolate (Markherb, Bandung, West Java, Indonesia), and Lipomed 20% MCT/LCT (Sanbe, Bandung, West Java, Indonesia) for the high-fat diet (HFD).

In vivo experimental design

Animal model and ethics approval

Male Swiss Webster mice, eight weeks old and weighing between 20 and 25 grams, were employed in the experiments. The mice were housed under standard laboratory conditions, including regulated temperature and light-dark cycles, with food and water provided *ad libitum*, in compliance with established guidelines for the care of experimental animals. The experimental protocol received approval from the Ethics Committee of the Bandung Institute of Technology (Approval No. 3/KEPHP-ITB/4-2022).

Body weight measurement

The body weight of the mice was measured using a digital scale, which was calibrated before each use to ensure precision. Measurements were recorded at baseline (day 0) and subsequently on a weekly basis throughout the study. These recorded weights were used to monitor changes over time and to evaluate the impact of the treatments on weight gain or loss. Body circumference was measured at the midsection using a flexible tape measure. The Lee Index was calculated by measuring the body length from the tip of the nose to the base of the tail using a caliper. This index serves as an indicator of relative body fat and overall health status, and in this study, the Lee index for the HFD group exceeded 0.31, indicating significant obesity.

Induction of obesity and diabetes

Obesity was induced by daily administration of 1 mL of Lipomed 20% MCT/LCT (HFD) for 28 days (Shcherbakova et al., 2022). Diabetes was subsequently induced by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 35 mg/kg body weight (Ghasemi and Jeddi, 2023). The control group, consisting of 25 mice, was fed a regular diet (BR511), while the HFD diabetes group, comprising 20 mice, received diet BR511 and the HFD supplement. Anthropometric measurements were conducted on the day prior to HFD administration (day 0) and 14 days post-HFD administration (day 14). The use of single daily doses in diabetes research, specifically for metformin (1.3 mg/20 g BW), oleuropein (20 mg/kg BW), and hydroxytyrosol (20 mg/kg BW), is supported by previous studies

Experimental groups and treatments

The mice were divided into several groups, each consisting of five mice. The Normal Control Group included mice that were neither induced with STZ nor subjected to any treatment. The Treatment Groups consisted of mice that were administered metformin (1.3 mg/20 g BW), oleuropein (20 mg/kg BW), hydroxytyrosol (20 mg/kg BW).

Fasting blood glucose levels were measured on days 0, 1, 3, 7, and 14 of the treatment period. Upon completion of the final measurements, the mice were sacrificed, and their liver and pancreas tissues were harvested for histological analysis.

Oral glucose tolerance test (OGTT)

Following a 7-day treatment with oleuropein or hydroxytyrosol, the mice underwent a 6-hour fasting period. Subsequently, they were administered glucose orally at a dose of 1.2 g/kg body weight, using a 20% glucose solution prepared by dissolving 20 g of glucose in 100 mL of distilled water. Blood glucose concentrations were measured (mg/dL) at intervals of 0, 15, 30, 60, 75, 90, and 120 minutes using a glucometer, with blood samples collected from the tail vein (Eyth et al., 2023).

Insulin tolerance test (ITT)

After 14 days of treatment with oleuropein or hydroxytyrosol, the mice underwent an insulin tolerance test. Following a 2-hour fasting period, the mice were injected intraperitoneally with 0.75 U/kg BW of insulin. Blood glucose levels were subsequently measured (mg/dL) using a glucometer (Cózar-Castellano and Perdomo, 2020). The insulin tolerance test results provided quantitative metrics that were used to calculate the constant insulin tolerance test (KITT), reflecting the rate at which blood glucose levels declined following insulin administration (Hayashishita et al., 2024).

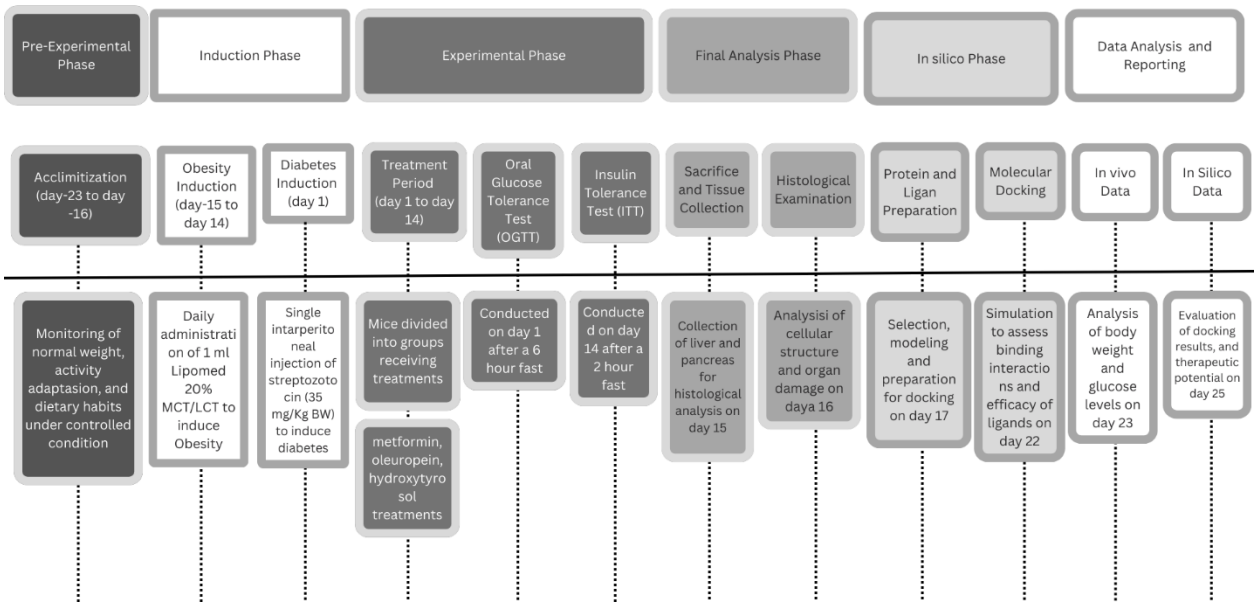

Figure 1. Research study timeline.

Histological analysis

All liver and pancreatic tissues were processed in accordance with standard histological protocols. The procedures included dehydration in a graded ethanol series, clearing with xylene, and embedding in molten paraffin wax. Tissue blocks were then sectioned into thin slices using a microtome. These sections were stained using the haematoxylin and eosin (H&E) protocol to highlight cellular structures. Microscopic examination was performed using an Olympus light microscope equipped with a 40× objective and 10× ocular magnification, with images captured using a high-resolution camera (specify camera type and resolution). Key parameters assessed during histological analysis included hepatocyte architecture, lobular organisation, sinusoidal integrity, inflammatory cell infiltration, and fibrosis in the liver. In the pancreas, the analysis focused on the morphology and quantity of beta cells within the islets of Langerhans, noting any degeneration, hypertrophy, or inflammatory changes. These assessments provided critical insights into the extent of organ damage and the effectiveness of therapeutic interventions in diabetic models (Al-Adsani and Al-Qattan, 2024; Lawrence House, 1958).

In silico experimental design

Protein preparation

Proteins involved in type 2 diabetes and obesity, particularly those related to adipose cells and glucose metabolism, were selected for the study. Their three-dimensional structures were obtained from the Protein Data Bank (PDB) of the Research Collaboratory for Structural Bioinformatics (RCSB; www.rcsb.org/pdb). The proteins were prepared for docking by isolating them from their native ligands, removing water content, and selecting potential chains based on the similarity between the original and experimental ligands using AutoDock Tools and Biovia (Al-Mohaya et al., 2024).

Ligand preparation

Experimental and comparative ligands, including oleuropein, hydroxytyrosol, and metformin, were sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) provided by the US National Center for Biotechnology Information (NCBI). These ligands were modelled and optimised in three dimensions using the Avogadro program and saved in PDB format using their respective PubChem CIDs (Alazmi and Motwalli, 2021).

Molecular docking analysis

Molecular docking simulations were conducted using AutoDock 4 (version 1.56) with a general evolutionary algorithm (rigid docking technique). The docking simulations were performed between each of the four target proteins and their respective ligands. The interactions were evaluated based on binding affinity, bond energy, and hydrogen bonding, and the results were visualised using Biovia. Ligand scores were compared with those of native ligands to assess binding efficiency (Ferrari and Patrizio, 2021).

Fig. 1 illustrates the timeline of the experimental design, providing a clear overview of the procedures and schedule.

In silico data analysis

The *in silico* experiment involved a series of detailed steps to analyse the interactions of oleuropein, hydroxytyrosol, and metformin with target proteins. Initially, the docking method was validated using Autodock, achieving a root-mean-square deviation (RMSD) of ≤2 Å, which confirmed the accuracy of the docking process. The ligands were assessed according to Lipinski's rule of five, meeting all criteria: fewer than five hydrogen bond donors, fewer than ten hydrogen bond acceptors, molecular weights under 500 Da, and a log P between -0.4 and +5.6. The target proteins were prepared by removing water molecules and adding hydrogen atoms to enhance ligand-receptor interactions, with energy minimisation performed to stabilise the protein bonds. Binding free energy calculations revealed that oleuropein had a lower binding free energy compared to hydroxytyrosol and metformin, indicating a stronger binding affinity. Specifically, oleuropein formed five hydrogen bonds with PPARG, while hydroxytyrosol and metformin formed fewer. Similarly, oleuropein formed five hydrogen bonds with the FBP1 receptor, compared to fewer hydrogen bonds for hydroxytyrosol and metformin. The stability of the ligand-protein interactions, as indicated by the more negative binding free energy values, suggested that oleuropein had a superior binding affinity. Data were processed and presented in tables and figures, with tables detailing binding free energy and the number of hydrogen bonds, and figures illustrating the ligand-protein interactions. Comparative analysis with native ligands provided further insights into the efficacy of the tested compounds.

Statistical analysis

In this study, statistical analysis was conducted using Minitab software version 19 and GraphPad Prism version 9. All group data are presented as mean ± standard deviation (SD), with the number of observations (n = 5) clearly indicated. Normal control and positive control (obese diabetic) groups were used as comparative references for each treatment group, with a 95% confidence level. ANOVA followed by Tukey's test was employed to analyse the results.

RESULTS

Effect of oleuropein and or hydroxytyrosol administration on body weight

Anthropometric measurements showed that the body weight of each experimental animal increased, which was particularly evident for mice in the HFD group, which had a Lee index (Adebayo et al., 2020) >0.31 and body mass index (BMI) >30 g/cm^2. The body length of all mice remained >10 cm for 14 days, with their body circumference remaining >9 cm (not shown). On the fourteenth day (Fig. 2), the body weight of the mice fed the HFD had increased by 40%–60% (>30 g), differing significantly from those in the normal control group (20.4 ± 0.5 g). The body weights of mice in the metformin, oleuropein, and hydroxytyrosol groups also differed significantly from those in the HFD diabetic control group after 14 days of treatment. The body weight of mice decreased even when administered metformin (23.1 ± 2.5 g), oleuropein (20.3 ± 1.4 g), or hydroxytyrosol (19.8 ± 2.9 g) with the HFD. However, the body weight of mice in the diabetic HFD group remained unchanged.

Effects of oleuropein or hydroxytyrosol administration on the oral glucose tolerance test

Glucose tolerance was assessed following a single administration of the compound, with glucose levels measured at 5, 30, 60, 90, and 120 minutes (Fig. 3). Blood glucose levels differed significantly after glucose induction between the normal group (80.6 ± 1.1 mg/dL) and the HFD diabetic groups, with or without treatment. After 15 minutes, all mice in the obese diabetic group exhibited a significant increase in glucose levels (>140 mg/dL), indicating impaired glucose tolerance. The blood glucose levels in the HFD diabetic control (165.8 ± 9.0 mg/dL), metformin (168 ± 7.0 mg/dL), oleuropein (170 ± 2.5 mg/dL), and hydroxytyrosol (162.4 ± 5.3 mg/dL) groups were also significantly higher than those in the normal control group. The metformin group demonstrated a decreasing trend in blood glucose levels at 30, 60, 90, and 120 minutes, whereas the hydroxytyrosol and oleuropein groups showed a decrease only at 120 minutes. Significant differences were observed in all HFD diabetes groups at 120 minutes.

Effects of oleuropein or hydroxytyrosol administration on fasting blood glucose level

The progression of the obese diabetic mouse model was monitored by regularly measuring fasting glucose levels, starting from day zero (D-0) and repeated on days 1, 7, and 14 after the commencement of treatment. Fasting blood glucose levels showed a marked difference between the normal and HFD diabetic groups at all measured time points (Fig. 4). Conversely, the normal control group remained normoglycaemic on day 0 (41.0 ± 1.7 mg/dL), day 1 (94.8 ± 4.8 mg/dL), day 3 (78.0 ± 4.6 mg/dL), day 7 (75.6 ± 2.9 mg/dL), and day 14 (81.6 ± 3.8 mg/dL). Each HFD diabetic group exhibited an increase in blood glucose due to the HFD and STZ induction. The fasting glucose levels progressively increased over time in the HFD diabetes group without therapy, indicating that

these mice remained hyperglycaemic. Blood glucose levels decreased by 140 mg/dL in the HFD diabetic groups treated with metformin, oleuropein, and hydroxytyrosol at all four time points. However, there were no significant differences between the treatment groups receiving metformin, oleuropein, or hydroxytyrosol. After 14 days of treatment with fluctuating blood glucose levels, the three treatment groups receiving metformin (103.8 ± 0.8 mg/dL), oleuropein (104.0 ± 9.3 mg/dL), and hydroxytyrosol (105.4 ± 3.8 mg/dL) exhibited similar blood glucose levels.

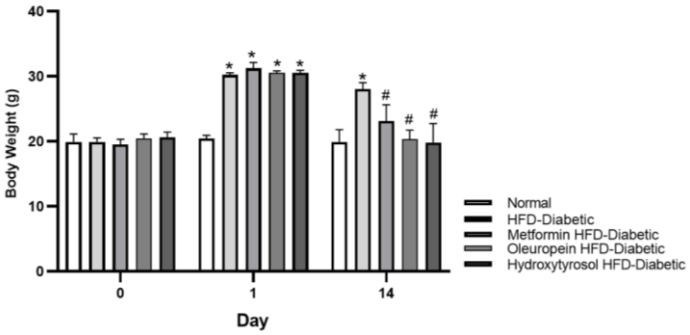

Figure 2. Effect of oleuropein and hydroxytyrosol on body weight

Body weight was assessed before (day 0), 14 days after HFD administration (day 1), and 14 days after treatment (day 14). All results are presented as mean ± SD of five mice per group. *Significantly different compared to normal control group ($p<0.05$); #Significantly different compared to HFD-diabetic control group ($p<0.05$).

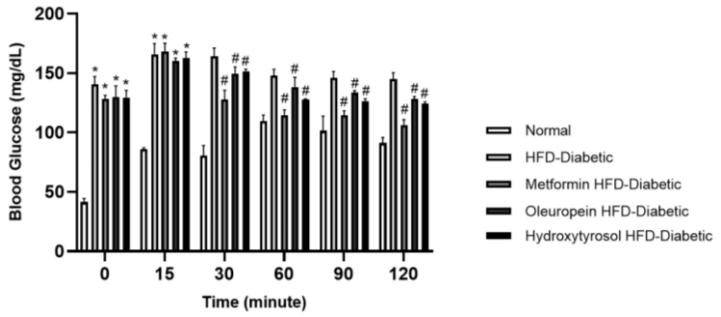

Figure 3. The Effects of oleuropein or hydroxytyrosol on the oral glucose tolerance test.

The effect of glucose loading on blood glucose in obese diabetic mice on the first day of treatment after obesity induction. Data are plotted as the mean ± SD of five mice per group. *Significantly different compared to normal control group ($p<0.05$); #Significantly different compared to HFD-diabetic control group ($p<0.05$).

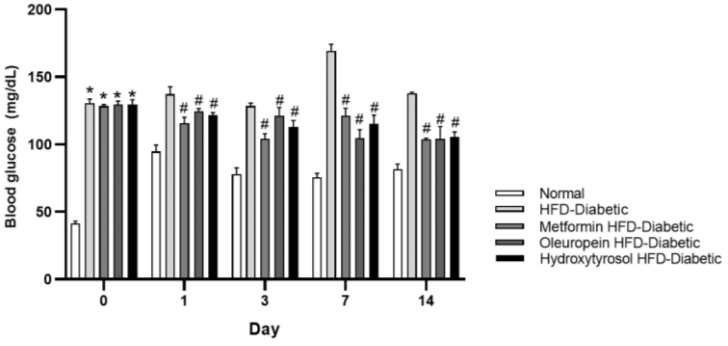

Figure 4. Effect of oleuropein or hydroxytyrosol on fasting blood glucose levels test.

Fasting blood glucose levels were monitored. Data are plotted as the mean ± SD of five mice per group. *Significantly different compared to the normal control group ($p<0.05$); #Significantly different compared to the HFD-diabetic control group ($p<0.05$).

Effects of oleuropein or hydroxytyrosol on the insulin tolerance test

All experimental groups were assessed for insulin tolerance after 14 days of treatment through intraperitoneal administration of insulin (Fig. 5). The normal control group's plasma glucose disappearance rate (KITT; 5.0 ± 1.9 mg/dL) indicated high insulin tolerance. KITT differed significantly between the HFD diabetes groups, with the HFD diabetes group without therapy (0.4 ± 0.1 mg/dL) displaying a lower value, suggesting insulin intolerance. In contrast, KITT did not differ significantly between the metformin (1.6 ± 1.0 mg/dL), oleuropein (2.5 ± 0.5 mg/dL), and hydroxytyrosol (1.5 ± 1.3 mg/dL) groups.

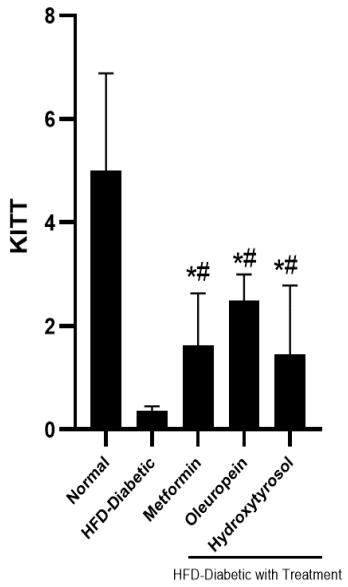

Figure 5. Effect of oleuropein or hydroxytyrosol on insulin tolerance test.

K_{ITT} data are plotted as the mean ± SD of five mice per group.
*Significantly different compared to normal control group ($p<0.05$);
#Significantly different compared to HFD-diabetic control group ($p<0.05$).

Histopathology

Liver histology (Fig. 6) in the metformin, oleuropein, and hydroxytyrosol groups exhibited normal features, including intact hepatocytes, eosinophilic cytoplasm, and basophilic nuclei surrounding the central vein. There was no evidence of hydropic or necrotic tissue or parenchymal degeneration. Each of these groups was assigned a score of one. The number of cells per view ranged from 16 to 20. In contrast, the HFD diabetes group was assigned a score of three, with observed dilated sinusoids and notable hydropic degeneration, characterised by enlarged and vacuolated hepatocytes. Pancreatic histology revealed pancreatic acinar cells across all experimental groups (Fig. 6), representing the functional units of the exocrine pancreas. The islets of Langerhans were discernible in the normal and treatment groups administered metformin, oleuropein, or hydroxytyrosol, but were absent in the HFD diabetes groups.

In silico experiment

This experiment utilised oleuropein and hydroxytyrosol analogues sourced from the NCBI PubChem database (PMID: 5281544 and 82755). Following the successful completion of the docking technique validation procedure (not shown), the Autodock application was employed to evaluate the suitability of the docking method, which exhibited a root-mean-square deviation (RMSD) of ≤2 Å, rendering it valid. All ligands were analysed in accordance with Lipinski's rule (Table 1). Oleuropein, hydroxytyrosol, and metformin were found to comply with the criteria, possessing no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, molecular weights of less than 500 Da, and an octanol-water partition coefficient (log P) within the range of −0.4 to +5.6, as most orally administered drugs are relatively small and moderately lipophilic.

The initial step in protein preparation involved the removal of water molecules (H_2O) to maintain the integrity of amino acids in the target protein and ensure that only the test compound interacted with these amino acids during the docking process (Mihoub et al., 2022). The addition of hydrogen atoms enhanced hydrogen bonding between the ligand and receptor. The energy minimisation procedure similarly affected the side chain, backbone, and alpha carbon atoms, thereby stabilising the protein bonds (Dwitiyanti et al., 2019).

Binding free energy was found to be lower for the interaction of PPARG with oleuropein than with hydroxytyrosol and metformin (Table 2). Oleuropein formed five hydrogen bonds (SER342, SER342, GLY284, GLY284, TYR473), hydroxytyrosol formed two hydrogen bonds (TYR327, TYR473), and metformin formed three hydrogen bonds (PHE282, PHE282, TYR473) (Fig. 7), indicating the superiority of oleuropein over hydroxytyrosol and metformin. PPARG formed four hydrogen bonds with oleuropein (THR264, ASP259, ASP259, ILE218, THR220), four hydrogen bonds with metformin (LEU217, GLN234, SER260), and two hydrogen bonds with hydroxytyrosol (PRO178, ALA172), further demonstrating the superior binding affinity of oleuropein. The FBP1 receptor (Fig. 7) was found to form one hydrogen bond with metformin (GLU20), three hydrogen bonds with hydroxytyrosol (LYS112), and five hydrogen bonds with oleuropein (GLY26, THR31, VAL160, ASP178, THR27), with oleuropein displaying the lowest binding energy with the FBP1 receptor (Table 2). The number of hydrogen bonds reflects the number of

amino acids within the receptor to which the ligand can bind to exert its function. This binding energy indicates that a more stable ligand-protein bond results from a more negative binding free energy value, correlating with lower energy requirements for the ligand-protein interaction.

Table 1. Predicted values of Lipinski Rule, absorption, distribution, and toxicity.

Characteristic	Ligand		Standard
	Oleuropein (P CID 5281544)	Hydroxytyrosol (P CID 82755)	
Psychochemical			
Topological polar surface area (TPSA) (Å^2)	201.67	60.69	
Log P (octanol-water partition coefficient)	-0.634200	0.632600	<5
Molecular weight (g/mol)	540.5	154	500
Number H-bond acceptors	6	3	<5
Number H-bond donors	13	3	<10
Molar refractivity	126.12	40.56	40-130
Pharmacokinetic properties			
Human intestinal absorption (HIA)	(-)20-40%	(+) 70-80 %	
Solubility (mg/l)	57248.77	101126.42	
Druglikeness			
Oral bioavailability (Veber)	Low	Good	
Oral bioavailability (Egan)	Good	Good	
In silico toxicity			
Carcinogenicity (trynary)	Not found	Not found	
Medicinal chemistry (PAINS)	One alert	One alert	
Mutagenicity (AMES Mutagenesis)	+	+	
Tumorigenicity	-	-	
Irritant	-	+	
Glucocorticoid receptor binding	+	-	

Table 2. Docking results between ligands and receptors.

Isolate	Ligand	Δ Gibbs (Kcal/mol)	IC (nM)	Number of H bonds	AAP
Metformin	PPARγ	-3.10	5.32	3	PHE^{282}, PHE^{282}, TYR^{473}
	HSD11B1	-5.05	197.5	4	LEU^{217}, GLN^{234}, SER^{260}
	FBP1	-2.86	8.07	1	GLU^{20}
Oleuropein	PPARγ	-8.99	255.7	5	SER^{342}, SER^{342}, GLY^{284} ·GLY^{284} TYR^{473}
	HSD11B1	-7.94	1.52	5	THR^{26}, ASP^{259}, ASP^{259}, ILE_{218}, THR_{220}
	FBP1	-6.83	9.77	5	GLY^{26}, THR^{31}, VAL^{160}, VAL^{160}, ASP^{178}
Hydroxytyrosol	PPARγ	-5.19	155.7	2	TYR^{327}, TYR^{473}
	HSD11B1	-3.80	1.64	2	PRO^{178}, ALA^{172}
	FBP1	-5.52	89.88	3	THR^{27}, THR^{31}, LYS^{112}

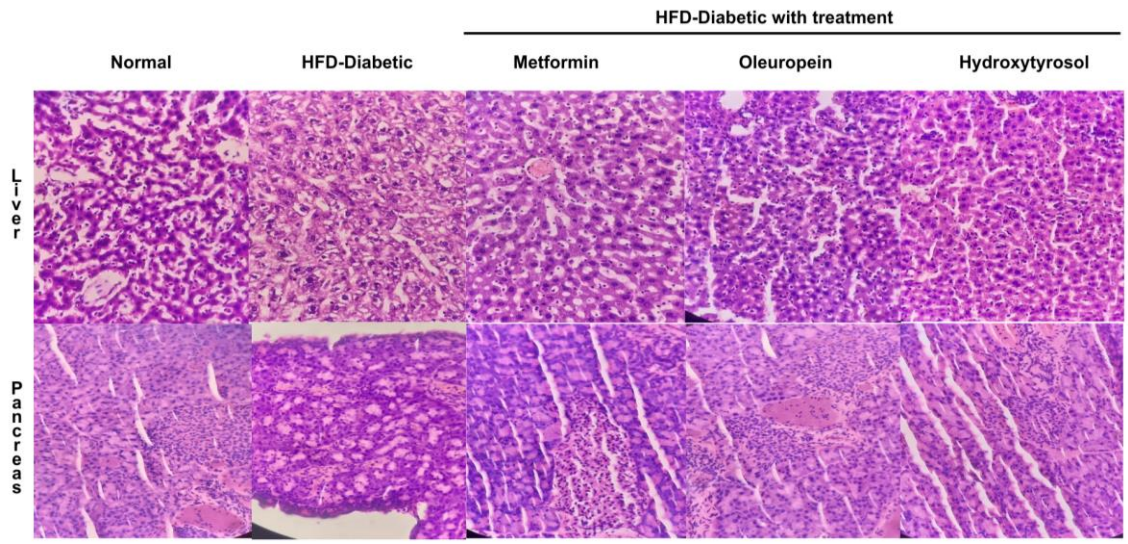

Figure 6. Liver and pancreas histology.

The liver and pancreas specimens were processed using standard methods, and sections were stained with haematoxylin and eosin. The images were taken at 40× objective and 10× ocular magnification. Scale: 1 cm = 100 µm.

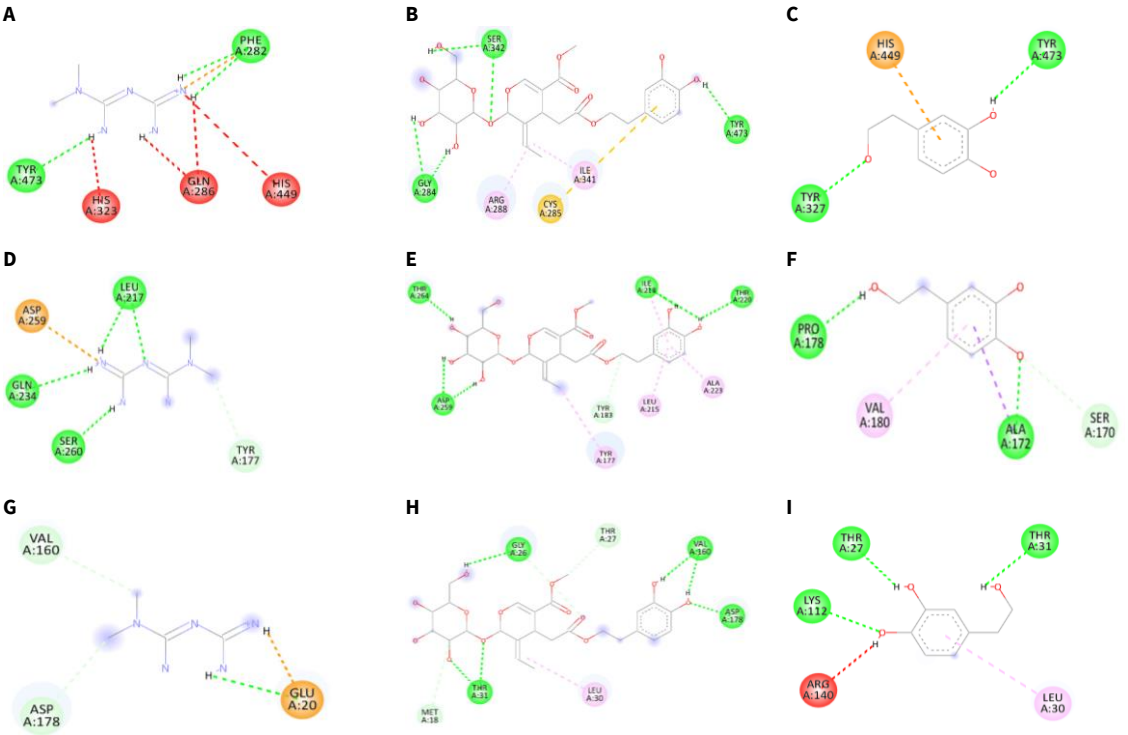

Figure 7. The interaction of the ligands with receptor biomarkers of diabetic obesity.

(A) The interactions of the ligands with PPARG and metformin. **(B)** The interactions of the ligands with PPARG and oleuropein. **(C)** The interactions of the ligands with PPARG and hydroxytyrosol. **(D)** The interactions of the ligands with HSD11B1 and metformin. **(E)** The interactions of the ligands with HSD11B1 and oleuropein. **(F)** The interactions of the ligands with HSD11B1 and hydroxytyrosol, **(G)** The interactions of the ligands with FBP1 and metformin. **(H)** The interactions of the ligands with FBP1 and oleuropein. **(I)** The interactions of the ligands with FBP1 and hydroxytyrosol.

DISCUSSION

This study explores the potential of olive oil in diabetes treatment, particularly focusing on the phytochemical components, oleuropein, and hydroxytyrosol. Previous research demonstrated that olive extract, rich in these compounds, facilitated weight reduction in obese diabetic mice, though the average weight loss was under 40% (Harun et al., 2021). Ongoing clinical trials are investigating how hydroxytyrosol and oleuropein regulate weight gain in individuals with type 2 diabetes, particularly in those with poor die-

tary habits. Metformin remains the first-line treatment for diabetes-associated obesity, given its ability to reduce blood glucose levels while preventing weight gain (Szymczak-Pajor et al., 2022). Bornstein et al. (2017) reported that metformin influences body mass by inducing significant alterations in metabolic profiles and the phenotype of adipose tissue. Our findings suggest that the weight loss effects of oleuropein and hydroxytyrosol are comparable to those of metformin, indicating similar efficacy

In this study, metformin was administered at a low single dose of 500 mg to prediabetic subjects to enhance insulin sensitivity and lower elevated blood glucose levels (Dahabiyeh et al., 2021). Initiating treatment with a low dose minimises the gastrointestinal side effects commonly associated with metformin, such as nausea, diarrhoea, and abdominal discomfort. This method enables the body to adjust gradually, improving tolerance. If necessary, the dosage can be increased incrementally to achieve the desired therapeutic effect while monitoring the patient's response (Nabrdalik et al., 2022). Oleuropein (20 mg/kg BW) and hydroxytyrosol (20 mg/kg BW) were administered over 14 days. Oleuropein, given at 0.037 mmol/kg BW, has demonstrated significant benefits in modulating metabolic and anti-inflammatory markers while maintaining a stable pharmacokinetic profile (Al-Azzawie and Alhamdani, 2006; Lepore et al., 2015). Hydroxytyrosol, which has previously been administered at 10 mg/kg/day, was increased to 20 mg/kg BW in this study to assess its comparative efficacy (Soylu and Karacor, 2023). These single-dose regimens, including the use of *O. europaea* leaf extract at 400 mg/kg BW, simplified the experimental design, reduced variability, and maintained therapeutic levels with minimal side effects, supported by previous research.

Several studies have indicated that lipid accumulation can lead to an increase in both the number and size of adipocytes in the liver and adipose tissue (Kojta et al., 2020; Longo et al., 2019). Our study demonstrated visible fat accumulation in the stomach and nape of all obesity models utilised in the research. The high-fat diet (HFD) consumed by individuals with diabetes can elevate their blood insulin (hyperinsulinaemia) and leptin (hyperleptinaemia) levels. This uncontrolled rise in leptin may result in increased leptin resistance, which exacerbates feelings of hunger (de Moura e Dias et al., 2021). Leptin exerts feedback to the hypothalamus by suppressing hunger signals, including agouti-related neuropeptide (AGRP), neuropeptide Y (NPY), and galanin (GAL) (Liu et al., 2023). Leptin reduces adipocyte differentiation in mice by decreasing the activity of peroxisome proliferator-activated receptors (PPARs). PPARs play crucial roles in regulating cell development, differentiation, metabolism (of carbohydrates, proteins, and lipids), and tumourigenesis in higher organisms. Suppression of PPARG expression, also known as the pioglitazone receptor, in the adipose tissue and liver of mice results in lipolysis rather than lipogenesis (Abbasi et al., 2017). Docking simulations demonstrated that oleuropein and hydroxytyrosol inhibit PPARG function with minimal binding energy, similar to metformin. Increased PPARG activation can reduce the levels of fatty acid synthase (FASN), lipoprotein lipase (LPL), acetyl-CoA carboxylase (ACC), glucose transporter type 4 (GLUT4), and phosphorylated adenosine monophosphate-activated protein kinase α/β (AMPKα/β) (Kim et al., 2016a).

Obese mice often exhibit a reduced capacity to manage blood glucose increases, particularly when their glucose load is substantial (Li et al., 2017), a condition exacerbated by diabetes, which increases insulin resistance. Lipid molecules, such as long-chain nonesterified fatty acids (FFAs), are more prevalent in obesity. FFAs may influence insulin secretion positively or negatively, depending on the conditions the day after administration. Consequently, obesity-induced increases in systemic FFAs may directly impact glucose homeostasis by affecting insulin production and systemic insulin sensitivity (Nagarajan et al., 2019).

Although the KITT values indicated equivalence, oleuropein and hydroxytyrosol conferred the same glucose tolerance ability as metformin, without surpassing it. The relationship between insulin and glucose tolerance in diabetic obese mice may be linked to an HFD, which impairs the body's ability to regulate inflammation manifesting in the liver and adipose tissues. Brancatelli et al. (2018) observed that liver histology in HFD mice showed extrahepatic inflammation with degrees of sinusoidal dilatation, hydropic degeneration, and vacuole formation in parenchymal cells. Therefore, the combination of an HFD and high levels of insulin resistance can lead to hepatocyte inflammation, which may promote liver fibrosis (Ramadan et al., 2022). However, the HFD groups receiving metformin, hydroxytyrosol, or oleuropein exhibited normal liver function without adverse effects.

Adipose, liver, and muscle cells express 11β-hydroxysteroid dehydrogenase isozymes, which are strongly associated with the subsequent inflammation (Chapman et al., 2013). Insulin resistance in the liver is a response to the activity of active glucocorticoids (Beaupere et al., 2021), primarily mediated by HSD11B1, which catalyses the interconversion of active cortisol and corticosterone with inert cortisone and 11-dehydrocorticosterone, predominantly in he-

patic cells (Kotelevtsev et al., 1997). Administration of oleuropein or hydroxytyrosol for 14 days consistently reduced the fasting glucose levels of HFD mice from their hyperglycaemic state. This finding raises questions regarding whether prolonged, regular use of these isolates might accelerate the recovery of pancreatic cells' insulin sensitivity (Da Porto et al., 2021). Histology revealed that the islets of Langerhans were preserved in the pancreas of mice treated with oleuropein or hydroxytyrosol. In contrast, the islets of Langerhans beta cells were damaged in diabetic mice subjected to chronic HFD administration without treatment (Eldeeb et al., 2015).

Docking simulations suggest that the binding of oleuropein, hydroxytyrosol, and metformin to HSD11B1, characterised by low binding energies, will inhibit its activity (Valentini et al., 2022). The activity of HSD11B1 in the liver can potentially enhance endogenous glucose synthesis, a necessary process, particularly during fasting (Zhao et al., 2015). This process can activate numerous genes, including glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK) (Beaupere et al., 2021), which are essential for non-carbohydrate metabolism to produce glucose as an energy source. One potential approach to limit hepatic gluconeogenesis and the increase in endogenous glucose during fasting is to inhibit the FBP1 regulator. Research suggests that elevated FBP1 activity, induced by excessive fat consumption, may reduce the activation of neuropeptides that stimulate hunger (NPY and AGRP) (Visinoni et al., 2012). The interactions of hydroxytyrosol and oleuropein with FBP1, demonstrated by low binding energies, support their potential efficacy as FBP1 inhibitors in obese diabetic mice.

Although extensive research has established the anti-diabetic effects of various compounds, including oleuropein and hydroxytyrosol, this study offers several novel contributions to the field. Firstly, while previous studies have highlighted the weight reduction effects of olive extract in obese diabetic mice, with an average weight loss of less than 40% (Harun et al., 2021), our research provides a direct comparison of the efficacy of oleuropein and hydroxytyrosol with metformin, the first-line treatment for diabetes-associated obesity. By demonstrating that oleuropein and hydroxytyrosol achieve comparable weight loss to metformin, our study offers valuable insights into their potential as effective alternatives or adjuncts to conventional treatments. Secondly, our study extends the current understanding of the molecular mechanisms underlying the anti-diabetic properties of these compounds. While existing literature reports the beneficial effects of oleuropein and hydroxytyrosol on glucose tolerance and insulin sensitivity, our research employs advanced docking simulations to elucidate their interactions with key metabolic receptors, including PPARG and HSD11B1, and their potential role as FBP1 inhibitors. This approach provides a more detailed molecular perspective, clarifying how these compounds might influence glucose metabolism and insulin sensitivity at the receptor level. Furthermore, our study introduces new data concerning the impact of oleuropein and hydroxytyrosol on liver function and pancreatic beta-cell preservation. While prior research has documented the effects of these compounds on weight and glucose levels, our findings reveal that oleuropein and hydroxytyrosol can preserve the islets of Langerhans in the pancreas, a crucial aspect of diabetes management. This preservation contrasts with the damage observed in diabetic mice subjected to a high-fat diet without treatment, underscoring the potential long-term benefits of these compounds.

Nevertheless, the study is limited by its focus on changes in blood glucose levels and histological outcomes, as well as the interpretations derived from docking simulations. Future research should specifically examine the effects of these compounds at the molecular level within cells expressing the relevant receptors. In summary, this study distinguishes itself by not only comparing oleuropein and hydroxytyrosol with metformin but also by providing novel insights into their molecular interactions and impact on pancreatic and liver function. These contributions advance our understanding of these compounds' therapeutic potential and their role in diabetes management.

CONCLUSION

Treatment with oral oleuropein or hydroxytyrosol led to reductions in body weight and plasma glucose levels in obese diabetic mice, while also protecting liver and pancreatic cells from damage. These effects may be attributed to the metabolic suppression of gluconeogenesis through the modulation of receptors such as PPARG, HSD11B1, and FBP1. Consequently, oleuropein and hydroxytyrosol hold potential as therapeutic agents for managing patients with comorbid obesity and diabetes.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Doctoral Dissertation Research Program 2021 (research grant number 2/E1/KP.PTNBH/2021), the collaborative research team at the School of Pharmacy, Bandung Institute

of Technology, and the Pharmacy Department at STIKes Muhammadiyah Ciamis for providing access to their facilities and support throughout this study.

REFERENCES

Abbasi A, Moghadam AA, Kahrarian Z, Abbsavaran R, Yari K, Alizadeh E (2017) Molecular effects of leptin on peroxisome proliferator activated receptor gamma (PPAR-γ) mRNA expression in rat adipose and liver tissue. Cell Mol Biol (Noisy-le-grand) 63(7): 89–93. https://doi.org/10.14715/cmb/2017.63.7.15

Adebayo AO, Akinloye AK, Oke BO, Taiwo VO (2020) Relationship between Body Mass Index (BMI) and testicular and hormonal parameters of sexually active male greater cane rats (*Thryonomys swinderianus*). Anim Reprod 17(1): e20190026. https://doi.org/10.21451/1984-3143-AR2019-0026

Al-Adsani AM, Al-Qattan KK (2024) Among other tissues, short-term garlic oral treatment incrementally improves indicants of only pancreatic islets of Langerhans histology and insulin mRNA transcription and synthesis in diabetic rats. Biology 13(5): 355. https://doi.org/10.3390/biology13050355

Alazmi M, Motwalli O (2021) *In silico* virtual screening, characterization, docking and molecular dynamics studies of crucial SARS-CoV-2 proteins. J Biomol Struct Dyn 39(17): 6761–6771. https://doi.org/10.1080/07391102.2020.1803965

Al-Azzawie HF, Alhamdani MSS (2006) Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sci 78(12): 1371–1377. https://doi.org/10.1016/j.lfs.2005.07.029

Al-Goblan AS, Al-Alfi MA, Khan MZ (2014) Mechanism linking diabetes mellitus and obesity. Diabetes Metab Syndr Obes 7: 587–591. https://doi.org/10.2147/dmso.s67400

Al-Mohaya M, Mesut B, Kurt A, Çelik YS (2024) *In silico* approaches which are used in pharmacy. J Appl Pharm Sci 14(04): 239–253. http://doi.org/10.7324/JAPS.2024.154854

Beaupere C, Liboz A, Fève B, Blondeau B, Guillemain G (2021) Molecular mechanisms of glucocorticoid-induced insulin resistance. Int J Mol Sci 22(2): 623. https://doi.org/10.3390/ijms22020623

Bornstein S, Moschetta M, Kawano Y, Sacco A, Huynh D, Brooks D, Manier S, Fairfield H, Falank C, Roccaro AM, Nagano K, Baron R, Bouxein M, Vary C, Ghobrial IM, Rosen CJ, Reagan MR (2017) Metformin affects cortical bone mass and marrow adiposity in diet-induced obesity in male mice. Endocrinology 158(10): 3369–3385. https://doi.org/10.1210/en.2017-00299

Brancatelli G, Furlan A, Calandra A, Dioguardi Burgio M (2018) Hepatic sinusoidal dilatation. Abdom Radiol 43(8): 2011–2022. https://doi.org/10.1007/s00261-018-1465-8

Camporez JP, Asrih M, Zhang D, Kahn M, Samuel VT, Jurczak MJ, Jornayvaz FR (2015) Hepatic insulin resistance and increased hepatic glucose production in mice lacking Fgf21. J Endocrinol 226(3): 207–217. https://doi.org/10.1530/joe-15-0136

Casamenti F, Stefani M 2017 Olive polyphenols: New promising agents to combat aging-associated neurodegeneration. Expert Rev Neurother 17(4): 345–358. https://doi.org/10.1080/14737175.2017.1245617

Chapman K, Holmes M, Seckl J (2013) 11β-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. Physiol Rev 93(3):1139–1206. https://doi.org/10.1152/physrev.00020.2012

Cózar-Castellano I, Perdomo G (2020) Assessment of Insulin Tolerance In Vivo in Mice. In: King A (ed.) Animal Models of Diabetes. Methods in Molecular Biology, vol 2128. New York, NY: Humana. https://doi.org/10.1007/978-1-0716-0385-7_15

Da Porto A, Brosolo G, Casarsa V, Bulfone L, Scandolin L, Catena C, Sechi LA (2021) The pivotal role of oleuropein in the anti-diabetic action of the Mediterranean diet: A concise review. Pharmaceutics 14(1): 40. https://doi.org/10.3390/pharmaceutics14010040

Dahabiyeh LA, Arafat T, Benabdelkamel H, Alfadda AA, Abdel Rahman AM (2021) A metabolic pattern in healthy subjects given a single dose of metformin: A metabolomics approach. Front Pharmacol 12: 705932. https://doi.org/10.3389/fphar.2021.705932

Darwish NM, Gouda W, Almutairi SM, Elshikh MS, Morcos GNB (2022) PPARG expression patterns and correlations in obesity. J King Saud Univ Sci 34(6): 102116. https://doi.org/10.1016/j.jksus.2022.102116

de Moura e Dias M, Dos Reis SA, da Conceição LL, Sediyama CMNO, Pereira SS, de Oliveira LL, Gouveia Peluzio MDC, Martinez JA, Milagro FI (2021) Diet-induced obesity in animal models: Points to consider and influence on metabolic markers. Diabetol Metab Syndr 13(1): 32. https://doi.org/10.1186/s13098-021-00647-2

Den Hartogh DJ, Vlavcheski F, Giacca A, Tsiani E (2020) Attenuation of free fatty acid (FFA)-induced skeletal muscle cell insulin resistance by resveratrol is linked to activation of AMPK and inhibition of mTOR and p70 S6K. Int J Mol Sci 21(14): 4900. https://doi.org/10.3390/ijms21144900

Dwitiyanti D, Rachmania RA, Efendi K, Septiani R, Jihadudin P (2019) *In vivo* activities and *in silico* study of Jackfruit seeds (*Artocarpus heterophyllus* Lam.) on the reduction of blood sugar levels of gestational diabetes rate induced by streptozotocin. Open Access Maced J Med Sci 7(22): 3819–3826. https://doi.org/10.3889/oamjms.2019.512

Eldeeb TA, Elmihi KA, Okasha EF, Abo Elnasr SE (2015) Evaluation of the role of olive leaf extract on the islets of Langerhans in adult male albino rats after induction of diabetes: a light and electron microscopic study. Egypt J Histol 38(4): 692–703. https://doi.org/10.1097/01.EHX.0000473761.65379.38

Eyth E, Basit H, Swift CJ (2023) Glucose Tolerance Test. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. https://www.ncbi.nlm.nih.gov/books/NBK532915/

Ferrari IV, Patrizio P (2021) Development and validation molecular docking analysis of human serum albumin (HSA). bioRxiv 2021.07.09.451789. https://doi.org/10.1101/2021.07.09.451789

Fischer AW, Albers K, Schlein C, Sass F, Krott LM, Schmale H, Gordts PLSM, Scheja L, Heeren J (2019) PID1 regulates insulin-dependent glucose uptake by controlling intracellular sorting of GLUT4-storage vesicles. Biochim Biophys Acta Mol Basis Dis 1865(6): 1592–1603. https://doi.org/10.1016/j.bbadis.2019.03.010

Gao F, Litchfield B, Wu H (2024) Adipose tissue lymphocytes and obesity. J Cardiovasc Aging 4: 5. https://doi.org/10.20517/jca.2023.38

Ghasemi A, Jeddi S (2023) Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. EXCLI J. 22: 274–294. https://doi.org/10.17179/excli2022-5720

Harun N, Adnyana IK, Damayanti S, Kurniati NF (2023) Oleuropein and hydroxytyrosol as antidiabetics: A review on extraction method, effectiveness, and toxicity effect. Mil Med Sci Lett 92: 1–9. https://doi.org/10.31482/mmsl.2023.037

Harun N, Herlina E, Siti Rahmah Kurnia R (2021) Antihyperglycemic of olive leaf (*Olea europaea* L.) ethanol extract in obesity diabetic male mice (*Mus musculus*). In: Proceedings of the 2nd International Conference on Contemporary Science and Clinical Pharmacy 2021 (ICCSCP 2021) [Internet]. Available from: https://www.atlantis-press.com/article/125962513

Hayashishita A, Watanabe T, Suzuki N, Nakaya T, Sugimoto A, Yokota I, Ohira H, Nishimura M, Tsujino I (2024) Insulin resistance assessed by short insulin tolerance test and its association with obesity and insulin resistance-related parameters in humans: A pilot randomized trial. PLoS One 19(6): e0297718. https://doi.org/10.1371/journal.pone.0297718

Kim JH, Kim OK, Yoon HG, Park J, You Y, Kim K, Lee YH, Choi KC, Lee J, Jun W (2016a) Anti-obesity effect of extract from fermented Curcuma longa L. through regulation of adipogenesis and lipolysis pathway in high-fat diet-induced obese rats. Food Nutr Res. 60: 30428. https://doi.org/10.3402/fnr.v60.30428

Kim Y, Kwon MJ, Choi JW, Lee MK, Kim C, Jung J, Aprianita H, Nam H, Nam TJ (2016b) Anti-obesity effects of boiled tuna extract in mice with obesity induced by a high-fat diet. Int J Mol Med 38(4): 1281–1288. https://doi.org/10.3892/ijmm.2016.2695

Kojta I, Chacińska M, Błachnio-Zabielska A (2020) Obesity, bioactive lipids, and adipose tissue inflammation in insulin resistance. Nutrients 12(5): 1305. https://doi.org/10.3390/nu12051305

Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson P, Best R, Brown R, Edwards CR, Seckl JR, Mullins JJ (1997) 11beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. Proc Natl Acad Sci U S A 94(26): 14924–14929. https://doi.org/10.1073/pnas.94.26.14924

Kovacova A, Shotliff K (2022) Eye problems in people with diabetes: more than just diabetic retinopathy. Pract Diabetes 39(1): 34–39a. https://doi.org/10.1002/pdi.2378

Lawrence House E (1958) A histological study of the pancreas, liver and kidney both during and after recovery from alloxan diabetes. Endocrinology 62(2): 189–200. https://doi.org/10.1210/endo-62-2-189

Lepore SM, Morittu VM, Celano M, Trimboli F, Oliverio M, Procopio A, Di Loreto C, Damante G, Britti D, Bulotta S, Russo D (2015) Oral administration of oleuropein and its semisynthetic peracetylated derivative prevents hepatic steatosis, hyperinsulinemia, and weight gain in mice fed with high fat cafeteria diet. Int J Endocrinol 2015: 431453. https://doi.org/10.1155/2015/431453

Li X, Yu X, Sun D, Li J, Wang Y, Cao P, Liu Y (2017) Effects of polar compounds generated from the deep-frying process of palm oil on lipid metabolism and glucose tolerance in Kunming mice. J Agric Food Chem 65(1): 208–215. https://doi.org/10.1021/acs.jafc.6b04565

Liu Z, Xiao T, Liu H (2023) Leptin signaling and its central role in energy homeostasis. Front Neurosci 17: 1238528. https://doi.org/10.3389/fnins.2023.1238528

Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, Beguinot F, Miele C (2019) Adipose tissue dysfunction as a determinant of obesity-associated metabolic complications. Int J Mol Sci 20(9): 2358. https://doi.org/10.3390/ijms20092358

Mihoub M, Hamri S, Bouchaour T, Popa M, Popa DM, Bedjaoui Alachaher L, Hamcerencu M (2022) An interpenetrating polymer network hydrogel based on cellulose, applied to remove colorant traces from the water medium: Electrostatic interactions analysis. Polymers (Basel) 14(23): 5090. https://doi.org/10.3390/polym14235090

Nabrdalik K, Skonieczna-Żydecka K, Irlik K, Hendel M, Kwiendacz H, Łoniewski I, Januszkiewicz K, Gumprecht J, Lip GYH (2022) Gastrointestinal adverse events of metformin treatment in patients with type 2 diabetes mellitus: A systematic review, meta-analysis and meta-regression of randomized controlled trials. Front Endocrinol 13: 975912. https://doi.org/10.3389/fendo.2022.975912

Nagarajan SR, Paul-Heng M, Krycer JR, Fazakerley DJ, Sharland AF, Hoy AJ (2019) Lipid and glucose metabolism in hepatocyte cell lines and primary mouse hepatocytes: A comprehensive resource for in vitro studies of hepatic metabolism. Am J Physiol Endocrinol Metab 316(4): E578-E589. https://doi.org/10.1152/ajpendo.00365.2018

Ramadan MA, Pramaningtyas MD, Lusiantari R (2022) Derajat fibrosis dan NAS pada hepar tikus diabetes mellitus tipe 2 remaja. J Kedokteran Kesehatan 17(1): 53-59. https://doi.org/10.32539/JKK.V9I1.151315

Reboredo-Rodríguez P, Varela-López A, Forbes-Hernández TY, Gasparrini M, Afrin S, Cianciosi D, Zhang J, Manna PP, Bompadre S, Quiles JL, Battino M, Giampieri F (2018) Phenolic compounds isolated from olive oil as nutraceutical tools for the prevention and management of cancer and cardiovascular diseases. Int J Mol Sci 19(8): 2305. https://doi.org/10.3390/ijms19082305

Shcherbakova K, Schwarz A, Apryatin S, Karpenko M, Trofimov A (2022) Supplementation of regular diet with medium-chain triglycerides for procognitive effects: A narrative review. Front Nutr 9: 934497. https://doi.org/10.3389/fnut.2022.934497

Soylu H, Karacor K (2023) The effects of hydroxytyrosol on Prdx6 and insulin expressions in diabetic rat pancreases. PREPRINT Research Square. https://doi.org/10.21203/rs.3.rs-2454142/v1

Szymczak-Pajor I, Wenclewska S, Śliwińska A (2022) Metabolic action of metformin. Pharmaceuticals 15(7): 810. https://doi.org/10.3390/ph15070810

Tang SCW, Chan ATP, Yiu WH, Lai KN (2021) Pathogenesis of diabetic kidney disease. Diabetic Nephropathy 1(1): 9–13. https://doi.org/10.2478/dine-2021-0004

Valentini M, Pieroni E, Concas A, Pisu M (2022) Docking and molecular dynamics simulations reveal a possible site of interaction of olive leaf extract hydroxytyrosol with polyunsaturated fatty acid 5-lipoxygenase human enzyme. Eur J Biol Biotechnol 3(2): 6–11. https://doi.org/10.24018/ejbio.2022.3.2.345

Visinoni S, Khalid NF, Joannides CN, Shulkes A, Yim M, Whitehead J, Tiganis T, Lamont BJ, Favaloro JM, Proietto J, Andrikopoulos S, Fam BC (2012) The role of liver fructose-1,6-bisphosphatase in regulating appetite and adiposity. Diabetes 61(5): 1122–1132. https://doi.org/10.2337/db11-1511

Vogel P, Kasper Machado I, Garavaglia J, Zani VT, de Souza D, Morelo Dal Bosco S (2014) Polyphenols benefits of olive leaf (*Olea europaea* L) to human health. Nutr Hosp 31(3): 1427–1433. https://doi.org/10.3305/nh.2015.31.3.8400

Yorek MS, Obrosov A, Shevalye H, Holmes A, Harper MM, Kardon RH, Yorek MA (2015) Effect of diet-induced obesity or type 1 or type 2 diabetes on corneal nerves and peripheral neuropathy in Swiss Webster mice. J Peripher Nerv Syst 20(1): 24–31. https://doi.org/10.1111/jns.12111

Zhao L, Pan Y, Peng K, Wang Z, Li J, Li D, Tong C, Wang Y, Liang G (2015) Inhibition of 11β-HSD1 by LG13 improves glucose metabolism in type 2 diabetic mice. J Mol Endocrinol 55(2): 119–131. https://doi.org/10.1530/jme-14-0268

AUTHOR CONTRIBUTION:

Contribution	Harun N	Adnyana IK	Damayanti S	Kurniati NF
Concepts or ideas	x			x
Design	x			x
Definition of intellectual content		x	x	
Literature search	x			
Experimental studies	x			
Data acquisition	x			x
Data analysis	x	x	x	x
Statistical analysis	x	x	x	x
Manuscript preparation	x	x	x	x
Manuscript editing	x	x	x	x
Manuscript review	x	x	x	x

Citation Format: Harun N, Adnyana IK, Damayanti S, Kurniati NF (2025) Administration of oleuropein or hydroxytyrosol improves diabetic markers in obese mice. J Pharm Pharmacogn Res 13(2): 513–526. https://doi.org/10.56499/jppres24.2043_13.2.513

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Open Access: This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/ licenses/by/4.0/), which permits use, duplication, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.