Juglans regia Linn: A Phytopharmacological Review

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ABSTRACT

In the last few decades there has been an exponential growth in the field of Herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. One such medicinal plant is Juglans regia (Juglandaceae), which is commonly known as walnut. All parts of plant are important viz. bark, leaves, flowers, seed, oil etc. Oil of this plant is extensively used in ayurveda, unani, homeopathic and allopathic system of medicines. Traditionally the plant is used as laxative, purgative, fertilizer and fungicide etc. whereas the plant possess beneficial effects such as anti-oxidant, antihistamic, antinociceptive, antiasthmatic, antiulcer, immunemodulatory, antidiabetic, hepatoprotective, antifertility, anti-inflammatory, antimicrobial, central nervous system stimulant, lipolytic, wound healing, insecticidal and larvicidal and many other medicinal properties. This activity of the plant possess due to the important phytochemical constituents like flavonoids, saponins, glycosides, alkaloids and steroids etc. The aim of this paper is to explain the details of phyto-pharmacological properties of Juglans regia for the future research work.

KEYWORDS: Juglans regia, phytoconstutents, traditional uses, bioactivity, clinical trial.

INTRODUCTION

Walnuts are the oldest tree food known to man, dating back to 7000 B.C. The Romans called walnuts Juglans regia, “Jupiter’s royal acorn.” Early history indicates that English walnuts came from ancient Persia, where they were reserved for royalty. Thus, the walnut is often known as the “Persian Walnut.” Walnuts were traded along the Silk Road route between Asia and the Middle East. Caravans carried walnuts to far off lands and eventually through sea trade, spreading the popularity of the walnut around the world. English merchant marines transported the product for trade to ports around the world. The bark is smooth, olive-brown when young and silvery-grey on older branches, and features scattered broad fissures with a rougher texture. Like all walnuts, the pith of the twigs contains air spaces; this chambered pith is brownish in color. The leaves are alternately arranged, 25–40 cm long, odd-pinnate with 5–9 leaflets, paired alternately with one terminal leaflet. The largest leaflets are the three at the apex, 10–18 cm long, and 6–8 cm broad; the basal pair of leaflets are much smaller, 5–8 cm long, with the margins of the leaflets entire. The male flowers are in drooping catkins 5–10 cm long, and the female flowers are terminal, in clusters of two to five, ripening in the autumn into a fruit with a green, semi-fleshy husk and a brown, corrugated nut (Fig. 1). The whole fruit, including the husk, falls in

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autumn; the seed is large, with a relatively thin shell, and edible, with a rich flavour.

**HABITAT**

*Juglans regia* is native to the mountain ranges of Central Asia, extending from Xinjiang province of 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, Turkmenistan and Iran to portions of Azerbaijan, Armenia, Georgia and eastern Turkey. In these countries, there is a great genetic diversity, in particular ancestral forms with lateral fruiting. During its migration to western Europe, the common walnut lost this character and became large trees with terminal fruiting. A small remnant population of these *J. regia* trees (Fig. 2) have survived the last glacial period in Southern Europe; but the bulk of the wild germplasm found in the Balkan peninsula and much of Turkey was most likely introduced from eastern Turkey by commerce and settlement several thousand years ago.

**TAXONOMICAL CLASSIFICATION**

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

**Walnut composition and nutritional value:**

Walnut has been used globally in human nutrition since ancient times. The high protein and oil contents of the kernels of *Juglans regia* L. (Juglandaceae) make this fruit indispensable for human nutrition. Therefore, the walnut is classified as a strategic species for human nutrition and is included in the FAO list of priority plants [1]. The seed part of the fruit (kernel) is consumed fresh, toasted, or mixed with other confectionaries. In the Middle East walnuts are added alone or along with almonds, date, and raisin as a special pastry preparation called Ma'moul. Walnuts are nutrient-rich food due to high contents of fats, proteins, vitamins and minerals. They are also good source of flavonoids, sterols, pectic substances, phenolic acids and related polyphenols. The nutritional contents differs from a cultivar to another which can be influenced by genotype, cultivator, different ecology and different soil [2-5]. The major components of walnut oil are triacylglycerols (980 g/kg oil), in which monounsaturated fatty acids (FAs) (mainly oleic acid) and polyunsaturated FAs (PUFAs; linoleic and α-linolenic acids) are present in high amounts in all genotypes (Table 1). Oil contents reported by [6] (78.83 to 82.4%) were higher than those reported by other researchers [7].

In general, the FA composition of walnut oil resembles that of soybean oil, but walnut oil contains a greater concentration of linolenic acid. In fact, among vegetable oils, walnut oil has one of the highest amounts of PUFAs (up to 78% of the total FA content). Walnuts have high amount of omega-6 and omega-3 PUFA, which are essential dietary fatty acids. Clinical studies suggest that omega-3 PUFA have significant role in prevention of coronary heart disease [8]. Oil rich in oleic acid displays greater oxidative stability therefore; it could be widely used as frying oil. According to an investigation conducted by several researchers, It was found that the average value for protein was 18.1% [9-11]. They are mainly composed of glutelins (about 70% of the total seed proteins) together with lesser amounts of globulins (18%), albumins (7%) and prolamins (5%).

The amino acid (AA) composition of walnut flour is dominated by the acidic AA residues of aspartate and glutamate together with relatively high levels of arginine. Walnut proteins contain all essential AAs required for the needs of a human adult. The lysine/arginine ratio in walnut proteins is lower than those observed in other common vegetable proteins, and this fact has been identified as a positive feature in the reduction of atherosclerosis development [12-13]. Walnut cultivars analyzed have recorded rich mineral composition, especially potassium, magnesium, and calcium. The minimum and maximum macro and micro nutrient contents of walnut are presented in Table 1 [14-17]. Walnuts contain high levels of potassium, phosphorus and magnesium and lower sodium. These elements play an important role for many enzymes activity especially as cofactor.

**ETHNOBOTANICAL USE**

*Juglans regia* leaves have been used mostly in worldwide traditional medicines as antimicrobial, antihelmintic, astringent, keratolytic, anti diarrhoeal, hypoglycaemic, depurative, tonic, carminative, and for the treatment of sinusitis, cold and stomach ache [18-20]. In Turkish folk medicine, fresh leaves applied on the naked body or forehead to reduce fever or on swelled joint to alleviate the rheumatic pain [21-22]. The kernel of *J. regia* has been used for the treatment of inflammatory bowel disease in Iranian traditional medicine [23]. In Palestine, it is used for treatment of diabetes and asthma [24-25] and to treat prostate and vascular disturbance [26]. 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 is used topically to treat scalp itching and
dandruff, sunburn and superficial burns as well as an adjunctive emollient in skin disorders [27-31]. It also has high anti-atherogenic potential and a remarkable osteoblastic activity that adds to the beneficial effect of a walnut enriched diet on cardio-protection and bone loss [32]. The bark, branches and exocarp of the immature green fruit of this medicinal plant have been used to treat gastric, liver and lung cancer a long time in China [33]. It is used by traditional healer in northeastern hophyton (H. pylori), inhibited by HpCG, HpFabD, and HpFabZ, with half maximal inhibitory concentration (IC50) values of 20±1, and 30±4 μmol/L, respectively. Therefore, HpCGS, HpFabD, and HpFabZ are considered to be the potential targets of juglone [47]. The antibacterial activity of Jordanian J. regia leaves extract to acne developing organism revealed that 12.5% S. epidermidis isolates were resistant to the leaf extract where as all Propionibacterium acnes isolates were sensitive even to 10% of the extract [42].

**ANTIFUNGAL ACTIVITY**

J. regia fruits, leaves and bark aqueous and solvents extract exhibited antifungal activity against wide range of fungi using disc diffusion method, agar dilution method, agar streak dilution and Raddish method. Pereira [6] reported that all the walnut varieties exhibited antifungal activity against Candida albicans and Cryptococcus neoformans when soxhleted with light petroleum ether (b.p. 40-60°C). The higher inhibition was observed with cv. Lara extract (MIC of 1 mg/mL). However, C. albicans and C. neoformans were only resistant to cv Mallanaise extract. Cold extraction of fruit, leaves and bark inhibited the growth of Microsporum canis, Trichophyton mentagrophytes, and Trichophyton violaceum [29]. On the other hand, the aqueous extract of green husks showed no antifungal activity against C. albicans and C. neoformans [40]. Methanol, acetone, chloroform and ethyl acetate bark extract revealed antifungal activity against A. niger, Alternaria alternata, Trichoderma viride, Fusarium solani, Pichia guilliermondii, Pichia jadinii and all Candida species tested [48-49].

**ANTIVIRAL ACTIVITY**

Mei-zhi et al [50] reported that 95% ethanol and ethyl acetate leaves extract of J. regia, inhibited tobacco mosaic virus (TMV). The methanol extract of J. regia inhibited Sindbis virus at a minimum concentration of 1.5 μg/ml [19].

**ANTIOXIDANT ACTIVITY**

The antioxidant potential of ethyl acetate, butanol, meta-nol, ether and aqueous methanol extract of walnut kernels, husks and leaves were measured by different methods such as reducing power, scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and lipid oxidation inhibition by β-carotene linolate system. All the extracts showed strong antioxidant activity [51-57]. Bullo et al. [58] reported a decrease in the antioxidant burden observed in enzymatic and non-enzymatic antioxidant systems after the consumption of a whole-walnut or a walnut-skin diet in C57BL/6 mice. The same author also reported that consumption of walnuts and walnut
skins have no deleterious effect on low-density lipoprotein (LDL) oxidizing capability, despite their higher contents of omega-6 PUFAs. Several phenolic compounds isolated from J. regia such as pyrogallol, p-hydroxybenzoic acid, vanillic acid, ethyl gallate, protocatechuic acid, gallic acid, 3,4,8,9,10-pentahydroxydibenzo pyran-6-one, tannins, glanssins, adenosine, adnine, etc, could provide a chemical basis for some of the health benefits claimed for J. regia in foods and folk medicine [59].

ANTIDIABETIC ACTIVITY

Fukuda et al. [60] demonstrated a strong inhibitory activity of walnut polyphenols and the polyphenolic components like Casuarictin, tellimagradin II and Tellimagradin I on different enzymes like glycoseidase, 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 200mg/kg/day. The consumption of walnut leaf pellets in alloxan induced diabetic rats at the dose of 185 mg/kg reduced fasting blood sugar significantly and the histomorphometric study of pancreas showed a sign of regeneration of β-cells in the treated group [61]. J. regia leaves methanolic extract at dose of 250 mg/kg decreases the postprandinal plasma blood glucose levels in both short and long term models. The plant extract significantly inhibited α-glucosidase activity in vitro for both maltase and sucrase enzymes and showed no changes in the insulin and glut-4 genes expression. The author attributed the inhibitory action of the plant extract to gallic acid and caffeoylquinic acid in the leaves.

ANTHELMINTIC ACTIVITY

Kale et al. [62] reported that stem park of J. regia Taha and Al-wadaan 5799 acetone extract exhibited significant activity at all dilution tested when compared to the Albendazole standard against Eicinia feotida. The benzene, methanol and ethanol soxhlet extracts of J. regia stem bark on adult Indian earthworm, Phoretima posthuma exhibited significant anthelmintic activity as comparable to that of standard drug Piperazine citrate [63]. The 95% ethanol, petroleum ether and ethyl acetate extract of green walnut hull have obvious anti-feeding effect on armyworm and the small vegetable-moth. The research group indicated that anti-feeding rate, death rates as well as growth inhibition rate of armyworm have correspondingly changed in dose dependant manner [64].

ANTI-INFLAMMATORY ACTIVITY

The ethanolic extracts of J. regia leaves exhibited potent anti-inflammatorv activity as potent as indomethacin against carrageenan-induced hind paw edema model in mice without inducing any gastric damage [65]. Mokhtari et al. [66] stated that the alcohol extract of walnut leaves in dose of 1.5 mg/kg caused a significant nociception decrease in acute phase of formalin test where as the aqueous (2.87 and 1.64 g/kg) and ethanolic (2.044 and 1.17 g/kg) extracts of leaves showed antinociceptive activity in hotplate test suggesting a promising analgesic and anti-inflammatory agents against diseases such as rheumatoid arthritis. On the basis of [51] result, a protective role of methanolic J. regia extract against CSE-induced acute lung toxicity in Wistar rats was suggested. The extract significantly decreased the levels of Lactate dehydrogenase (LDH), total cell count, total protein and increased the glutathione (GSH) level in bronchoalveolar lavage fluid. It also significantly restored the levels of Glutathione reductase (GR), catalase and reduced the xanthine oxidase (XO) activity in lung tissue.

ANTIDEPRESSANT ACTIVITY

The macerated hexane extract of J. regia fruit produced significant antidepressant activity at both doses of 100 and 150 mg/kg body weight when compared with standard drug fluoxetine on male Wistar rats. The antidepressant activity was evaluated by forced swimming and tail suspension test [67].

ANTITYROSINASE ACTIVITY

Ozer et al. [68] suggested that gel formulation containing ellagic acid and plant leaves extract of J. regia is effective in treating uneven skin pigmentation. The ethanolic leaves extract could be suggested as new sources of skin-whitening agents. Aitani and Shimoda [69] reported that melanin formation was inhibited at concentration 1 to 30 μg/ml in Pre-cultured B16 melanoma cells incubated with medium containing walnut polyphenols and their result indicated that walnut polyphenols is more superior to the popular skinlightening agent, ascorbic acid and arbutin upon data comparison.

HEPATOPROTECTIVE ACTIVITY

Orally fed Walnut polyphenols prepared from the kernelpellicle demonstrated a dose dependent lowering effect in glutamyl oxaloacetic transaminase (GOT) and glutamyl pyruvic transaminase (GPT) in carbon tetrachloride (CCl4)
induced liver damage in mice model after a single oral administration (200 g/kg). Result indicated that walnut polyphenols is more superior to Curcumin, a commonly used hepatoprotective agent. The effect of each active component of in vitro evaluation of walnut polyphenols on CCl4-induced cytotoxicity in primary cultured rat hepatocytes showed that tellimagrandin I, casuariatin, tellimagrandin II, and rugosin C (Figure 3) are inhibitory on CCl4-induced cytotoxicity in primary cultured rat hepatocytes however, tellimagrandin I of walnut polyphenols is believed to be the most important active compound responsible for hepatoprotective effect [70]. The same author, Hiroshi et al. [71] reported that 50% EtOH extract from endocarps of walnuts on mouse liver injury models induced by carbon tetrachloride at the dose of 100 and 200 mg/kg significantly suppressed GOT and GPT deviations. Polyphenolic constituents, tellimagrandins I and II, rugosin C and casuariatin were found to be principal constituents with hepatoprotective activity against oxidative damage.

HYPOTRIGLYCERIDEMIC ACTIVITY

Oral administration of a polyphenol-rich extract (WP) from walnuts (100 and 200 mg/kg) in high fat diet fed mice significantly reduced liver weight and serum triglycerides (TG) where as hepatic β-oxidation in cytosol, including peroxisome, was enhanced by WP (50-200 mg/kg). A polyphenol-rich extract was found to possess hypotriglyceridemic activity via enhancement of peroxisomal fatty acid β-oxidation in the liver. These results suggest that tellimagrandin I is involved in the hypotriglyceridemic mechanism [72].

ANTICANCER ACTIVITY

Juglone has been reported to inhibit intestinal carcinogenesis induced by azoxymethane in rats and might be a promising chemopreventive agent in human intestinal neoplasia [73]. Juglone was also proven to be a potent cytotoxic agent in vitro in human tumor cell lines, including human colon carcinoma (HCT-15) cells, human leukemia (HL-60) cells and doxorubicin-resistant human leukemia (HL-60R) cells [74–75]. In a recent study, Juglone inhibited the growth and induce apoptosis of sarcoma and 180 SGC-7901 cells in vivo. The mechanism is mediated by the activation of the mitochondrial death pathway, which requires the generation of reactive oxygen species (ROS), down-regulation of Bcl-2 protein expression and up-regulation of Bax protein expression [76]. Walnut methanolic extracts obtained from J. regia seed, green husk and leaf showed concentration dependent growth inhibition against human renal cancer cell lines A-498, 769-P and the colon cancer cell line Caco-2. Concerning A-498 renal cancer cells, all extracts exhibited similar growth inhibition activity (IC50 values between 0.226 and 0.291 mg/mL), while 769-P renal and Caco-2 colon cancer cells, walnut leaf extract showed a higher antiproliferative efficiency (IC50 values of 0.352 and 0.229 mg/mL, respectively) than green husk or seed extracts [52]. The tested dried fine powder of J. regia light petroleum seed extract in cancer induced in Swiss albino mice with the help of 7,12-Dimethylbenz(a)anthracene (DMBA) and croton oil showed the petroleum extract was significant in reducing the cancer cells [77].

OTHER MEDICINAL USES

Willis et al. [78] examined the effects of walnut diet on motor and cognitive ability in aged rats for 8 weeks. The three treated groups (2, 6 and 9%) revealed that the 2% walnut diet improved performance on rod walking, while the 6% walnut diet improved performance on the medium plank walk; the higher dose of the 9% walnut diet impaired reference memory, however the researcher attributed this to the number of polyphenolic compounds that could be negatively effecting reference memory at a higher dose. A 2004 study by the NYS Institute for Basic Research in Developmental Disabilities (OMRDD) revealed that methanolic extract of walnut was able to inhibit and defibrillize fibrillar amyloid β-protein (the principal component of amyloid plaques in the brains of patients with Alzheimer's). It is proposed that polyphenolic compounds present in walnuts may be responsible for its anti-amyloidogenic activity [79]. Similarly, it was found that two of its major components in walnuts, gallic and ellagic acid, act as "dual-inhibitors" of the enzyme acetylcholinesterase which, in association with amyloid inhibits protein aggregation, and inhibit the site of acetylcholinesterase responsible for the breakdown of acetylcholine. These results suggest that walnuts may reduce the risk or delay the onset of Alzheimer's disease by maintaining amyloid-protein in the soluble form and prevent the breakdown of acetylcholine [80].

CLINICAL STUDY

A daily intake of 43 to 57g of walnuts incorporated into Japanese diet for 4 weeks to 40 healthy Japanese men and women lowered blood cholesterol, particularly in women [81]. In double-blind case with either plasma triglyceride (TG) concentration more that 350 mg/dl or total cholesterol concentration more that 250 mg/dl were randomized into two groups, group A subject were
administered 6 capsules, each filled with 500 mg of the extracted walnut oil, per day for 45 days, group B individual serve as control and received placebo for 45 days. The result of this lowered plasma triglyceride level by 19 to 33%. [82-83] reported that substituting walnuts for monounsaturated fat in a Mediterranean diet improves endothelium-dependent vasodilation (EDV) in hypercholesterolemic subjects. A daily intake of 8-15 walnuts for 4 weeks significantly improves the EDV of 21 hypercholesterolemic males and females. On the other hand, walnut-enriched meals effectively prevented post prandial lipidemia where triacylglycerol was significantly reduced. [84] assessed the effect of walnuts on markers of prostate cancer between 45 and 75 years of age. Results suggest that walnuts improved serum γ-T and α-T; γ-T, two biomarkers that are important in prostate and vascular health. Total bilirubin, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), leucine aminopeptidase (LAP), gamma-glutamyltransferase (γ-glutamyltransferase), cholinesterase, γ-glutamyltranspeptidase (γ-GTP), amylase, lipase, Lecithin: cholesterol acyltransferase (L-CAT), LDL-cholesterol, total cholesterol, triglyceride, phospholipid, free fatty acid (FFA), high-density lipoprotein (HDL)-cholesterol, Na, K, serum Fe, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), urea nitrogen, uric acid, glucose, hemocyes revealed no abnormal reading for four male volunteers were given oral walnut polyphenols at the dose of 50 mg/day for 4 weeks [85].

**TOXICITY**

A review of the literature showed that juglone can cause irritant reactions as well as skin hyper pigmentation but, although it has been found to be a strong sensitizer in guinea pigs, contact allergy is considered a very rare event in man [86-87]. However, a case report of 65-year-old woman complaints of skin hyper pigmentation and large tense blisters involving the palms and fingers caused by the cumulative effect of 15 kilos of walnuts shelled in the 3 days was reported by [88]. Haque et al. [89] investigated the modulatory effects of walnut aqueous extract on the toxicity of an anticancer drug, cyclophosphamide (CP) with special reference to protection against disruption of drug metabolizing and antioxidant enzymes during the chemotherapy. The extract showed a significant increase in the activity and level of glutathione and glutathione peroxidase in both liver and kidney tissues and catalase in liver only. While the extract CP treated group showed a significant decrease in the lipid peroxidation in liver and kidneys when compared with the CP-treated group. Aqueous extract from *J. regia* leaves reduced 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) formation by about 60% at concentration of 500 μl/ml on HepG2 cell. Additionally, the co-culture of HepG2 with THP1 revealed no sign of any negative effect at all concentration tested after exposure to the extract. The investigator also reported no significant changes of LDH and albumin levels on the culture medium after 24 h of exposure to the extract [90]. Hosseinzadeh et al. [91] calculated the half-maximal lethal dose (LD50) values of intraperitoneal injection of *J. regia* aqueous and ethanolic leaves extract and found it to be 5.5 and 3.3 g/kg, respectively. Acute dermal toxicity studies showed that petroleum ether extract of *J. regia* gives lethal effect at 2000 mg/kg [77].

**OTHER USES**

The seeds contain unusual fatty acids which are industrially important, as they are used in protective coatings, dispersants, pharmaceuticals, cosmetics, soaps and a variety of synthetic intermediates as stabilizers in plastic formulations [92-93]. The wood is of very high quality, and is used to make furniture, and gunstocks. The dye is used as a coloring and tonic for dark hair [94]. The unripe fruits are pickled in vinegar [95].

**CONCLUSIONS**

The present review article documents the publications on walnut and its constituents in the recent and last few years. The paper highlights the traditional use of this plant and some scientific validation of the claimed biological activity *in vivo* as well as *in vitro*. To best of our knowledge and internet survey only one case of contact dermatitis was reported after shelling 15 kilos of walnuts. The toxicological studies of various secondary metabolites which contribute to its medicinal value are still in its infancy and are becoming an important limiting factor for utilizing the metabolites as therapeutic agent. Besides, isolation and characterization of active secondary metabolites responsible for various biological activities have not yet been structurally elucidated, mode of action, target organ of toxicity and molecular mechanism also need to be investigated. Further trials in humans are required to determine the efficacy of walnut extract or one or more of its constituents and to establish what, if any, adverse effects are observed.

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Fig: 1. *J. regia* seed

Fig: 2. *J. regia* tree

Juglone (Kong et al, 2008)

Juglanin (Liu et al, 2008)

Ellagic acid (Martine Z et al, 2010)

tellimagrandin I (1)
Tajamul et al., World J Pharm Sci 2014; 2(4): xxxx

Fig. 3: Structure of isolated compounds of *J. regia*

![Structure of isolated compounds of *J. regia*](image)

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