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JUGLANS REGIA: A REVIEW OF ITS TRADITIONAL USES PHYTOCHEMISTRY AND PHARMACOLOGY

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ABSTRACT

Walnut (Juglans regia L.) is the most widespread tree nut in the world. The tree is commonly called as the Persian walnut, white walnut, English walnut or common walnut. It belongs to Juglandaceae and has the scientific name Juglans regia (*J. regia*). The array of human health benefits, derived from walnut is primarily due to the abundant presence of phytochemical components such as flavonoids, carotenoids, alkaloids, nitrogen-containing compounds, as well as other polyphenolic. All parts of the plant are important *viz*. kernel, bark, leaves, flowers, green husk, septum, oil etc. Oil of this plant is extensively used in ayurveda, unani, homeopathic and allopathic system of medicines. Many health benefits claimed for the consumption of *J. regia* includes antioxidant, antihistaminic, analgesic, bronchodilator, antiulcer, immunomodulatory, antidiabetic, hepatoprotective, antifertility, anti-inflammatory, antimicrobial, antihypertensive, neuroprotective, anticancer, lipolytic, wound healing, insecticidal and several other therapeutic properties. This review article attempts, bring to light the available literature on *J. regia* with respect to traditional, ethnobotany, phytoconstituents and summary of various pharmacological activities on animal and humans.

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INTRODUCTION

Juglans regia Linn is commonly known as Walnut tree. *J. regia* is well-known as Akhort in India, a native of Eastern Europe to North Asia i.e. China, Iraq, Mexico, Spain, Turkey, Nepal, India (forests in the Himalayas) is a member of Juglandaceae family. It is a large, deciduous tree, reaching a height up to 25-35 m and exceptionally a maximum trunk diameter up to 2 m. It is long-lived: normally 100-200 years, but some specimens may reach 1000 years old [1].

In Ayurvedic medicine system *J. regia* is known as Aksoda or Aksota were used by both Charaka and Sushruta, together with Vatama (almond), Abhisuka (pista) and other dry fruits. Literature based on Ayurveda *J. regia* is used for wounds, phthisis and diseases of the nervous system (therapeutic uses based on texts from 1000 BC to sixteenth century). Charaka and Sushruta (1000BC) used the edible nut kernel in prescriptions for anemia, phthisis, debility, senility and as a vitalizing tonic. Sushruta gave oil of the seeds as a digestive tonic [2].

Walnut is a crop of high economic interest to the food industry. The palatable part of the fruit (the seed or kernel) is consumed fresh or toasted alone or in other edible products. It is worldwide popular and valued for its nutritional, health and sensory attributes. The fresh natural kernels are consumed mainly as whole nuts or used in various confectioneries. They are a nutrient-dense food mainly owing to their fat content, protein, vitamin and mineral profiles. Also, walnut kernels serve as a good source of a wide variety of flavonoids, phenolic acids and related polyphenols [3].

J. regia, contain many potent chemical constituents, have been continuously used since antiquity to treat diverse ailments, including diarrhea, hyperglycemia, cancer, infectious disease, anorexia, eczema, asthma, antihypertensive, neuroprotective, helminthiasis, arthritis, sinusitis, stomach-ache, skin disorders, among others [4].

Habitat and Ecology

J. regia is native to the mountain ranges of Central Asia, extending up to western China, parts of Kazakhstan, Uzbekistan and southern Kirghizia and from lower ranges of mountains in Nepal, Bhutan, Tibet, northern India, Pakistan and Sri Lanka through Afghanistan [5]. The common walnut is a demanding species and requires special site conditions. Usually grown in pure stands or as individual trees, rather than within mixed woodland, it needs a warm and sheltered site and a long growing season. It also prefers deep and rich soils, with pH values of between 6 and 7.5. It is light-demanding, highly susceptible to competition and sensitive to winter and late spring frosts. Older trees are however able to withstand winter temperatures as low as -30 °C. Germination is improved in mild winters, indicating that a changing climate with warmer winters may prove beneficial to its establishment [6].

Taxonomical Classification

Kingdom : Plantae
Order : Fagales
Family : Juglandaceae
Genus : Juglans
Species : *J. regia*

Phytoconstituents

J. regia contain a number of potential neuroprotective compounds such as gamma tocopherol (vitamin E), folate, melatonin, flavonoids, and phenolic acid (ellagic acid) and a significant amount of n-3 α-linolenic acid (ALA) (a plant-based omega-3 fatty acid) [7]. *J. regia* seeds are commonly contain the phenolic compounds such as phenolic acids, namely gallic, ellagic, syringic, caffeic, p-coumaric, ferulic and sinapic acids and tannins, such as glansrins A, B and C, casuarinin, stenophyllarin, between others. In relation to walnut leaves, its phenolic composition has already been studied by some researchers [8]. Another researcher determined the phenolic profile of walnut leaves of several cultivars. This variety was characterized by the presence of at least nine phenolic compounds: three hydroxycinnamic acid derivatives, the 3-O-caffeoylquinic, 3-O-p-coumaroylquinic and 4-O-p-coumaroylquinic acids, and six flavonol heterosides, the quercetin 3-O-galactoside (its major compound), a quercetin 3-O-pentoside derivative, quercetin 3-O-arabinoside, quercetin 3-Oxyloside, quercetin 3-O-ramnoside and a kaempferol 3-Opentoside [9]. Pereira and colleagues identified another two hydroxycinnamic acid derivatives, the 5-Ocaffeoylquinic and p-coumaric acids [10]. More recently it is also reported that 10 compounds in methanol and petroleum ether walnut extracts: 3- and 5-caffeoylquinic acids, 3- and 4-pcoumaroylquinic acids, p-coumaric acid, quercetin 3-galactoside, quercetin 3-pentoside derivative, quercetin 3-arabinoside, quercetin 3-xyloside and quercetin 3-rhamnoside [11].

Traditional and Ethnobotanical uses

The plant is used as a topical remedy for dermal inflammation and excessive perspiration of the hands and feet. It is also a common home remedy for the treatment of chronic eczema and scrofula. The leaves of this plant are used topically to treat scalp itching and dandruff, sunburn, and superficial burns as well as an adjunctive emollient in skin disorders [12]. The kernel of *J. regia* has been used for the treatment of inflammatory bowel disease in Iranian traditional medicine [13].

In Turkish folk medicine, fresh leaves of *J. regia* are used on the forehead and body to alleviate fever and on joints to reduce pain from rheumatism [14]. In Palestine, *J. regia* has been used to treat diabetes, cardiac disease and inflammatory conditions [15], as well as to improve vascular and prostate health in elderly males [16].

The Angami, Lotha and Sumi tribes of Kohima (Nagaland) used to bark and unripe fruit for Piscicidal activity, they also used leaves as astringent, anthelmintics, used in eczema and herpes [17].

It is also a common home remedy for the treatment of chronic eczema and scrofula. The leaves are used topically to treat scalp itching and dandruff, sunburn, and superficial burns as well as good for adjunctive emollient in skin problems [18-19]. Exocarp of the immature green fruit of this medicinal plant has been used to treat gastric, liver and lung cancer a long time in China [20].

In Palestine, *J. regia* has been used to treat diabetes and cardiac disease [16, 21]. It also improves vascular and prostate health in elderly males [22].

PHYTO-PHARMACOLOGICAL ACTIVITY

ANTIOXIDANT ACTIVITY

Aqueous and ethanolic extracts were tested for free radical scavenging ability against 2, 2 diphenyl-1-picryl hydrazyl (DPPH) radical, ferric reducing and iron (II) chelating ability. Generally, ethanolic extract was found to exhibit a significantly higher antioxidant activity than do aqueous in all parameters determined. The result showed that both extracts demonstrated potent free radical scavenging and ferric reducing ability and iron chelating activity *in vitro*. The phenolic content of *J. regia* was estimated to be 35.22 ± 0.75 mg/g gallic acid equivalents (GAE) for ethanolic and 20.26 ± 0.55 mg/g (GAE) for aqueous extract whereas the flavonoids content was estimated to be 20.02 ± 0.12 mg/g quercetin equivalent (QE) for ethanolic and 14.82 ± 0.15 mg/g (QE) [23].

The reducing power capacity of the green hull of *J. regia* was evaluated in the ethanolic extract at a concentration of 500 mg/ml, the absorbance of the ethanolic extract was found to be 5.2. The reduction power activity of ethanolic extract expressed the absorbance of 700 nm at a concentration of 400 mg/mL which is almost near the standard BHT. This indicates that the extract seems to have antioxidant capacity due to the presence of polyphenols, which may act on a similar fraction reduction by donating the electrons. The higher values of reducing power indicate that some components are electron donors which react with the free radicals [24].

Leaves of walnut obtained from 14 different sources were investigated for their antioxidant potency and the availability of total phenolic compounds. From the different sources, total phenolic content ranged from 17.7-39.6 mg (GAE)/g. The methanolic extract possessed the most potent DPPH-scavenging activity [25].

Carey *et al* investigated the beneficial ability of polyunsaturated fatty acids (PUFAs) from Walnut extract to slow down the damage of hippocampal cells, mediated by inflammation and oxidative stress. The outcome of this study revealed that walnut extract delivered noteworthy defence against cell death and calcium dysregulation in a concentration-dependent manner [26].

ANTIBACTERIAL ACTIVITY

The antibacterial activity of *J. regia* hull extracts was determined by the disc diffusion method (zone of inhibition) and it exhibits good antibacterial activity against all the bacterial species *E. coli*, *B. subtilis*, *K. aerogenosa* and *S. aureus*. Result confirmed that *J. regia* green hull extract may be beneficial in treating acne especially when they are known to have anti-inflammatory activities [27]. In a recent study, Juglone was shown to potently inhibit the three key enzymes from *Helicobacter pylori*, cystathionine γ-synthase (HpCGS), malonyl-CoA-acyl carrier protein transacylase (HpFabD), and β-hydroxy acyl-ACP dehydratase (HpFabZ) with the half maximal inhibitory concentration (IC_{50}) values of 7.0±0.7, 20±1, and 30±4 μmol/L, respectively [28]. The antimicrobial activity against gram-negative bacteria was selective since not all the fruit extract of *J. regia* cultivator inhibited the growth of *Pseudomonas aeruginosa* and *E. coli.* cv. Lara inhibited the growth of *K. pneumonia* minimum inhibitory concentrations (MIC of 100 mg/mL), cv. Mayette inhibited the development of *P. aeruginosa* and *E. coli* with (MICs) of 50 and 10 mg/mL, respectively, and cv. Mellanaise inhibited the growth of *E. coli* and *K. pneumonia* at the concentration of 100 mg/mL [29, 30].

ANTIVIRAL ACTIVITY

Mouhajir *et al* investigated the antiviral activity of methanol extracts of *J. regia*. The 2 mg/ml concentration of extract was evaluated against herpes simplex virus (HSV), Sindbis virus (SINV) and poliovirus (polio) at noncytotoxic concentrations [31]. Shailima and Vardhini explore the antiviral activity of Juglone by computational method [32]. Other researcher investigated the Phytochemical and chromatographical techniques and they were used to isolate compounds from *J. regia*; MT4 cells and HIV III B virus were used to study the effect of anti-HIV activity *in vitro*. BIACORE 3000 molecule coupled equipment was used for the target research. Two extractions (B & E) were isolated from *J. regia* which possess the effect of anti-HIV activity. Targets study found that extraction B showed the anti HIV activity by affecting on HIV-1 gp-41 fusing protein and extraction E could affected on HIV-1 integrase respectively [33].

ANTIDIABETIC ACTIVITY

J. regia leaf extract 200 and 400 mg/kg body weight for 28 days given to streptozotocin treated rat and it ameliorating hyperglycemia by decreasing glycosylated hemoglobin while enhancing Insulin [34]. Fukuda *et al.* proved strong inhibitory activity of walnut polyphenolic components like casuarictin, tellimagradin II and Tellimagradin I on different enzymes like glycosidase, sucrose, maltase and amylase. In addition to the above findings, researchers also noticed that walnut polyphenol rich fraction has triglyceride lowering effect and urine peroxide lowering effect in genetically inherited Type II diabetes mellitus (db/db) mice at the dose of 200 mg/kg/day [35]. Treatment by both leaf and ridge extracts decreased blood glucose and liver phosphoenolpyruvate carboxykinase activity and increased blood insulin and liver glycogen phosphorylase activity [36].

Some researcher also used bark ethanolic extract at a dose of (200 mg/kg b.w.) over 10 days, and blood glucose was monitored and compared with a control group. The glucose AUC showed a hypoglycemic effect in normal rats due to the high concentration of phenolic compounds like phenolics, flavonoids and proanthocyanidins [37]. Hydroalcoholic extract (100 mg/kg) of *J. regia* green husk for 10 days significantly reduced glucose level in alloxan treated rat [38].

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY

Hosseinzadeh and colleagues have recently described that aqueous (2.87 and 1.64 g/kg) and ethanolic (2.044 and 1.17 g/kg) extracts of *J. regia* showed antinociceptive activity in hot-plate test. The extracts exhibited antinociceptive activity in writhing test, which was not blocked by naloxone. In xylene test, both extracts showed anti-inflammatory activity in some doses. The extracts showed anti-inflammatory activity against the chronic inflammation. *J. regia* leaves demonstrated antinociceptive effect through non-opioid receptors and anti-inflammatory effect against acute and chronic inflammation so it could be considered as a promising analgesic and anti-inflammatory agents against diseases such as rheumatoid arthritis [39]. Qamar also proved the protective role of methanolic extract of *J. regia* against cigarette smoke extract (CSE) induced acute lung toxicity in rats. The extract significantly decreased the levels of lactate dehydrogenase (LDH), total cell counts, total protein and increased the glutathione (GSH) level in broncho-alveolar lavage fluid. It also significantly restored the levels of glutathione reductase (GR), catalase and reduced the xanthine oxidase (XO) activity in lung tissue [40]. Kshitij and colleagues evaluated the anti-inflammatory property of the aqueous, chloroform and alcoholic extracts of the bark by *in vitro* methods. *In vitro* method was estimated by human red blood cell membrane stabilization (HRBC) method. Results showed significant anti-inflammatory property of the different extracts tested. The aqueous extract at a concentration of 200 mg/ml showed potent activity on comparing with the standard drug diclofenac sodium [41].

ANTIDEPRESSANT ACTIVITY

Depression is an extremely complex and heterogeneous condition. The therapeutic approach to the treatment of depression includes a long-term use of antidepressants, either in the form of monotherapy or as a combination of several antidepressants with various mechanisms of action. The *J. regia* fruit extract was administered in doses of 100 and 150 mg/kg body weight. Both the doses significantly decreased the duration of immobility in Forced swimming and tail suspension test models of depression. The effect of the extract was less significant than standard drug fluoxetine. *J. regia* fruit may exert antidepressant activity due to the presence of omega 3 fatty acid in extract [42].

LEARNING AND MEMORY ACTIVITY

The brain requires a sufficient amount of water, vitamins (such as folate, thiamine, vitamins B6, and B12), α-lipoic acid, lutein, and n-3 fatty acids. Walnuts contain a number of potential neuroprotective compounds such as gamma tocopherol (vitamin E), folate, melatonin, flavonoids, and phenolic acid (ellagic acid) and a significant amount of n-3 α-linolenic acid (ALA) (a plant-based omega-3 fatty acid). When 1,113 different food items were analyzed for antioxidant content, walnuts ranked second [43].

Memory is the process by which a learning experience is maintained over time. A single memory can be recalled by presenting a proper stimulus. Polyphenols have been shown to modulate critical neuronal signaling pathways involved in processes of learning and memory. The performance of C57BL/6J mice for learning and memory was done by Morris water maze test. Polyphenolic extracts from Walnut testa (42%) improved learning and memory functions in hypercholesterolemic mice based on obesity, hypercholesterolemia and oxidative stress [44]. Another researcher describes the neuroprotective activity of a 6% walnut diet against neurotoxicity in male rats induced by the anticancer drug cisplatin. The results showed that administration of walnut improved the cognitive and motor functions, demonstrating the potential benefits of including walnut in the diet for combating chemotherapy-induced disruptions of the motor and cognitive function [45]. Previously it is studied that walnut extract can inhibit amyloid-β fibrillization, can solubilize its fibrils and has a protective effect against Aβ induced oxidative stress and cellular death. Recently researcher proved that dietary supplementation with walnuts 6% or 9% improved learning skills, memory, anxiety, locomotor activity, and motor coordination in the Tg2576 transgenic mouse model of Alzheimer's disease [46].

ANTICANCER ACTIVITY

The free radical oxidations in humans can be a caused several diseases such as cancer. Breast Cancer is the most common malignancy in females. Walnut hydrolysates protein tested against the viability of human breast (MDA-MB231) and colon (HT-29) cancer cell lines. MTT, [3-(4, 5dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide], an assay was used to assess *in vitro* cancer cell viability upon treatment with the peptide fractions. The peptide fractions showed cell growth inhibition of 63 ± 1.73 % for breast cancer and 51 ± 1.45 % for colon cancer cells [47]. Shah and colleagues find that leaf methanolic extracts showed concentration dependent growth inhibition activity (IC50 0.234 and 0.304mg/ml) against B16F10 mice melanoma and A375 human melanoma cell line (IC50 0.298 and 0.350mg/ml) respectively. The extracts proved least toxic when treated with normal lymphocytes. The results indicate that walnut leaves are an excellent source of antioxidant and anti-cancerous agents [48]. Oguzhan *et al* studied the anticancer activity of walnut milk (WM) in DU145 (human prostate cancer cell) and MCF-7 cells. Results showed a remarkable reduction in cell viability and selective induction of caspase-dependent apoptosis in both cell lines, through stimulation of a constitutive apoptotic pathway and facilitation of ROS generation [49].

The extract of *J. regia* exhibited a potent and dose-dependent anti-proliferative activity against human prostate cancer cells *in vitro*. The extract also induced significant apoptosis in these PC3 cancer cells as revealed by annexin V binding assay as well as inverted phase contrast microscopy. It triggered a significant formation of apoptotic bodies after treatment with varying concentrations of the extract. Within 48 h of incubation, approximately 9.5, 15.5 and 26.3 % of the cells underwent early apoptosis after treatment with 5, 50 and 100 µg/mL of the extract, respectively. Similarly, 5.2, 11.2 and 18.9 % of the cells underwent late apoptosis after treatment with 5, 50 and 100 µg/mL of the extract, respectively. Treatment with different concentrations of the extract for 48 h induced an increase in the population of cells in the sub-G1 phase and a slight decrease in the G2/M phase [50].

Alshatwi and colleagues found that green husk extracts of *J. regia* suppressed proliferation and induced apoptosis in a dose and time dependent manner by modulating expression of apoptosis-related genes. This involved DNA fragmentation and significant changes in levels of mRNA and the expression of corresponding proteins. Authors suggested that presence of bioactive compounds in walnut green husks that are capable of killing prostate carcinoma cells by inducing apoptosis and that the husks are responsible for anticancer property [51].

HEPATOPROTECTIVE ACTIVITY

Treatments with walnut leaf extract (ranging from 0.2 to 0.4 g/kg b.w.) significantly lowered serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase levels in CCl4-treated rats. Walnut leaf extracts increased antioxidant enzymes, including superoxide dismutase and catalase [52]. A single oral administration of polyphenol-rich fraction of Walnut kernel pellicles (200 mg/kg) significantly suppressed serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) elevation in liver injury induced by carbon tetrachloride (CCl4). According to author the ellagitannins, tellimagrandin I is responsible for walnut hepatoprotective effect [53]. Walnuts isolated orally Juglone 0.25 mg/kg or 1 mg/kg for 70 days, protected HFD-induced liver damage in rats by inhibiting various inflammatory cytokines, like TNF-α, IL-1β and IL- 6, through down-regulation of toll-like receptor 4 and activation of NF-κB activity [54]. Walnut showed the hepatoprotective effect at various doses against ethanol-induced oxidative stress in rats by evaluating the serum marker enzymes of liver injury. Serum biochemistry results revealed a significant rise in AST, gamma glutamyl transpeptidase, ALT and lactate dehydrogenase (LDH) in the 20% ethanol-fed group, compared with the control group (without ethanol) [55].

CARDIOVASCULAR ACTIVITY

Walnuts are a rich source of ω-3 and ω -6 PUFA. While some workers have suggested that ω -6 PUFAs may be associated with an increased proinflammatory vascular response, most researchers find that consumption of these has no adverse effects on cardiovascular health in humans. Several studies have concluded that inclusion of ω-3 and ω-6 PUFAs through regular consumption of walnuts (30-100 g/day) lowers CVD risk factors in non-hyperlipidaemic individuals [56].

Davis and colleagues monitored concentrations of aortic endothelin 1 (ET-1) and other CVD risk markers in hamsters fed with high fatty acids diets with the inclusion of walnuts. The authors concluded that ET-1 regulator levels decreased as Walnut consumption increased, thereby producing beneficial effects on CVD risk in part *via* the ET-1-related effects on endothelial processes [57]. Overall, the current state of play relating fatty acid intake to risk of concludes that a proportionally high intake of saturated fatty acids (SFA) over monounsaturated fatty acids (MUFA) and PUFA increases CVD risk, although the authors do note the limited presence of studies that have produced conflicting outcomes [58, 59]. An *in vitro* study revealed that walnut green hull extract at a concentration of 50 mg/mL inhibited thrombin-induced platelet aggregation and protein secretion by 50%, without any cytotoxic effects on platelets. The examined extract suppressed reactive oxygen species generation and also caspase activation in thrombin stimulated platelets. Presumably, the antiplatelet activity of walnut green hull extract is related to its polyphenolic compounds and their antioxidant properties. Therefore it can be considered as a candidate for thrombotic disorders [60].

TOXICITY STUDIES ON ANIMALS AND HUMANS

In the oral acute toxicity study, female Wistar rats were treated with various dose intervals of 10 to 5000 mg/ kg of the *J. regia* septum of methanol extract (14 days). In sub chronic study, the extract was given orally at a dose of 1000 mg/kg daily in Wistar rats for 28 days. The extract did not produce any toxic signs or deaths; even at a dose of 5000 mg/kg. In sub chronic study, No significant morphological and histopathological changes were observed in the studied tissues [61]. Yang and colleagues study the effects of walnut polyphenol extract (WPE) on immunotoxicity induced by 4-pentylphenol (PP) and 3-methyl-4-nitrophenol (PNMC) in murine splenic lymphocytes. Co-treatment with WPE significantly enhance proliferation of splenocytes exposed to PP or PNMC, characterized by increases in the percentages of splenic T lymphocytes (CD3+ T cells) and T cell subsets (CD4+ and CD8+ T cells), as well as the production of T cell-related cytokines and granzymes (interleukin-2, interleukin-4, and granzyme-B) in cells exposed to PP or PNMC. The author suggests that walnut polyphenols significantly attenuated PP and PNMC-mediated immunotoxicity and improved immune function by inhibiting oxidative stress [62].

Previous studies have reported irritation and skin hyper pigmentation associated with topical walnut use. Although, in guinea pigs, it has been reported to be a strong sensitizer, in humans contact allergy is very rare [63]. In a case report, the palms and fingers of a 65-year-old woman showed large blisters and skin hyper pigmentation following the consumption of 15 kg of walnuts in 3 days [64]. Walnut aqueous extract modulated the toxicity of cyclophosphamide and protected metabolizing and antioxidant enzymes during the chemotherapy [65]. Calabro and colleagues find that juglone induced the suicidal death of erythrocyte by increasing the abundance of ceramide and reducing energy level and protein kinase C (PKC) activation. In this study, treatment of human erythrocytes with juglone for 24 h, at a dose of 5µmol/L, caused the significant reduction on erythrocyte forward scatter. Furthermore, juglone at doses from 1-5 µmol/L significantly raised the annexin V binding percentage.

Likewise, at a dose of 5µmol/L, juglone significantly reduced the concentration of ATP in erythrocytes, and also increased the abundance of ceramide at the surface of erythrocytes. In contrast, the activity of 10 µmol/L juglone on the binding of annexin V was considerably inhibited by removing extracellular Ca^{2+} as well as by treating with PKC inhibitor, staurosporine (1 µmol/L) [66]. There are also some reports of the harmful effects of walnut on animals, specifically, horses. Walnut heartwood can cause an inflammatory condition in horses called laminitis. For this reason, the black walnut extract model was developed to study the various parameters associated with laminitis in horses [67, 68].

CLINICAL STUDY

Iwamoto *et al* randomly assigned 20 men and 20 women to two mixed natural diets, each to be consumed for 4 weeks in a crossover design. Both diets conformed to the average Japanese reference diet and contained identical foods. Total cholesterol concentration was 0.16 mmol/L lower for men and 0.21 mmol/L lower for women when they consumed the walnut diet than when they consumed the reference diet. The LDL cholesterol concentrations were 0.18 mmol/L lower for men and 0.22 mmol/L lower for women when they consumed the walnut diet. The ratio of LDL cholesterol to HDL cholesterol and the apolipoprotein B concentration were also lowered by the walnut diet [69]. Sixty hyperlipidemic subjects were randomized into 2 groups; group A patients (n = 29) received walnut oil encapsulated in 500 mg capsules, 3 g/day, for 45 days. Group B patients (n = 31) received placebo and served as the control group. After the end of the study, Walnut oil decreased plasma TG concentrations [70]. 21 Hypercholesterolemic individuals were given a cholesterol-reducing Mediterranean diet or walnut-enriched (100 g/d) diet. Compared to the Mediterranean diet, the walnut diet improved endothelium-derived vasorelaxation and reduced vascular cell adhesion molecule-1 expression. Walnut consumption significantly decreased the concentration of total cholesterol and LDL cholesterol [71].

Female volunteers were given walnut (43 g/d) first and then a control diet lasting 8 weeks. Compared to the control diet, walnut significantly decreased non-HDL cholesterol and apolipoprotein B levels. The Walnut diet also reduced total cholesterol. However, the walnut diet did not show significant differences in the levels of fasting very low-density lipoprotein, LDL, HDL, triglyceride, glucose, insulin, HOMA-IR or HbA1c [72]. Thirteen studies representing 365 participants. Diets lasted 4-24 weeks with walnuts providing 10–24% of total calories. High Walnut enriched diets significantly decreased total and LDL cholesterol for the duration of the short-term trials [73]. The potential anti-hypertensive effects associated with the administration of aqueous extract of the Walnut fruit was investigated using 130 hypertensive subjects. Short term meal of walnut normalizes high blood pressure, high cholesterol and serum electrolytes. Walnut meal has no effect on hemoglobin concentration, white blood cell count, packed cell volume and platelet counts compared with their corresponding controls. The author concludes from the study that walnut contains anti-hypertensive active constituents [74]. Eight patients with diabetes mellitus (DM) type 1 were enrolled in the study. They administered insulin and 250 mL Walnut hydrosol after meals twice a day for four weeks. Their baseline level was measured and their insulin dose was changed according to their baseline. The average daily baseline level and insulin dose decreased in seven subjects. Two subjects developed a generalized pruritic erythematous skin rash. One patient presented hypoglycemic coma. Seven compounds were identified in the Walnut essential oil and the rate of monoterpenoid and sesquiterpenes hydrocarbons were 53.45% and 5.95%, respectively [75].

CONCLUSIONS

J. regia are amongst the most highly consumed tree nuts by humans globally. Many documented evidence concentrating on their novel fatty acid profile, several polyphenols, stilbenes, green husk unique constituents juglone, and benefits against the several diseases like diabetes, hepatic problems, CVD-related diseases, age-related neurological disorders, and also in cancer afforded through regular consumption of *J. regia* as part of a healthy diet. Moreover, purification, isolation, and characterization of active secondary metabolites responsible for various pharmacological activities have not still been structurally elucidated, and require much further well designed and collaborative research.

Conflict of Interest:
No conflict of interest

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List of abbreviations and acronyms:

BC : Before Christ
DPPH : 2, 2 diphenyl-1-picryl hydrazyl
GAE : Gallic Acid Equivalent
BHT : Butylated Hydroxy Toluene
QE : Qurcetin Equivelent
PUFA : Poly Unsaturated Fatty Acids
HRBC : Human Red Blood Cell
SFA : saturated fatty acid
PKC : Protein Kinase C
DM : Diabetes Melltus

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