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REVIEW ARTICLE

OLEUROPEIN AND HYDROXYTYROSOL AS ANTIDIABETICS: A REVIEW ON EXTRACTION METHOD, EFFECTIVENESS AND TOXICITY EFFECT

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

[1] Department of Pharmacology-Clinical Pharmacy, School of Pharmacy, Institut Teknologi Bandung, Jl. Ganesa No.10, Bandung 40132, Indonesia; harunnurhidayati@gmail.com, ketut@itb.ac.id, nfkurniati@itb.ac.id.
[2] Department of Pharmacochemistry, School of Pharmacy, Institut Teknologi Bandung, Jl. Ganesa No.10, Bandung 40132, Indonesia; sophi.damayanti@itb.ac.id.
[3] University Center of Excellence on Artificial Intelligence for Vision, Natural Language Processing & Big Data Analytics (U-CoE AI-VLB). Jalan Ganesa 10, Bandung 40132, Indonesia; sophi.damayanti@itb.ac.id.
[4] STIKes Muhammadiyah Ciamis, Jl. K.H.A. Dahlan No. 20 Ciamis, Indonesia; harunnurhidayati@gmail.com.

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Summary

Introduction: Olive (*Olea uropeae*) is a traditional plant containing oleuropein and hydroxytyrosol, which are useful and used empirically for treating diabetes mellitus.
Objective: To review the potential of oleuropein and hydroxytyrosol as an evidence base for diabetes potential treatment and safety.
Methods: This chapter summarizes several studies available on Pubmed and Google Scholar regarding the characteristic method and extraction method as well as the effectiveness and toxicity of oleuropein and hydroxytyrosol *in vitro* and *in vivo*.
Result: Oleuropein and hydroxytyrosol are effective antihyperglycemics for treating T2D. They can reduce body weight, basal glycemia, and insulin resistance by stopping the liver from making glucose and stopping the body from absorbing glucose. Several studies have shown that both isolates can control glycemic levels equivalent to free fatty acids and are safe to use.
Conclusion: Oleuropein and hydroxytyrosol are extracted by several methods and can be used as potential anti-diabetics with obesity risk factors. Evidence shows that both isolates are safe for both acute and chronic use.

Key words: Type 2 diabetes mellitus; obesity; oleuropein; hydroxytyrosol

INTRODUCTION

Insulin resistance, preceded by Langerhans beta cell dysfunction in the pancreas, is the main cause of diabetes mellitus (1). This pancreatic disorder results in hyperglycaemia, which if not treated, may lead to microvascular

✉ Institut Teknologi Bandung, School of Pharmacy, Department of Pharmacology-Clinical Pharmacy, Jl. Ganesa No.10, Bandung 40132, Indonesia
nfkurniati@itb.ac.id

or macrovascular disease. The cause of this disease is a combination of genetic, environmental and lifestyle factors (2), but several reports indicate that obesity is the most common trigger of insulin resistance in urban communities. The prevalence of type 2 diabetes (T2D) is higher in urban areas (10.8%) than in rural areas (7.2%), and in high-income areas (10.4%) than in low-income areas (4.0%). It is also known that 50.1% of people with diabetes are unaware that they have the disease (3). Research and Development of the Indonesian Ministry of Health stated that from 2013 to 2018, the prevalence of diabetes mellitus increased along with the prevalence of obesity, with a body mass index (BMI; body mass divided by square of body height) of 25 kg/m^2 as a risk factor (89%) (4).

The increase in body weight in obesity (5) is in line with a chronic increase in free fatty acids (FFA), which significantly affects the function of adipose tissue as a fat storage site. Infiltrating macrophages (6) cause adipose dysfunction, which in turn controls the release of hormones that cause inflammation (cytokines) (7). Inflammatory effects occur in adipose tissue and the liver, muscle, pancreas and brain because of high cytokine secretion. Fatty acids are released into the circulatory system and blood sugar increases because of disruption of cellular glucose uptake by GLUT4 due to malfunction of insulin receptors, resulting in toxicity in vessels (8).

The intestine's microbial community interacts with the body, leading to imbalances in metabolism. Diabetic's patients often have abnormal glucose and lipid metabolism, leading to obesity, hypertension, and insulin resistance. Low-fat diets, which increase dietary fiber and reduce lipid intake, are effective treatments. A study used high-throughput sequencing technology to analyze microbial community changes. Liu *et.al's* (1) study found that a low-fat diet effectively controlled blood glucose and BMI in diabetic's patients, while altering intestinal flora, with the structure gradually resembling healthy individuals after 6 months.

Managing lifestyle factors and taking oral hypoglycaemic drugs are still the main ways to treat diabetes (9). However, the majority of patients experience frequent relapses due to non-adherence to therapeutic strategies. Some reports say that hypoglycaemic effects are common with some oral antidiabetics, such as the sulfonylurea group, which can worsen the patient's condition. Meanwhile, the biguanide group is preferred in diabetic patients with obesity because it does not cause weight gain. However, long-term use can cause neuromusculoskeletal disorders due to decreased vitamin B12.

For treating T2D in overweight or obese people, a very low calorie diet (800 kcal/day) and ample physical activity (200–300 minutes/week) are recommended (10). In addition, good glycaemic control can prevent the development of microvascular complications in diabetics. Checking the HbA1C value every 3–6 months can be used for glycaemic monitoring, with a value of less than 7% indicating that glycaemic stability has been maintained (11). The American Diabetes Association (ADA) provided a guideline for the treatment of diabetes with obesity in 2015, stating that suitable glycaemic-lowering drugs in diabetic cases with obesity are sodium–glucose cotransporter-2 (SGLT2) inhibitors and GLP-1 receptor agonists (GLP-1 RA) (12), which may lead to an average weight loss of about 2–3 kg when used at approved doses (13). SGLT2 inhibitors (dapagliflozin) are thought to help obese people lose weight and lower their systolic blood pressure (14). On the other hand, GLP-1 RA (liraglutide) control blood sugar and weight loss better than a DPP-4 inhibitor and cause less hypoglycaemia (15).

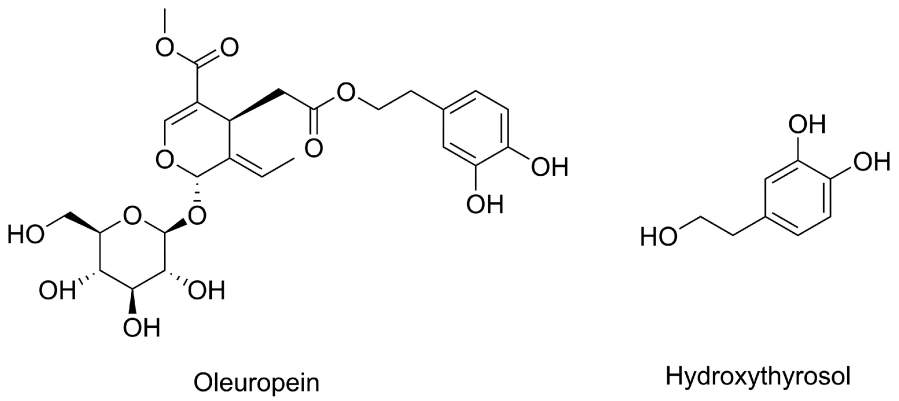

Figure 1. The structure of oleuropein and hydroxytyrosol of *Olea europeae*.

Oleuropein and hydroxytyrosol (Figure 1) are secondary metabolites in olive fruit and leaves (*Olea uropeae*), belonging to the *Oleaceae* family (16). Several studies have shown that olive leaf extract with both isolates has a wide range of biological and pharmacological properties, such as anti-obesity, anti-diabetic, anti-cancer, anti-inflammatory, antioxidant and heart-protecting effects. As pure isolates, both have also been shown to reduce blood sugar and lipid levels in diabetes and obesity, with a low risk of side effects in human and animal models.

Characteristics and extraction methods of oleuropein and hydroxytyrosol

Oleuropein is a phenolic secoiridoid with an oleoside framework, and its active metabolite is hydroxytyrosol (Figure 1). Secoiridoids are glycosides that are precursors to alkaloids (17). Oleuropein contains oleanolic acid, and has a characteristic bitter taste (18). When olives are ripe, the oleuropein breaks down into hydroxytyrosol (4-(2-hydroxyethyl)-1,2-benzenediol), which has an extra hydroxyl in the meta position in the aromatic ring (19). With regard to its chemical composition, oleuropein has two pi bonds, two esters, two acetals, one catechol and four hydroxyl hexoses (20). If the glycosidic bond is broken, glucose is released, and oleuropein aglycone, with the help of water, is quickly converted into the dialdehyde form of decarboxymethyl oleanolate acid (21). Numerous crucial parameters, such as pH, the type of organic acid and time, as well as combinations of these factors, related to the quantity of purification of hydroxytyrosol isolates, affect the hydrolysis of oleuropein to hydroxytyrosol (22). Various methods for the isolation of oleuropein (Table 1) and hydroxytyrosol (Table 2) have been developed, including standard and modified versions.

Table 1. Review isolation method of oleuropein compounds.

Method Extraction	Extraction solvent	Purity	Literature
Reflux-assisted adsorbed by a boric acid–functionalized resin	Ethyl acetate, ethanol, and water at the ratio of 10:1:9 (v/v)	68.30 ± 2.08 %	(2)
Soxhlet	Methanol	37.84 mg/g dried olive leaf	(3)
Ultrasound-assisted extraction (USAE)	Water–ethanol, 30:70 [v/v]	37.6 ± 0.6 mg/g dried olive leaf	(4)
Microwave–assisted extraction and frozen was frozen at –20 °C for a week	Water	15,6 mg/g freezing olive leaf	(5)
Homogenization-Assisted extraction	80% ethanol	4,3 mg of /100g dried olive leaf	(6)

Table 2. Review isolation method of hydroxytyrosol compounds.

Method Extraction	Method hydrolysis	Purity	Reference
Ethanol followed by distilled water	Oleuropein bioconversion with enzymatic three yeasts at room temperature	317 ± 14 mg/l, 210 ± 14 mg/l, and 149 ± 21 mg/l	(7)
Ultrasound-assisted solid–liquid extractions	Enzymatic reaction and using (Natural deep eutectic solvents (NADES) (LA:G (5:1) and CA:Gly:W (2:1:1))	74 and 87 ppm	(8)
Solid–liquid extraction using water–ethanol mixtures	After adjusting the pH, the hydrolyzed method (HCl) and purity of the lysate with ethyl acetate	Increased 60% or or 10–15 g of hydroxytyrosol per kg of dry leaves	(9)
Ultrasound extraction using methanol:water 80:20 (v/v)	Hydrolysis using strongly-acid aqueous steam, 10%HCl (v/v) at 100°C	92%	(10)

The Soxhlet, supercritical, ultrasonic and microwave methods are all types of extraction. In general, water, methanol and ethanol are used as polar solvents, and hexane and dichloromethane as nonpolar solvents. The results of dynamic desorption experiments using boric acid resin increased the purity of oleuropein by 5.55 fold, especially with nonpolar solvents (ethyl acetate:ethanol:water = 10:1:9) (23). In practical applications, the resin must absorb many target components while maintaining a high desorption rate to ensure effective component recovery.

The Soxhlet method can be used in heat extraction of chemicals that are soluble in both polar and nonpolar solvents. This method reduces production costs because it uses less solvent (24). Ultrasound-assisted extraction uses sound waves with frequencies higher than 20 kilohertz (kHz) to break up plant tissue and facilitate penetration of the solvent (25). Microwaves are a form of non-ionizing radiation with a frequency range of 300 MHz to 300 GHz, which can stimulate the rotational energy levels of molecules (26). This method's advantages include reduced solvent quantity and extraction time and increased yield (27). The investigation results in Table 1 show that these methods have good results and can be used to isolate oleuropein.

Acid hydrolysis has been investigated as a new cost-effective process for extracting phenolic compounds from olive leaves (28), and the resulting extract contained an optimal amount of hydroxytyrosol (29). However, the production of hydroxytyrosol remains problematic. One proposed solution has been application of an efficient enzymatic cascade using commercial hydrolytic enzyme preparations consisting of lipase, esterase, cellulase and xylanase (30), with use of glucosidase from yeast increasing the yield of oleuropein hydrolysis (17). This method is considered by some researchers to be more selective and environmentally friendly.

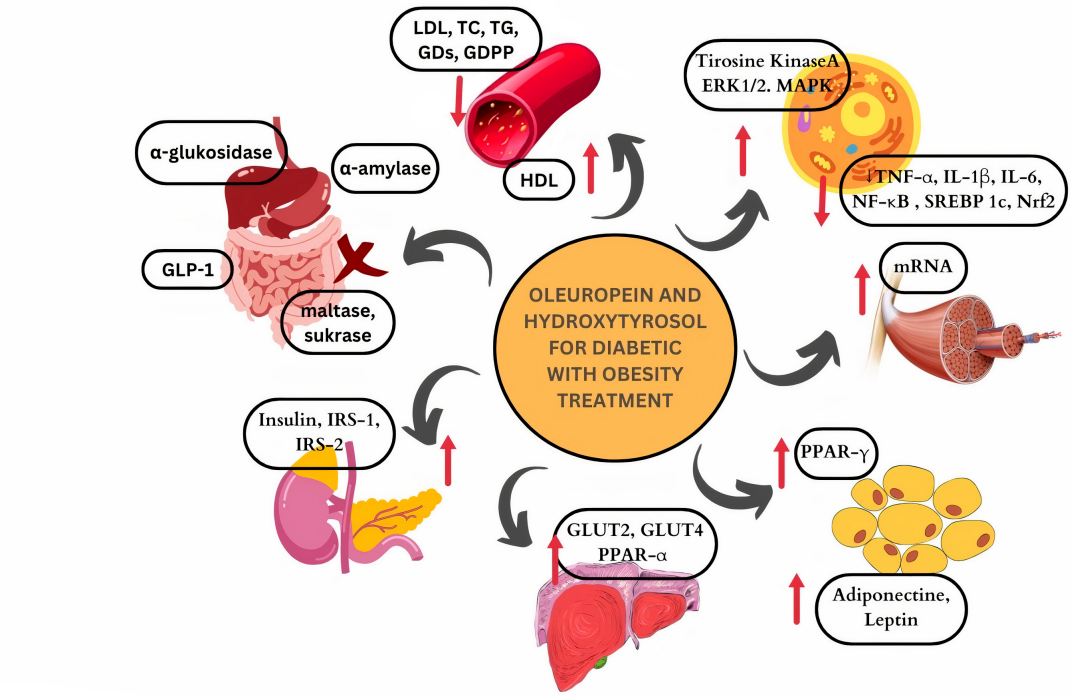

Figure 2. Oleuropein and hydroxytyrosol can be found as powders, extracts, and isolated compounds in olive leaves. These two chemicals may be used to treat diabetes, improve lipid profiles, and reduce inflammation caused by obesity. IRS-1: insulin receptor 1; IRS-2: insulin receptor 2; GLUT2: glucose transporter 2; GLUT4: glucose transporter 4; PPAR-γ: receptor peroxisome proliferator-activated receptor gamma; PPAR-α: receptor peroxisome profilerator-activated receptor alpha; mRNA: messenger ribonucleic acid; GLP-1: glucagon-like peptide-1; ERK 1/2: reticulum endoplasmic regulated kinase 1/2; MAPK: the nmitogen-activated protein kinase; NF-kB: nuclear factor kappa B; SREBP-1c: bsterol regulatory element-binding protein-1c; Nrf2: the nuclear factor erythroid 2-related factor 2; TNF α: tumor necrosis factor α; TG: triglycerides; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; IL-1β: interleukin 1 β; IL-6: interleukin 6.

Effectiveness of oleuropein and hydroxytyrosol as antidiabetics in diabetes and obesity

The main way to prevent hyperglycaemic effects in diabetic patients (Figure 2) (31) is to control their intake of carbohydrates. However, this is hard because of the abundance of processed foods. Postprandial hyperglycaemic events are more common in T2D because of the high gastric emptying rate (32); in addition, postprandial hyperglycaemia is more pronounced if a meal is taken at night (33). In the digestive process, the enzyme amylase,

found in saliva, breaks the bonds between the monomer sugar units of disaccharides, oligosaccharides and starch (34), and 30 minutes after the stomach contents empty into the intestine, glucose is absorbed as a monosaccharide. Digestive enzymes in the epithelial tissue of the small intestine, such as glucosidase, play an important role in this process, and inhibition of these enzymes is believed to slow down the breakdown of dietary polysaccharides into simple saccharides (35) in the gastrointestinal tract, thereby reducing postprandial hyperglycaemia. Inhibitors of glucosidase, such as the drugs acarbose, voglibose and miglitol, may be used for this purpose. Of note, functioning of the enzymes glucosidase and amylase is often abnormal in people with diabetes, which can be problematic if they do not control what they eat.

Table 3. Review Model intervention administer Oleuropein and Hydroxytyrosol.

Model	Oleuropein Concentration/Duration	Effect	Reference
	In vitro		
The inhibitory effect of the hydroxytyrosol and the oleuropein against α-amylase and α-glucosidase	Hydroxytyrosol 150 µM Oleuropein 400 µM	Inhibited α-glucosidase with an IC50 value of 150 µM	(11)
Giving olive oil containing hydroxytyrosol to rats that get exercise for 5 weeks	Administration of 0.31-10 mg/kg/BW	↑ Expression mRNA	(12)
Administration of hydroxytyrosol for 10 days to Human omental pre-adipocyte cells	Hydroxytyrosol 5, 10, 30, and 70 µg/mL	↑GATA2, GATA3, WNT3A, SFRP5, HES1, and SIRT1. Promoting adipogenesis such as LEP, FGF1, CCND1, and SREBF1	(13)
Administering a combination of fatty acid oleic acid (OA) and hydroxytyrosol to human and murine adipocytes	1-100 µmol/L OA, 0.1-20 µmol/L hydroxytyrosol or OA plus hydroxytyrosol	Activation of JNK, ↓ PPAR-γ, ↓ TNF-α	(14)
Cell viability test by incubating HepG2 cells using olive leaf extract for 24 hours	Administration of olive leaf extract 10 - 320 µg/mL	↑IRS-1, tyrosine kinase (TK), GLUT-2, and GLUT-4	(15)
	In vivo		
Twenty healthy subjects were randomly allocated in a cross-over design	20 mg oleuropein before lunch	Improves postprandial glycaemic	(16)
Separate randomized, controlled, crossover intervention study in healthy volunteers	Providing olive extract capsules (certain brands) containing 0.4 mg oleuropein/100 g oleuropein	OLE inhibits intestinal maltase, human sucrase, and GLUT 2	(17)
Administration of hydroxytyrosol to rats fed the HFD diet	Hydroxytyrosol 50 mg/kg/day	↓TNF-α, IL-1β, IL-6 repair IR through the JNK/IRS line	(18)
Postprandial glycemic and lipid profiles on olive oil administration to 25 healthy subjects cross design randomized in a Mediterranean-type diet without/with diet	10 g of olive oil	↓ GD Post pandrial, ↑insulin and improve lipid profile	(19)
Administration of a combination of olive leaves and glyburide to rats	Olive leaf extract 250-500 mg/kgBW and glyburide 5 mg/kgBW	↑Insulin receptor (INR), ↑GLUT2 and ↑PPAR-α in the liver	(20)
3-week supplementation in male Wistar rats with phenolic-rich olive leaf extract	Olive leaf extract orally 100 mg/kg/BW	↑Insulin, ↑PPAR-α, ↓PPAR-γ in adipose	(21)
Giving olive leaf powder to mice fed a high-fat and high-cholesterol for 9 weeks	Olive leaf powder 0.8% (w/w)	↓ TC and ↓ alanine aminotransferase, a lower concentration of hepatic cholesterol. ↑ Adiponectine	(22)
Mice HFD received hydroxytyrosol for 12 week	HT (daily doses of 5 mg kg /BW	Up-regulation of NF-κB and SREBP 1c, ↓ Nrf2, and ↓ PPAR-γ	(23)
Mice fed HFD olive leaf extract administered for 12 weeks	150 mg/kg/BW olive leaf extract	↓ Weight gain, visceral fat accumulation, and serum lipid composition	(24)

Although both oleuropein and hydroxytyrosol are more concentrated in olive leaf extract, the efficacy of oleuropein and hydroxytyrosol isolates as anti-diabetic candidates has been investigated in studies (Table 3). Investigations of inhibition of the enzymes amylase and glucosidase, with the use of both leaf extract (36) and olive oil preparations (37), have progressed to vitro testing. Both isolates have a relatively high level of alpha-glucosidase inhibition, compared with amylase inhibition. Further, maltase inhibition in the saliva is lower than in the intestines (38). As described below, studies have revealed that oleuropein and hydroxytyrosol can be used to reduce the effects of glucosidase and amylase. In clinical trials, the time at which the doses are administered can help counteract the effect of high blood sugar after a meal. After food has been ingested, hormones are released in the intestinal tract, stimulating vagal activity. Consumption of oleuropein before lunch was shown to lower blood sugar levels by stimulating GLP-1 (39). GLP-1, in addition to glucose-dependent insulinotropic polypeptide (GIP), is one of the incretin hormones, which are rapidly secreted within 10–20 minutes of food intake and stimulate the secretion of insulin. GLP-1 is secreted by L cells, which are abundant in the distal part of the small intestine. Its effect is to limit blood glucose after a meal and induce the effect of satiety, such that it reduces appetite (40) and, in the long run, can reduce body weight (41). In sum, because of its inhibition of glucosidase and stimulation of GLP-1, oleuropein may be useful as an antihyperglycaemic agent.

Another way of preventing hyperglycaemia in T2D is by suppressing hepatic glucose production; metformin, a biguanide, has been used for this purpose. Blood glucose level plays a significant role in the body's homeostasis. Early in the development of T2D, postprandial cell activity becomes disturbed, resulting in the abrupt loss of insulin sensitivity after meals. In addition, disruptive regulation of hepatic glucose production can result in adverse clinical outcomes (42). In the fasting state, the liver produces glucose through gluconeogenesis and glycogenolysis (43). With the help of GLUT4, GLUT2 and glucose absorption, glucose is able to enter hepatocytes from peripheral tissues, especially fat and muscles. A study showed that GLUT2 in *Xenopus* oocytes could be inhibited by oleuropein in olive leaf extract (38). Oleuropein was also able to increase the expression of GLUT2 and GLUT4 transport proteins up to 10 g/mL, activating insulin receptor phosphorylation by binding intracellular signal molecules, phosphatidylinositol 3-kinase (PI3K) and serine/threonine kinase PI3K-linked protein kinase B (Akt/PKB) (44).

On the other hand, hydroxytyrosol prevented the expression of GLUT4 protein in muscle by increasing mRNA expression in the endoplasmic reticulum (ER) (45). Insulin-regulated GLUT4 translocation on PI3K in adipose cells involves insulin receptor I (IRS-1) and insulin receptor II (IRS-2) capabilities, and hydroxytyrosol is thought to improve insulin signalling (46). In obese mice, its mechanism of action was via the JNK/IRS pathway (Serum 307) through IRS-1 phosphorylation and ACT deactivation (47). Sulfonylurea therapy, such as glibenclamide, has been used but has a tendency to increase body weight and induce severe hypoglycaemic events, which may occur without typical symptoms (48). In addition, several reports state that long-term use can worsen insulin secretion, characterised by hyperinsulinaemia (49). Wu *et al*. demonstrated that oleuropein and hydroxytyrosol could stimulate insulin secretion in beta cells by activating the extracellular signal-regulated kinase 1/2 (ERK 1/2) belonging to the MAPK signalling pathway. Mixing olive oil with food can increase insulin levels and provide a hypo-glycaemic effect (50). Mixing olive oil with food can increase insulin levels and provide a hypoglycaemic effect (51). Compared with glyburide, it was more effective in controlling fasting blood sugar levels and increasing insulin levels (52).

Obesity and diabetes may occur simultaneously or separately, depending on the amount of adiponectin secreted by adipocytes. Adiponectin acts directly on the liver, skeletal muscles and blood vessels. In obesity, an olive extract containing oleuropein and hydroxytyrosol can reduce body weight, basal glycemia and insulin resistance, as well as improve plasma lipid profiles by increasing expression of PPAR-γ (53, 54), adiponectin (55) and leptin, especially in adipose tissue (56). PPAR-γ is responsible for adipose differentiation and lipid metabolism (57). Thiazolidinediones, such as rosiglitazone and pioglitazone, are a synthetic form of PPAR-γ, and can increase insulin sensitivity in line with increased adiponectin. It was reported that hydroxytyrosol in olive oil could modulate adipocyte gene expression profiles through a mechanism that involved the reduction of oxidative stress and inhibition of NF-kB, SREBP-1c and Nrf2 (58), occurring as a result of cardiometabolic complications in obesity due to inflammation (59). Further, suppressed activity of adiponectin and PPAR-γ by activation of the JNK pathway by cytokine TNF α could be controlled by hydroxytyrosol (60). Recent studies demonstrated that a long-term high-fat diet (HFD) increases TG, FFA, glucose, TC, HDL and LDL, and decreases cholesterol levels, leading

to an imbalance in serum lipid composition. Oleuropein effectively lowers serum TG, FFA, glucose and TC levels, and stimulates adipogenesis in pre-adipocytes (and development into mature adipocytes) and thermogenesis in visceral adipose tissue (61).

Toxicity effect of oleuropein and hydroxytyrosol

Several studies have stated that oleuropein and hydroxytyrosol are safe to use, and that weight loss may occur as a side effect, especially with hydroxytyrosol. Further, several studies have shown that olive leaf extract is generally safe and unlikely harmful. Camila *et al*. reported that acute and subacute use of olive leaf ethanol extract at doses of 2000 mg/kg (single) and 100, 200 and 400 mg/kg (28 days) was safe to use (62). Repeated use over 90 days at doses of 360, 600 and 1000 mg/kg/day did not have a genotoxic effect. Likewise, the administration of pure aqueous olive oil extract (VOO), rich in hydroxytyrosol, acutely and chronically at a dose of 100–2000 mg/kg is safe and can be used as a functional food (63).

Oleuropein is an antioxidant, and was shown to progressively inhibit proliferation of cancer cells and the migration of advanced tumour cell lines for 9–12 days (64). Further, olive leaf extract, which is high in oleuropein, protected the liver by reducing the damage caused by oxidative stress from cadmium poisoning (65). Hydroxytyrosol has also shown anti-cancer effects, especially in human hepatocellular carcinoma (HCC) cells, by inhibiting proliferation, inducing G2/M cell cycle arrest and inducing apoptosis *in vitro* (66).

Pharmacokinetic profiles of oleuropein and hydroxytyrosol

There is no research has been found that explains the pharmacokinetics of oleuropein. Meanwhile, in a study conducted by D'Angelo (1), regarding the pharmacokinetics of hydroxytyrosol found in intestinal and renal excretion, ninety percent of the radioactivity given is found in urine taken up to five hours after the injection, showing that renal excretion is the preferred method for getting rid of hydroxytyrosol. Additionally, 5 minutes after injection, 9% of the injected radioactivity can be found in the gastrointestinal tract. During the other time intervals, this value is nearly steady before dropping to 2.5% at 5 hours, and 300 minutes after treatment, 3.2% of the administered dose is retrieved in the stool.

CONCLUSION

Oleuropein and hydroxytyrosol are suitable treatments for T2D because they lower blood sugar by reducing glucose production by the liver and decreasing glucose absorption by the body. Both isolates can reduce body weight, basal glycaemia and insulin resistance, and improve plasma lipid profiles by increasing the expression of PPAR-γ. Furthermore, oleuropein and hydroxytyrosol are safe to use.

Oleuropein and hydroxytyrosol's effectiveness in treating individuals with diabetes mellitus has not been studied; instead, research has only focused on *in vivo* and preclinical investigations. Oleuropein's pharmacokinetics have not been discovered by research. In order to ensure that further research on this subject is required.

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AUTHOR CONTRIBUTION

Conception/Design of study:	Neng Fisheri K/ Sophi Damayanti/ I Ketut Adyana
Data Acquisition:	Nuhidayati Harun
Data Analysis/Interpretation:	Neng Fisheri K / Nuhidayati Harun
Drafting Manuscript:	Nuhidayati Harun
Critical Revision of Manuscript:	Neng Fisheri K/ Sophi Damayanti/ I Ketut Adyana
Final Approval and Accountability:	Neng Fisheri K/ Sophi Damayanti

Technical or Material Support:	Nuhidayati Harun
Supervision:	I Ketut Adyana

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CONFLICT OF INTEREST

The authors state that there are no conflicts of interest regarding the publication of this article.

ADHERENCE TO ETHICAL STANDARDS

This article does not contain any studies involving animals performed by any of the authors. This article does not contain any studies involving human participants performed by any of the authors.

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