Research Journal of Pharmacognosy (RJP) 7(2), 2020: 81-92
Received: 29 Sep 2019
Accepted: 7 Jan 2020
Published online: 14 Jan 2020
DOI: 10.22127/rjp.2020.203451.1522

Review article

Internal Septum of Walnut Kernel: a Neglected Functional Food

Zahra Ghiravani[1], Mohammadmehdi Hassanzadeh-Taheri[2], Mahsa Hassanzadeh-Taheri[3], Mehran Hosseini[2*]

[1]Department of Physiology, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran.
[2]Cellular and Molecular Research Center, Department of Anatomical Sciences, Birjand University of Medical Sciences, Birjand, Iran.
[3]Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran.

Abstract

Walnut (*Juglans regia* L.) is a well-known member of the Juglandaceae family and its kernel is widely consumed around the world for both unique nutritional characteristics and health-related benefits. Even though several studies investigated the composition and biological activities of different parts of the walnut tree, the internal septum of the walnut kernel is less evaluated. In the last two decades, some studies investigated phytochemical and pharmacological aspects of the walnut septum. Their results showed a wide range of biological properties along with safety of walnut septum constituents convincing us to shift our view to walnut septum as a useless by-product to a natural herbal material with valuable properties. The purpose of this review was to summarize the currently available investigations on chemical composition and biological activities of the walnut septum. Several phytochemical studies showed that the walnut septum is a rich source of secondary metabolites like polyphenols are which estimated to be responsible for its high antioxidant property. Further experimental studies confirmed many biological activities of this by-product such as radical scavenging, food preservative, antibacterial, antitumor, hypoglycemic, hypolipidemic, and hepatorenal protective properties.

Keywords: Juglandaceae; *Juglans regia*; walnut

Citation: Ghiravani Z, Hassanzadeh-Taheri M, Hassanzadeh-Taheri M, Hosseini M. Internal septum of walnut kernel: a neglected functional food. Res J Pharmacogn. 2020; 7(2): 81-92.

Introduction

Persian or common walnut tree (*Juglans regia* L.) is a widespread species in the world and its kernel has been consumed for a long time. Walnut tree is commonly cultivated for nut production and it is one of the oldest food sources known dating back to 7000 BC. Since 2012, walnut world production exceeded three million tons, mostly provided by China, the USA, and Iran. One of the unique features of walnut is its compositions. Most of others nuts contain a high level of monounsaturated fatty acids while the walnut kernel is rich in polyunsaturated fatty acids such as linoleic (n-3) and linolenic (n-6) acids [1]. Walnut not only for its high nutritional value but also due to many health benefits has always been of great interest.

Several clinical studies demonstrated that walnut fruit posed high antioxidant activity [2], anti-inflammatory potential [3], glucose and lipid lowering efficiency [4-6], and antidepressant effects [7,8]. Besides, walnut consumption by decreasing low density lipoprotein cholesterol, blood pressure and also by improving endothelial function increases cholesterol efflux and consequently, confers many benefits on cardiovascular disease risk factors [9].

As the walnut tree has a long history of medicinal use, its other parts such as green walnuts, flowers,

*Corresponding author: mehranhosseiny@bums.ac.ir, Mehranhosseiny@yahoo.co.in

bark, shells and leaves have been employed in different traditional medicine systems [10]. Some studies investigated specific aspects of biologic activities of walnut parts. For example, methanolic extract of walnut flower showed remarkable antihypoxic, anti-inflammatory, antioxidant and antidepressant activities [11]. The bark extract of the walnut tree (prepared using different solvents) exhibited antimicrobial activity against various bacteria [12,13]. Moreover, walnut shells have been considered as an important bio-resource for pyroligneous acid with antioxidant, antimicrobial, and plant growth stimulating properties [14]. Several experimental and clinical studies have evaluated walnut leaves in diabetic animals or patients [15-21].

The other part of walnut which has been prescribed in the traditional Iranian and Chinese medicine the is internal septa of the fruits, the dry wood diaphragm tissue that grows inside the walnut (figure 1), which has gained considerable attention in folk prescriptions to treat various illnesses, such as diabetes, insomnia, diarrhea, kidney diseases and reproductive disorders [10,22]. Despite the fact that several beneficial effects of this walnut by-product have been mentioned in the traditional medicine literatures, so far, few scientific studies have taken it into account. We have previously reported that ethanol extract of the internal septum of walnut fruit exhibited profound hypoglycemic activity in diabetic rats and also effectively prevented the progress of diabetic nephropathy in hyperglycemic animals [23,24]. Over the last few years, some studies have focused on biological activities of walnut fruit septum and their results provide further support for the need to increase the level of research on this topic.

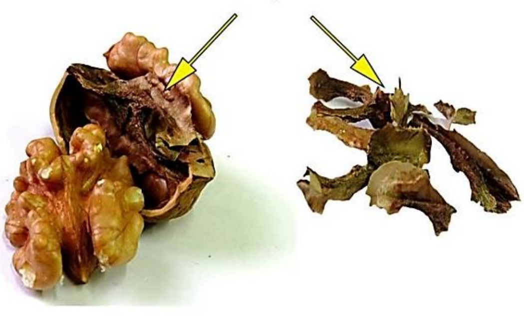

Figure 1. Walnut septum (photo taken by the authors)

Methods

The purpose of this review was to summarize currently available investigations relating to biological activities of the internal septum of walnut fruit. We have searched the topics "Diaphragma *Juglandis* fructus", *"Juglans regia* septum", "septa of the fruit of *Juglans regia*", "kernel septum membranes", "walnut septum" and "walnut membrane septum" in the two popular electronic databases including PubMed and Google Scholar. Datasets were outlined from 1980 to 2019.

In this paper, we have collected all the articles related to the internal septum of walnut fruit from tow popular databases including PubMed and Google Scholar.

Results and Discussion

Traditional background of internal septum use

Through the literature, several names for internal septum of walnut fruit have been used. Diaphragma *Juglandis* fructus, *Juglans regia* septum, septa of the fruit of *Juglans regia*, Kernel septum membranes, walnut septum and walnut membrane septum are the names that have been used in various sources that refer to this part of walnut fruit [22-36].

Some of the early published Iranian articles have mentioned that walnut septum has been used in Iranian traditional medicine for its anti-diabetic potential and most of them referred to the book titled "Knowledge of Plants" written by Hossein Mirheidar and book titled "Medicinal Plants" written by Ali Zargari [27,28,37]. However, considering these books we could not find any description regarding walnut septum and only anti-diabetic potential of walnut leaves had been claimed [36,38]. Anyway, there are numerous articles directly or indirectly referring these books [23,24,33,34,39].

In Chinese traditional medicine, the walnut septum had been used to treat different illnesses such as insomnia, renal diseases, enuresis, gonorrhea, and reproductive disorders [22,40].

Phytochemical constituent

To the best of our knowledge, for the first time, Bezhuashvili and Kurashvili evaluated the chemical composition of the walnut septum. They reported that the septa of *Juglans regia* fruit was a rich raw material containing several compounds including lipids, low-molecular-mass phenolic carboxylic acids, phenolic aldehydes, catechins, proanthocyanidins, oligomeric and polymeric phenolic substances. They highlighted the high content of polyphenol compounds which were found in the walnut septum [26]. Wang et al. evaluated chemical constituents of ethyl acetate extract of the walnut septum by preparative high-performance liquid chromatography and high-speed counter-current chromatography. They reported 14 compounds isolated from the walnut septum. The isolated compounds were gallic acid, dihydrophaseic acid, blumenol B, quercitrin, protocatechuic acid, taxifolin-3-O-α-L-arabinofuranoside, ρ-hydroxybenzoic acid, vanillic acid, ethyl gallate, dihydroquercetin, (4s)-4-hydroxy-1-tetralone, (+)-dehydrovomifoliol, (6R,9S)-9-hydroxymegastigman-4-en-3-one, and (6R,9S)-9-hydroxymegastigman-4-en-3-one [35].

In a study conducted by Popovici, total phenols content and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of ethanol extracts of walnut leaves, husk and the membrane septum were investigated. Surprisingly, the total phenol content of the internal septum was markedly higher than both leaves and husk extracts and its antioxidant activity was superior to only husk extract [41].

In a comprehensive study, Rusu and colleagues investigated the walnut septum phytochemical constituents to optimize the suitable extraction method for obtaining high content of biologically active compounds from the plant material. They also aimed to investigate the phytochemical profile of walnut septum extract using HPLC-MS/MS (high-performance liquid chromatography-tandem mass spectrometry). In addition, they tested the biological potential of the richest polyphenol extract of the walnut septum by FRAP (ferric reducing antioxidant power) and DPPH antioxidant capacity and the inhibition of tyrosinase methods. Accordingly, the best solvent and extraction method in which the highest content of biologically active compounds was obtained were acetone and Ultera-Turrax extraction method, respectively. However, the content of total phenolics, total flavonoids, condensed tannins and total antioxidant activity in extracts which were prepared via maceration method (ethanol used as the solvent) were considerable [34]. Another important activity of walnut septum which was firstly reported by Rusu and co-workers was its amazing tyrosinase inhibitory activity which was 129.98±3.03 mg kojic acid equivalent per gram of the dry lyophilized extract (mg KAE/g dw extract). Tyrosinase is the key enzyme involving in the production of melanin. It is well documented that tyrosinase plays a pivotal role in dopamine neurotoxicity as well as in contribution to the neurodegenerative Parkinson’s disease. Therefore, inhibitors of tyrosinase may alleviate skin hyperpigmentation, wrinkle formation and also inhibit neurodegeneration and slow down aging [42]. To highlight the high level of tyrosinase inhibitory activity of the walnut septum extract we can compare its activity with other medicinal plants that are known to have valuable tyrosinase inhibitory activity. For example, *Achillea sivasica* exhibited tyrosinase inhibitory activity which varied from 2.9 to 6.2 mg KAE/g extract [42], and *Lycium barbarum* L. leaves showed the potential 12.84-16.81mg KAE/g extract [43].

In a study conducted by Liu et al. polyphenol profile of the walnut septum was explored. They found 75 individual phenolic compounds belonging to flavonoids, tannins and phenolic acids. Among them, quercetin-3-O-glucoside, quercetin-rhamnose-hexoside, kaempferol-rhamnoside, and two isomers of quercetin-rhamnoside were identified for the first time in walnut. They also reported that the total polyphenol content of walnut septum ethanol extract was 122.78± 2.55 mg gallic acid equivalent (GAE)/g dry weight of walnut septum (after taking into account the extraction yield it may be around 45 mg GAE/g dry weight) [30]. We previously found that the polyphenol content of walnut septum ethanol extract was 21.64 ± 1.44 mg GAE/g dw [23]. Rusu et al. also found that the total phenol content of walnut septum was 67.03 ± 9.76 mg GAE/g dw in Ultra-Turrax extraction method and 31.27 ± 5.24 mg GAE/g dw in maceration method [34]. In a study conducted by Akbari et al., phenolic compounds of different parts of walnut fruit including hull, shell, pellicle (brown skin covering the kernel) and kernels were investigated. They reported the mean total phenolic content to be 24.68 ± 4.28mg GAE/g, 18.04± 4.20 mg GAE/g, 52.05± 1.27 mg GAE/g and 1.45±0.12 mg GAE/g for the hull, shell, pellicle and kernel, respectively [44]. In a comprehensive review article by Jahanban-Esfahalan and co-authors, it has been mentioned that the methanol extract of walnut leaves contained the highest polyphenol content (94.39 ± 5.63 mg GAEs/g extract) as compared to aqueous extract (27.92 ± 1.40 mg GAEs/g extract) [45]. Johnson et al. investigated total phenolics content of 11 highly pigmented medicinal plants collected from Kenya, Nigeria, and the USA. They found that total polyphenol content of those plant were 55.14 mg GAEs/g (*Prunus africana)*, 42.11 mg GAEs/g *(Acacia tortili)*, 17.54 mg GAEs/g *(Khaya grandifoliola)*, 17.23 mg GAEs/g *(Curcuma longa)*, 14.9 mg GAEs/g (*Vernonia amygdalina)*,14.03 mg GAEs/g (*Russelia equisetiformis)*, 7.96 mg GAEs/g *(Calendula officinalis)*, 7.09 mg GAEs/g (*Phragmites australis*), 6.69 mg GAEs/g *(Rauwolfia vomitoria*), 6.21 mg GAEs/g (*Phragmites australis)* (industrial), and 5.6 mg GAEs/g (*Cnidoscolus aconitifolius)*. [46]. The amounts of phenolic compounds in different parts of walnuts, especially its septum is considerable compared to other famous medicinal plants such as *Curcuma longa*; however, it is difficult to compare the results of these studies because different extraction methods have been used.

Recently, Hu et al. published an article in which phenolic composition and nutritional attributes of the internal septum of the walnut kernel were investigated. They reported that dietary fiber was the main constituent found in the internal septum (73.66 ± 0.73 g/100 g dw). The other most prevalent compounds were total phenolic compounds (4.84 ± 0.01 g/100g dw), the crude protein (2.02 ± 0.11 g/100g dw.), crude ash (2.68±0.08 g/100g dw), crude lipid (2.49±0.36 g/100g dw) and total sugar (0.13 ± 0.002 g/100g dw). Also they found that the internal septum of walnut fruit possessed several mineral compounds such as K (9362.87 ± 21.90 mg/kg dw), Na (479.17 ± 6.29 mg/kg dw), Ca (3526.37 ± 15.71 mg/kg dw), Mg (636.80 ± 12.94 mg/kg dw), Fe (36.93 ± 0.90 mg/kg dw) and few amount of elements such as Cu, Zn, Mn, and Se [29]. They identified twenty-six fatty acids in the walnut septum (13 saturated and 13 unsaturated). Among saturated fatty acids palmitic acid (C16:0) (700.55 ± 8.80 mg/kg dw) and stearic acid (C21:0) (143.96 ± 4.02 mg/kg dw) were highest while linoleic acid (C18:3N6) (1314.06 ± 10.71 mg/kg dw) and oleic acid (C18:1N9C) (549.92 ± 18.98 mg/kg dw) accounted for the most unsaturated fatty acids. Amino acid profile of walnut septum exhibited six essential amino acids and eleven non-essential amino acids. Lysine, an essential amino acid and glutamate, a non-essential amino acid were the most abundant in the walnut septum (2.41 ± 0.02 and 3.86 ± 0.02 mg/g dw, respectively). The findings of these researches revealed that there were nine types of monosaccharide compounds in walnut septum mainly trehalose (223.76 ± 10.01 mg/g dw), xylose (44.79 ± 2.08mg/g dw) and mannose (11.45 ± 0.52 mg/g dw). In the mentioned study, 14 phenolic compounds in the walnut septum including gallic acid, phthalic acid, catechin, vanillin, ethyl gallate, dihydroquercetin, kaempferol, taxifolin-3-o-α-L-arabinofuranoside, quercetin-3-rhamnoside, quercetin-3-o(4-o-acetyl)-α-L-rhamnopyranoside, blumenol B, propyl gallate and vanillic acid were also reported [29].

According to the results of the aforementioned studies, the phytochemical profile of walnut septum warrants the need of further research in order to deeply evaluate its probable pharmacological activities.

Pharmacological activities

Some studies investigated biological activities of walnut septum and their results have been summarized in table 1. We categorized as well as discussed these results in the following lines.

Safety

Ravanbakhsh et al. investigated acute and sub-acute oral toxicity of walnut septum methanol extract on female rats. In the acute phase, oral doses of 10-5000 mg/kg extract were tested on rats and in sub-acute assay daily dose of 1000 mg/kg of walnut septum extract was administrated to rats for 28 consecutive days. It was found that the extract did not produce any toxic sign or death and therefore the medium lethal dose of walnut septum extract must be higher than the tested dose (5000 mg/kg). The results of the sub-acute test in which a high dose of the extract (1000 mg/kg) was investigated for 28 days showed that significant increase in liver malondialdehyde concentration, and significant reduction in serum urea, and kidney xanthine dehydrogenase were observed. Generally, not only the extract had no important acute and sub-acute adverse effects, but also, it may have beneficial effects mainly for kidney function [33].

Table 1. Pharmacological investigations of internal septum of walnut kernel

Pharmacological activity	Sample	Result	Ref.
Safety	Methanolic extract (100-5000 mg/kg in acute test and 1000mg/kg in sub-acute test)	No acute and sub-acute toxicity In sub-acute test: hepatic malondialdehyde↑, serum urea↓, renal xanthine dehydrogenase↓ and renal aldehyde oxidase↑	[33]
Antioxidant effect	Different extracts prepared with different extraction methods (Ultra-turrax or maceration) as well as solvents (different percentages of acetone or ethanol)	- Antioxidant activity in the ABST method (174.28±9.68 mg TE/g dw walnut septum). - Radical scavenging activity in DPPH test (255.89 mg TE/g dw walnut septum) - Reducing power in FRAP test (400.97 mg TE/g dw walnut septum)	[34]
	Polysaccharides from internal septum of walnut fruit	- Radical scavenging activity increased in a dose dependent manner in the DPPH, ABST, hydroxyl radical scavenging, and FRAP methods.	[32]
Anti-bacterial effect	Polysaccharides from internal septum of walnut fruit	- Dose dependent (0.2-1.2 mg/mL) antibacterial activity against Gram-negative and also Gram-positive strains	[32]
	Adding ethanol extract to traditional butter at concentrations of 0.05%, 0.1% and 0.5%	- Butters with 0.5% walnut septum extract showed less bacterial growth (except *Staphylococcus aureus*) and more oxidative stability than untreated butters	[60]
Anti-tumor effect	polysaccharides from the internal septum of walnut fruit (8-500 µg/mL)	- Dose dependently suppressed the proliferation of human hepatocellular carcinoma cell line (HepG-2) and human gastric carcinoma cell line (BGC-823)	[65]
Anti-diabetic effect	Single aqueous extract (100-1000 mg/kg oral) in diabetic rats	- No hypoglycemic activity	[37]
	Single dose of ethanol extract (200-400 mg/kg oral) in diabetic rats after glucose loading and 28 days repeated administration	- Dose dependently decreased hyperglycemia after 3g glucose loading - Markedly decreased blood glucose concentrations in repeated administration	[23]
	28 days oral administration of aqueous extract of walnut septum (200-800 mg/kg) in diabetic mice	- Decreased blood glucose only at the maximum dose (800 mg/kg)	[27]
	14 days oral administration of ethanol extract of walnut septum (100-1000 mg/kg) in diabetic rats	- Decreased blood glucose at 200-1000 mg/kg doses.	[28]
	15 days oral aqueous extract of the walnut septum (200-400 mg/kg) in diabetic mice	- Significantly decreased blood glucose in both 200 and 400 mg/kg doses	[39]
	pure polysaccharide fraction from the ethanol extract of walnut septum (50-200 mg/kg oral for 10 days) in diabetic mice	- Dose dependently decreased blood glucose levels - Dose dependently showed α-amylase and α-glucosidase activities (0.125-4 mg/mL)	[31]
	Ethanol extract (200-400 mg/kg) 28 days oral administration in diabetic rats	- Blood glucose ↓, Blood urea↓, Blood creatinine↓, urine total protein↓, kidney histological alterations↓	[24]

↓: decrease; ↑: increase

Antioxidant activity

Several endogenous and exogenous processes produce reactive oxygen and nitrogen species (RONS).

In the normal condition, reactive oxygen species are neutralized by the antioxidant defense system. However, oxidative damage occurs when the balance between the production of RONS and the ability of the antioxidant defense system is disturbed. Oxidative damage is involved in the pathogenesis of many chronic diseases such as cardiovascular, neurodegenerative and chronic kidney diseases, and also various cancers [47-51]. There are debates on whether antioxidant-rich food or supplements have beneficial effects on prevention of diseases, but numerous experimental studies have shown that several natural products through the antioxidant activity could inhibit the progression of many diseases and their complications [39,43,52-56]. Rusu et al. evaluated the antioxidant activity of walnut septum extract using ABST (2,2′-azino-bis-3-

ethylbenzthiazoline-6-sulphonic acid), DPPH and FRAP methods and their results showed good antiradical effects. The highest antioxidant activity was reported for the 75% acetone extract of the walnut septum with 174.28 ± 8.22 mg trolox equivalents (TE) per gram of the dried extract in the ABST method, 255.89 mg TE/g in DPPH method and 400.97 mg TE/g extract in FRAP test [34]. In a review article written by Neslihan GöncüoğluTaş and VuralGökmen the antioxidant activity of kernels and skins of almond, Brazil nut, cashew, hazelnut, macadamia nut, pecan, pine nut, pistachio, and walnut were summarized. Accordingly, their antioxidant activities were reported as follow: peanut (*Arachis hypogaea* L.) (10.76-28.86 mmol TE/g), pecan nut (*Carya illinoinensis*) (78.3 mg TE/g dw), pistachio (*Pistachia vera* L.) (8.05 mmol TE/g fresh w), walnut kernel (*Juglans regia* L.) (83.46-93.08%), almond skin (*Prunus dulcis*) (69.52 mmol TE/g fw), and hazelnut skin (309-1375 mmol TE/g fw) [57]. Meng et al. also reported that the half-maximal inhibitory concentration (IC_{50}) of walnut septum extract was 1.06 mg/mL, which was much lower than that of the polysaccharide from potato peels (11.57 mg/mL) and higher than that of ascorbic acid (0.077mg/mL) [32]. Although it is impossible to compare these values due to the use of different methods as well as different ways of expressing the antioxidant activity, in general, all shows that walnut septum has high antioxidant activity. High phenolic compounds can be responsible for the high antioxidant activity of walnut septum. Wang and colleagues found that walnut septum through inhibiting nitric oxide production poses anti-inflammatory activity. They attributed this activity due to the presence of gallic acid, ethyl gallate and (+)-dehydrovomifoliol on walnut septum [35].

Antibacterial and preservative potential

Numerous herbal extracts with antimicrobial properties have been reported. Since ancient time, walnut was widely used in traditional medicine because of having different potentials such as antimicrobial, anti-inflammatory, and anti-atherogenic effects [25]. There is evidence showing that walnut leaves extract selectively inhibited the growth of Gram-positive bacteria, [58]. Meng and co-workers in an experimental study found that water-soluble polysaccharide fraction isolated from walnut septum exhibited a significant as well as dose dependent (0.2-1.2 mg/mL) antibacterial activity against two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and also two Gram-positive strains (*Staphylococcus aureus* and *Listeria monocytogenes*) [32].

One of the applications of antioxidant compounds is their protective role against oxidative damage which occurs during the food production and storage process. In this regard, using synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene is common in food industries. However, nowadays, their usage has been limited due to their toxicity; hence, searching for natural antioxidant has increased [59]. Mehdizadeh et al. assessed the antimicrobial and antioxidant effects of walnut kernel septum ethanol extract on the shelf life of traditional butter. They added the ethanol extract at the concentrations of 0.05%, 0.1% and 0.5% to the traditional butter immediately after production. They found that after 90 days of production, the 0.5% concentration was more effective for inhibiting the growth of bacteria (except for *Staphylococcus aureus*) and oxidative stability of traditional butter. They concluded that the walnut septum extract could be an excellent natural antimicrobial and antioxidant agent which can be used in butter [60].

Antitumor activity

Cancer is a public health problem that affects many people worldwide. Despite the great advances in cancer diagnosis and treatment, the need for new, more effective and low complicated strategies in the prevention and treatment of this disease is felt. For many years medicinal plants have been used and are still in use in many societies for a variety of therapeutic purposes. Many researchers investigate the claimed potential properties and uses of different herbal extracts in different diseases including cancer [61]. There is evidence showing the walnut leaves extract exhibited anti-proliferative effects on several cancerous cell lines including prostate cancer (PC-3), breast adenocarcinoma (MCF-7), colon carcinoma (HCT-15 and Caco-2), cervical carcinoma (HeLa), hepatocellular carcinoma (HepG-2), and renal cell carcinoma (A-498 and 769-P) [62-64].

In a recent study conducted by Meng et al., the anti-proliferative effect of polysaccharides from the internal septum of walnut fruit has been investigated on human hepatocellular carcinoma cell line (HepG-2) and human gastric carcinoma cell line (BGC-823) based on MTT assay. They found that the polysaccharides markedly suppressed the proliferation of HepG-2 and BGC-823 cell lines in a dose-dependent manner (8-500 µg/mL) and at the maximum concentration the antitumor activity was 67.39% and 61.25% in HepG-2 and BGC-823 cell lines, respectively [65].

Anti-diabetic activity

One of the most investigated properties of medicinal plants, especially those with high levels of phenolic compounds, is the hypoglycemic potential. There are several scientific evidences showing hypoglycemic and anti-diabetic activities of various medicinal plants [66]. As mentioned before, walnut especially its leaf and shell, has been used in traditional medicine for the treatment of diabetes [36,38]. There are several experimental studies that have investigated aqueous, methanol or ethanol extracts of walnut leaf in diabetic animals and almost in most of them, hypoglycemic activity has been reported [15,19,21,67-69]. However, clinical studies show contradictory results. In 2014, Hosseini et al reported that 2-month oral consumption of aqueous extract of walnut leaves tablet (200 mg two times per day) significantly reduced blood sugar (from 165 to 144 mg/dL) and increased insulin levels in type 2 diabetes patients (n=30) [16]. On the other hand, the results of a double-blind clinical study in which 40 type two diabetic patients were treated with 200 mg ethanol extract of walnut leaf (n=20) or placebo (n=20) for two-month showed no significant effects on blood glucose (191.7±36.6 mg/dL vs. 179.5±49.0 mg/dL, *p*=0.309) and insulin levels (6.4 ± 3.7 vs. 7.3 ± 3.9 , *p*=0.44) [70].

Sarahroodi et al. investigated the hypoglycemic potential of the walnut septum in diabetic rats. They performed oral glucose tolerance test on normal and diabetic rats which were orally treated with a single dose of 50-750 mg/kg (normal animals) or 100-1000 mg/kg (diabetic animals) aqueous extract of the walnut septum. They couldnt find any hypoglycemic activity neither in normal nor in diabetic animals treated with the extract [37]. However, our previous investigation showed that while oral glucose loading (3 g/kg) significantly increased blood glucose levels in diabetic rats with a peak at 30 min, ethanol extract of walnut septum dramatically decreased glucose levels in 90 min and 120 min at concentrations of 400 mg/kg and 200 mg/kg respectively. We also found that 28 days daily treatment of diabetic rats with ethanol extract of walnut fruit at doses of 200-400 mg/kg exhibited strong hypoglycemic and hypolipidemic activities in diabetic rats [23]. Similarly, Dehghani and colleagues found that 4-week oral treatment of diabetic mice with four different doses of aqueous extract of walnut septum (200-800 mg/kg) could decrease glucose levels only at the maximum dose [27]. It seems that the ethanol extract of walnut septum exhibited more considerable hypoglycemic potential. Hajikhani et al. also reported that 14 days of oral administration of walnut septum ethanol extract (100-1000 mg/kg) resulted in significant blood glucose reduction in diabetic rats [28]. Zangeneh et al. found that 15 days oral administration of aqueous extract of the walnut septum (200-400 mg/kg) could ameliorate blood glucose levels and also inhibit hepatic damage progress in diabetic mice [39]. The recently reported results of Meng and co-workers study showed that DJP-2, a pure polysaccharide fraction from the ethanol extract of walnut septum had clear in vivo and in vitro hypoglycemic activities and antiglycation potential [31]. In our previous study, we found that the ethanol extract of walnut septum significantly reduced blood glucose, urine total protein, blood urea nitrogen, and creatinine concentrations in a dose independent manner. Beside, 4-week administration could ameliorate kidney histological alterations such as mesangial matrix expansion and glomerular tuft-to-capsule adhesion in diabetic rats [24].

Conclusion

In summary, phytochemical evaluation of walnut septum confirmed the presence of high amount of phenolic compounds and desirable radical scavenging and tyrosinase inhibitory activities. Moreover, some experimental works have demonstrated its antibacterial, anti-tumor, anti-diabetic and food preservative potentials. Taken together, these properties are opening up new possibilities regarding beneficial effects of walnut septum on neurodegenerative diseases (like Parkinson disease) and skin disorders (hyper pigmentation due to melanin as well as skin wrinkle formation). Nevertheless, further studies are required to investigate other probable pharmacological activities of walnut septum.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Zahra Ghiravani and Mohammadmahdi Hasanzadeh-Taheri searched and summarized articles. Mahsa Hassanzadeh-Taheri searched Persian traditional books and contributed to writing the manuscript. Mehran Hosseini wrote the first draft of the manuscript. All authors read and approved the final version of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Maguire LS, O'Sullivan SM, Galvin K, O'Connor TP, O'Brien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. *Int J Food Sci Nutr*. 2004; 55(3): 171-178.

[2] Haddad EH, Gaban-Chong N, Oda K, Sabate J. Effect of a walnut meal on postprandial oxidative stress and antioxidants in healthy individuals. *Nutr J*. 2014; 13(1): 1-9.

[3] Hayes D, Angove MJ, Tucci J, Dennis C. Walnuts (*Juglans regia*) chemical composition and research in human health. *Crit Rev Food Sci Nutr*. 2016; 56(8): 1231-1241.

[4] Bamberger C, Rossmeier A, Lechner K, Wu L, Waldmann E, Stark RG, Altenhofer J, Henze K, Parhofer K. A walnut-enriched diet reduces lipids in healthy Caucasian subjects, independent of recommended macronutrient replacement and time point of consumption: a prospective, randomized, controlled trial. *Nutrients*. 2017; 9(10): 1-14.

[5] Zibaeenezhad M, Aghasadeghi K, Hakimi H, Yarmohammadi H, Nikaein F. The effect of walnut oil consumption on blood sugar in patients with diabetes mellitus type 2. *Int J Endocrinol Metab*. 2016; 14(3): 1-8.

[6] Zibaeenezhad MJ, Farhadi P, Attar A, Mosleh A, Amirmoezi F, Azimi A. Effects of walnut oil on lipid profiles in hyperlipidemic type 2 diabetic patients: a randomized, double-blind, placebo-controlled trial. *Nutr Diabetes*. 2017; 7(4): 1-5.

[7] Arab L, Guo R, Elashoff D. Lower depression scores among walnut consumers in NHANES. *Nutrients*. 2019; 11(2): 1-13.

[8] Pribis P. Effects of walnut consumption on mood in young adults-a randomized controlled trial. *Nutrients*. 2016; 8(11): 1-9.

[9] Kris-Etherton PM. Walnuts decrease risk of cardiovascular disease: a summary of efficacy and biologic mechanisms. *J Nutr*. 2014; 144(4S): 547-554.

[10] Ebrahimi S, Jamei R, Nojoomi F, Zamanian Z. Persian walnut composition and its importance in human health. *Int J Enteric Pathog*. 2018; 6(1): 3-9.

[11] Nabavi SF, Ebrahimzadeh MA, Nabavi SM, Mahmoudi M, Rad SK. Biological activities of *Juglans regia* flowers. *Rev Bras Farmacogn*. 2011; 21(3): 465-470.

[12] Bakht J, Khan S, Shafi M, Iqbal A. Fractionation of crude extracts from controlled dried and commercially available stem bark of *Juglans regia* and their antimicrobial effects. *Pak J Pharm Sci*. 2017; 30(5): 1581-1588.

[13] Zakavi F, Golpasand Hagh L, Daraeighadikolaei A, Farajzadeh Sheikh A, Daraeighadikolaei A, Leilavi Shooshtari Z. Antibacterial effect of *Juglans regia* bark against oral pathologic bacteria. *Int J Dent*. 2013; Article ID 854765.

[14] Jahanban-Esfahlan A, Amarowicz R. Walnut (*Juglans regia* L.) shell pyroligneous acid: chemical constituents and functional applications. *RSC Adv*. 2018; 8(40): 22376-22391.

[15] Asgary S, Parkhideh S, Solhpour A, Madani H, Mahzouni P, Rahimi P. Effect of ethanolic extract of *Juglans regia* L. on blood sugar in diabetes-induced rats. *J Med Food*. 2008; 11(3): 533-538.

[16] Hosseini S, Huseini HF, Larijani B, Mohammad K, Najmizadeh A, Nourijelyani K, Jamshidi L. The hypoglycemic effect of *Juglans regia* leaves aqueous extract in diabetic patients: a first human trial. *Daru J Pharm Sci*. 2014; 22(1): 1-5.

[17] Hosseini S, Jamshidi L, Mehrzadi S, Mohammad K, Najmizadeh AR, Alimoradi

H, Huseini HF. Effects of *Juglans regia* L. leaf extract on hyperglycemia and lipid profiles in type two diabetic patients: a randomized double-blind, placebo-controlled clinical trial. *J Ethnopharmacol.* 2014; 152(3): 451-456.

[18] Javidanpour S, Fatemi Tabtabaei SR, Siahpoosh A, Morovati H, Shahriari A. Comparison of the effects of fresh leaf and peel extracts of walnut (*Juglans regia* L.) on blood glucose and beta-cells of streptozotocin-induced diabetic rats. *Vet Res Forum*. 2012; 3(4): 251-255.

[19] Mohammadi J, Delaviz H, Malekzadeh JM, Roozbehi A. The effect of hydro alcoholic extract of *Juglans regia* leaves in streptozotocin-nicotinamide induced diabetic rats. *Pak J Pharm Sci*. 2012; 25(2): 407-411.

[20] Mollica A, Zengin G, Locatelli M, Stefanucci A, Macedonio G, Bellagamba G, Onaolapo O, Onaolapo A, Azeez F, Ayileka A, Novellino E. An assessment of the nutraceutical potential of *Juglans regia* L. leaf powder in diabetic rats. *Food Chem Toxicol.* 2017; 107(PtB): 554-564.

[21] Nasiry D, Khalatbary AR, Ahmadvand H, Talebpour Amiri F, Akbari E. Protective effects of methanolic extract of *Juglans regia* L. leaf on streptozotocin-induced diabetic peripheral neuropathy in rats. *BMC Complement Altern Med*. 2017; 17(1): 1-11.

[22] Prasad R. Walnuts and pecans. In: Encyclopedia of food science and nutrition. 2nd ed. London: Academic Press, 2003.

[23] Ghiravani Z, Hosseini M, Taheri MMH, Fard MH, Abedini MR. Evaluation of hypoglycemic and hypolipidemic effects of internal septum of walnut fruit in alloxan-induced diabetic rats. *Afr J Tradit Complement Altern Med*. 2016; 13(2): 94-100.

[24] Ghiravani Z, Zardast M, Hassanpour Fard M, Hosseini M. Effects of hydroalcoholic extract of the internal septum of walnut on diabetic nephropathy in rats. *J Birjand Univ Med Sci.* 2015; 22(2): 104-114.

[25] Acquaviva R, D'Angeli F, Malfa GA, Ronsisvalle S, Garozzo A, Stivala A, Ragusa S, Nicolosi D, Salmeri M, Genovese C. Antibacterial and anti-biofilm activities of walnut pellicle extract (*Juglans regia* L.) against coagulase-negative staphylococci. *Nat Prod Res*. In press.

[26] Bezhuashivili M, Kurashvili N. Chemical characteristics of the septa of the fruit of *Juglans regia*. *Chem Nat Compd*. 1998; 34(2): 128-130.

[27] Dehghani F, Mashhoody T, Panjehshahin M. Effect of aqueous extract of walnut septum on blood glucose and pancreatic structure in streptozotocin-induced diabetic mouse. *Iran J Pharmacol Ther*. 2012; 11(1): 10-14.

[28] Hajikhani R, Solati J. Effects of walnut alcoholic extract (*Juglans regia* L.) septum on serum glucose, insulin and activities of aminotransferase enzymes. *J Appl Chem Res*. 2010; 12(4): 17-23.

[29] Hu Q, Liu J, Li J, Liu H, Dong N, Geng YY, Lu Y, Wang Y. Phenolic composition and nutritional attributes of diaphragma juglandis fructus and shell of walnut (*Juglans regia* L.). *Food Sci Biotechnol*. 2019; 29(2): 187-196.

[30] Li L, Song L, Sun X, Yan S, Huang W, Liu P. Characterisation of phenolics in fruit septum of *Juglans regia* Linn. by ultra performance liquid chromatography coupled with orbitrap mass spectrometer. *Food Chem.* 2019; 286(1): 669-677.

[31] Meng Q, Chen F, Xiao T, Zhang L. Inhibitory effects of polysaccharide from diaphragma juglandis fructus on alpha-amylase and alpha-d-glucosidase activity, streptozotocin-induced hyperglycemia model, advanced glycation end-products formation, and H_2O_2-induced oxidative damage. *Int J Biol Macromol*. 2019; 124(1): 1080-1089.

[32] Meng Q, Li Y, Xiao T, Zhang L, Xu D. Antioxidant and antibacterial activities of polysaccharides isolated and purified from diaphragma juglandis fructus. *Int J Biol Macromol.* 2017; 105(Pt1): 431-437.

[33] Ravanbakhsh A, Mahdavi M, Jalilzade-Amin G, Javadi S, Maham M, Mohammadnejad D, Rashidi MR. Acute and subchronic toxicity study of the median septum of *Juglans regia* in Wistar rats. *Adv Pharm Bull*. 2016; 6(4): 541-549.

[34] Rusu M, Gheldiu AM, Mocan A, Moldovan C, Popa DS, Tomuta I, Vlase L. Process optimization for improved phenolic compounds recovery from walnut (*Juglans regia* L.) septum: phytochemical profile and biological activities. *Molecules*. 2018; 23(11): 1-28.

[35] Wang D, Mu Y, Dong H, Yan H, Hao C, Wang X, Zhang L. Chemical constituents of

the ethyl acetate extract from diaphragma juglandis fructus and their inhibitory activity on nitric oxide production in vitro. *Molecules*. 2017; 23(1): 1-12.

[36] Zargari A. Medicinal plants. 4th ed. Tehran: Tehran University of Medical Sciences, 1997.

[37] Sarahroodi S, Rasekh H, Kamali-Nejad M, Safaeian L. Water extract of *Juglans regia* L. and diabetes mellitus in Iranian traditional medicine. *Qom Univ Med Sci J.* 2009; 3(3): 19-24.

[38] Mirheidar H. Knowledge of plants. 1st ed. Tehran: Daftar-e- Nashr Farhang Eslami, 1995.

[39] Zangeneh A, Zangeneh M, Goodarzi N, Najafi F, Hagh Nazari L. Protective effects of aqueous extract of internal septum of walnut fruit on diabetic hepatopathy in streptozotocin-induced diabetic mice. *Sci J Kurdistan Univ Med Sci.* 2018; 23(1): 26-37.

[40] Bi D, Zhao Y, Jiang R, Wang Y, Tian Y, Chen X, Bai S, She G. Phytochemistry, bioactivity and potential impact on health of *Juglans*: the original plant of walnut. *Nat Prod Commun*. 2016; 11(6): 869-880.

[41] Popovici C. Soxhlet extraction and characterisation of natural compounds from walnut (*Juglans regia* L.) by-products. *Food Technol*. 2013; 2(3): 328-336.

[42] Haliloglu Y, Ozek T, Tekin M, Goger F, Baser KHC, Ozek G. Phytochemicals, antioxidant, and antityrosinase activities of *Achillea sivasica* Çelik and Akpulat. *Int J Food Prop*. 2017; 20(S1): 693-706.

[43] Mocan A, Zengin G, Simirgiotis M, Schafberg M, Mollica A, Vodnar DC, Crişan G, Rohn S. Functional constituents of wild and cultivated Goji (L. *barbarum* L.) leaves: phytochemical characterization, biological profile, and computational studies. *J Enzyme Inhib Med Chem.* 2017; 32(1): 153-168.

[44] Akbari V, Jamei R, Heidari R, Esfahlan AJ. Antiradical activity of different parts of walnut (*Juglans regia* L.) fruit as a function of genotype. *Food Chem.* 2012; 135(4): 2404-2410.

[45] Jahanban-Esfahlan A, Ostadrahimi A, Tabibiazar M, Amarowicz R. A comparative review on the extraction, antioxidant content and antioxidant potential of different parts of walnut (*Juglans regia* L.) fruit and tree. *Molecules*. 2019; 24(11): 1-40.

[46] Johnson CE, Oladeinde FO, Kinyua AM, Michelin R, Makinde JM, Jaiyesimi AA, Mbiti WN, Kamau GN, Kofi-Tsekpo WM, Pramanik S, Williams A. Comparative assessment of total phenolic content in selected medicinal plants. *Niger J Nat Prod Med.* 2008; 12(1): 40-45.

[47] Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. *Clin Interv Aging*. 2018; 13(1): 757-772.

[48] Habybabady RH, Mortazavi SB, Khavanin A, Mirzaei R, Arab MR, Mesbahzadeh B, Hoseini M, Mohammadi M. Protective effects of n-acetyl-l-cysteine on the density of spiral ganglion cells and histological changes induced by continuous noise exposure in rats. *Malays J Med Sci*. 2018; 25(5): 48-58.

[49] Hami J, Hosseini M, Nezhad SV, Shahi S, Lotfi N, Ehsani H, Sadeghi A. Beneficial effects of L-arginine on 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced neuronal degeneration in substantia nigra of Balb/c mice. *Adv Biomed Res*. 2016; Article ID 27656609.

[50] Hami J, Hosseini M, Shahi S, Lotfi N, Talebi A, Afshar M. Effects of L-arginine pre-treatment in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced Parkinson's diseases in Balb/c mice. *Iran J Neurol*. 2015; 14(4): 195-203.

[51] Hassanzadeh-Taheri M, Hosseini M, Hassanpour-Fard M, Ghiravani Z, Vazifeshenas-Darmiyan K, Yousefi S, Ezi S. Effect of turnip leaf and root extracts on renal function in diabetic rats. *Orient Pharm Exp Med*. 2016; 16(4): 279-286.

[52] Dourandishan M, Hossieni M, Malekaneh M, Bagherzade G. Effect of *Otostegia persica* root extract on the blood biochemical factors in diabetic hyperlipidemic rats. *Horizon Med Sci*. 2014; 20(1): 17-21.

[53] Fard MH, Naseh G, Lotfi N, Hosseini SM, Hosseini M. Effects of aqueous extract of turnip leaf (*Brassica rapa*) in alloxan-induced diabetic rats. *Avicenna J Phytomed*. 2015; 5(2): 148-156.

[54] Hassanzadeh-Taheri M, Hassanpour-Fard M, Doostabadi M, Moodi H, Vazifeshenas-Darmiyan K, Hosseini M. Co-administration effects of aqueous extract of turnip leaf and

metformin in diabetic rats. *J Tradit Complement Med*. 2018; 8(1): 178-183.
[55] Kiani Z, Hassanpour-Fard M, Asghari Z, Hosseini M. Experimental evaluation of a polyherbal formulation (Tetraherbs): antidiabetic efficacy in rats. *Comp Clin Path*. 2018; 27(6): 1437-1445.
[56] Serki E, Vazifeshenas Darmiyan K, Ezi S, Bayat J, Shahamat F, Ghiravani Z, Hosseini M. Effects of colostrum on sperm parameters, sex hormones and testes histopathological changes in diabetic rats. *J Mazandaran Univ Med Sci*. 2016; 26(141): 83-94.
[57] Taş NG, Gökmen V. Phenolic compounds in natural and roasted nuts and their skins: a brief review. *Curr Opin Food Sci*. 2017; 14(1): 103-109.
[58] Pereira JA, Oliveira I, Sousa A, Valentão P, Andrade PB, Ferreira IC, Ferreres F, Bento A, Seabra R, Estevinho L. Walnut (*Juglans regia* L.) leaves: phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. *Food Chem Toxicol*. 2007; 45(11): 2287-2295.
[59] Nadeem M, Mahud A, Imran M, Khalique A. Enhancement of the oxidative stability of whey butter through almond (*Prunus dulcis*) peel extract. *J Food Process Preserv*. 2015; 39(6): 591-598.
[60] Mehdizadeh T, Mohammadipour N, Langroodi AM, Raeisi M. Effect of walnut kernel septum membranes hydroalcoholic extract on the shelf life of traditional butter. *Heliyon*. 2019; 5(3): 1-20.
[61] Greenwell M, Rahman PKSM. Medicinal plants: their use in anticancer treatment. *Int J Pharm Sci Res*. 2015; 6(10): 4103-4112.
[62] Carvalho M, Ferreira PJ, Mendes VS, Silva R, Pereira JA, Jerónimo C, Silva BM. Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L. *Food Chem Toxicol*. 2010; 48(1): 441-447.
[63] Li W, Li DY, Wang HD, Zheng ZJ, Hu J, Li ZZ. *Juglans regia* hexane extract exerts antitumor effect, apoptosis induction and cell circle arrest in prostate cancer cells in vitro. *Trop J Pharm Res*. 2015; 14(3): 399-405.
[64] Santos A, Barros L, Calhelha RC, Dueñas M, Carvalho AM, Santos-Buelga C, Ferreira IC. Leaves and decoction of *Juglans regia* L.: different performances regarding bioactive compounds and in vitro antioxidant and antitumor effects. *Ind Crops Prod*. 2013; 51(1): 430-436.
[65] Meng Q, Wang Y, Chen F, Xiao T, Zhang L. Polysaccharides from diaphragma juglandis fructus: extraction optimization, antitumor, and immune-enhancement effects. *Int J Biol Macromol*. 2018; 115(1): 835-845.
[66] Bindu J, Narendhirakannan RT. Role of medicinal plants in the management of diabetes mellitus: a review. *3 Biotech*. 2019; 9(1): 1-17.
[67] Nasiry D, Khalatbary AR, Ahmadvand H. Therapeutic potential of *Juglans regia* L. leaf extract against diabetic retinopathy in rat. *Iran J Basic Med Sci*. 2017; 20(11): 1275-1281.
[68] Nasiry D, Khalatbary AR, Ahmadvand H, Talebpour Amiri F. *Juglans regia* L. leaf extract attenuates diabetic nephropathy progression in experimental diabetes: an immunohistochemical study. *Iran J Med Sci*. 2019; 44 (1): 44-52.
[69] Teimori M, Montasser Kouhsari S, Ghafarzadegan R, Hajiaghaee R. Study of hypoglycemic effect of *Juglans regia* leaves and its mechanism. *J Med Plants*. 2010; 9(S6): 57-65.
[70] Rabiei K, Ebrahimzadeh MA, Saeedi M, Bahar A, Akha O, Kashi Z. Effects of a hydroalcoholic extract of *Juglans regia* (walnut) leaves on blood glucose and major cardiovascular risk factors in type 2 diabetic patients: a double-blind, placebo-controlled clinical trial. *BMC Complement Altern Med*. 2018; 18 (1): 1-7.

Abbreviations

DPPH: 1,1-diphenyl-2-picrylhydrazyl; HPLC-MS/MS: high-performance liquid chromatography-tandem mass spectrometry; FRAP: ferric reducing antioxidant power; mg KAE/g dw extract: mg kojic acid equivalent per gram of the dried lyophilized extract; mg GAE/g dw: mg gallic acid equivalent per gram of the dried weight; ABST: 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; mg TE/g dw: mg trolox equivalents per gram of the dried extract; RONS: oxygen and nitrogen species; PC-3: prostate cancer cell line; MCF-7: breast adenocarcinoma cell line; HCT-15 and Caco-2:

colon carcinoma cell line; HeLa: cervical carcinoma cell line; HepG-2: hepatocellular carcinoma cell line; A-498 and 769-P: renal carcinoma cell lines; BGC-823: human gastric carcinoma cell line