World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.com/
Review Article

Juglans regia Linn: A Phytopharmacological Review

Tajamul Islam Shah[1*], Ekta Sharma[2], Gowhar Ahmad[3]

[1]Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal-India
[2]Department of Botany, Banezeer College Bhopal-India
[3]Department of Zoology, Banezeer College Bhopal-India

Received: 07-11-2013 / Revised: 07-02-2014 / Accepted: 10-03-2014

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 *Juglana 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*, phytoconstituents, 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 and they became known as "English Walnuts." England, in fact, never grew walnuts commercially. The outer shell provided a natural protective layer helping to maintain the quality of the nut. Today the nut trade continues to be a well-established, ordered, and structured business, and the California walnut is well known as the top quality walnut for the world.

MORPHOLOGY

Juglans regia is a large, deciduous tree attaining heights of 25–35 m, and a trunk up to 2 m diameter, commonly with a short trunk and broad crown, though taller and narrower in dense forest competition. It is a light-demanding species, requiring full sun to grow well. 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, semifleshy husk and a brown, corrugatednut (Fig. 1). The whole fruit, including the husk, falls in

***Corresponding Author Address:** Tajamul Islam Shah, Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal-India; E mail: taju.zoology@gmail.com*

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 westernChina, parts of Kazakhstan, Uzbekistan and southern Kirghizia and from lower ranges of mountains in Nepal, Bhutan, Tibet, northern India, Pakistan and SriLanka, 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. (Juglandacea) 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, antidiarrhoeal, 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 cardioprotection 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 region of Mexico to protect against liver damage [34]. The bark is used as miswaks for teeth cleaning [35]. In Nepal the bark paste is useful in arthritis, skin diseases, toothache, and hair growth. Seed coat is used for healing wounds [36]. The shell of *Juglans regia* is used in Calabria folk medicine to heal malaria [37].

PHYTO-PHARMACOLOGY

ANTIBACTERIAL ACTIVITY

Hot and cold solvent and aqueous extract of leaves, barks, fruits and green husks of *J. regia* from different countries revealed broad spectrum antibacterial activity against gram-positive and gram-negative bacteria viz. *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus epidermidis, Micrococcus luteus, Salmonella typhimurium, Enterococcus faecalis, Bacillus thuringiensis, Protomonas extroquens,* and Proteus sp. using agar streak method and disc diffusion method [38-44]. 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. pneumoniae* (MIC of 100 mg/mL), cv. Mayette inhibited the development of *P. aeruginosa* and *E. coli* with minimum inhibitory concentrations (MICs) of 50 and 10 mg/mL, respectively, and cv. Mellanaise inhibited the growth of *E. coli* and *K. pneumoniae* at concentration of 100 mg/mL [29]. Mexican aqueous bark and leaves extract exhibited no antimycobacterial activity. Only the hexane and methanol extract showed antimycobacterial activity with MIC of 100 and 125 mg/ml, respectively using Soxhlet extractor [45]. Over 45% of Iranian clinical isolates of *Helicobacter pylori* strain were inhibited by *J. regia* aqueous and equal mixture of methanol, diethyl ether and petroleum benzene extract [46]. In a recent study, juglone was shown to potently inhibit the three key enzymes from *Helicobacter pylori*, cystathionine γ-synthase (HpCGS), malonyl-CoAacyl carrier protein transacylase (HpFabD), and β-hydroxyacyl-ACP dehydratase (HpFabZ) with the half maximal inhibitory concentration (IC50) values of 7.0±0.7, 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, Trihoderma viresn, fusarium solani, Pichia guiliermondii, Pichia jadinii* and all Candida speices 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 linoleate 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, glansrins, adenosine, adenine, 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 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 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, *Pheretima 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-inflammatory 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 skin-lightening 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, casuarictin, 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 mice 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 casuarictin 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 8-13 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-glutamyltranspeptidase (γ-GTP), cholinesterase, 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, hemocytes 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-2yl)-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.

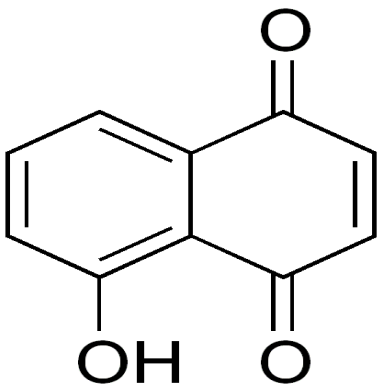
ACKNOWLEDGEMENT

The authors extend their appreciation to the Head, Deptt. Of Research Jawaharlal Nehru Cancer

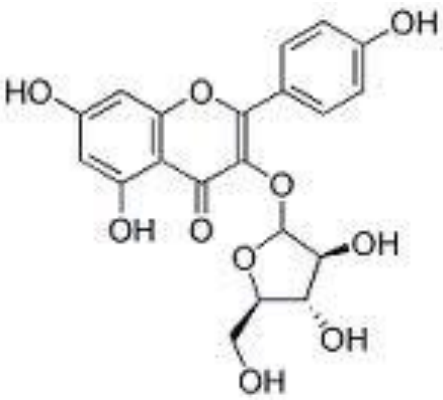
Hospital & Research Centre Bhopal-India for being supportive in every aspect.

Fig: 1. *J. regia* seed

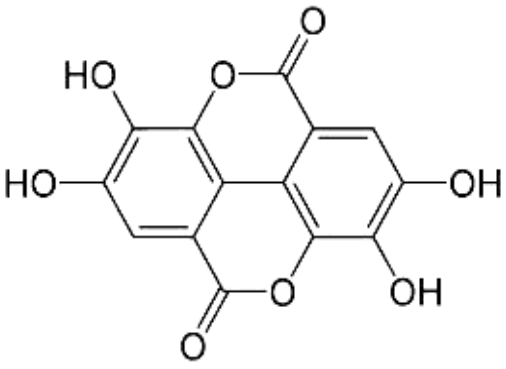
Fig: 2. *J. regia* tree



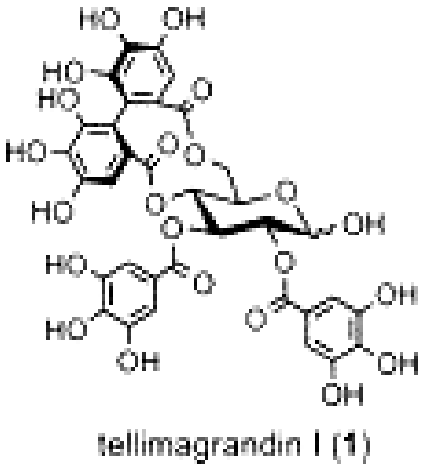
Juglone (Kong et al, 2008)



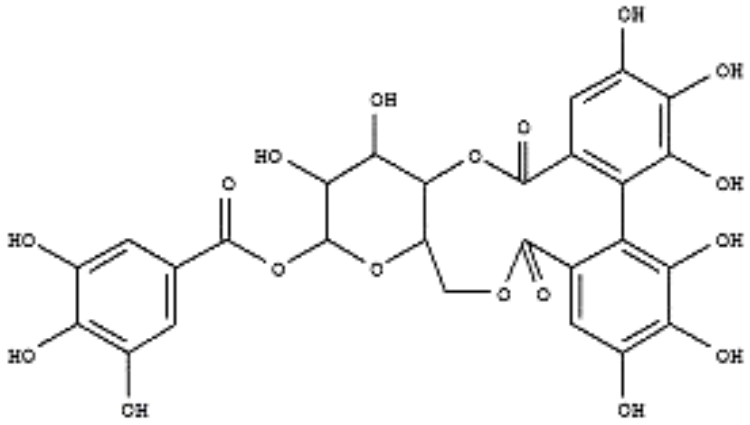
Juglanin (Liu et al, 2008)



Ellagic acid (Martine Z et al, 2010)



tellimagrandin I (1)



Strictinin (Shimoda et al, 2009)

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

REFERENCES

1. Gandev, S. Budding and grafting of the walnut (Juglans regia L.) and their effectiveness in Bulgaria (Review). Bulgar. J. Agri. Sci., 2007, 13:683-689.
2. Caglarirmak N. Biochemical and physical properties of some walnut genotypes (Juglans regia L). Nahrung Food. 2003, 47:28–32.
3. Crews C, et al. Study of the main constituents of some authentic walnut oils. J. Agric. Food. Chem., 2005, 53:4853-4860.
4. Martinez ML, et al. Walnut (Juglans regia L.): genetic resources, chemistry, by-products. J. Sci. Food. Agric., 2010, 90: 1959-1967.
5. Muradoglu FH, et al. Some chemical composition of walnut (Juglans regia L.) selections from Eastern Turkey. Afr. J. Agric. Res., 2010, 5: 2379-2385.
6. Pereira JA, et al. Bioactive properties and chemical composition of six walnut (Juglans regia L.) cultivars. Food Chem. Toxicol., 2008, 46: 2103-2111.
7. Savage GP. Chemical composition of walnuts (Juglans regia L.) grown in New Zealand. Plant Foods Hum. Nutr., 2001, 56: 75-82.
8. Davis L, et al. The effects of high walnut and cashew nut diets on the antioxidant status of subjects with metabolic syndrome. Eur. J. Nutr., 2007, 46: 155-164.
9. Amaral JS, et al. Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (Juglans regia L.) cultivars grown in Portugal. J. Agric. Food Chem., 2003, 51: 7698-7702.
10. Muradolu F. Selection of promosing genotypes in native walnut (Juglans regia L.) populations of Hakkari central and Ahlat (Bitlis) districht, and genetic diversty. PhD dissertation, University of Yuzuncu Yil, Turkey. 2005.
11. Mitrovic M, et al. Biochemical composition of fruits of some important walnut cultivars and selections. Proceeding of the third International walnut held at Alcobaca, Portugal, Congress. Acta. Hortcult; 1997, 442: 205- 207.
12. Sze-Tao and Sathe SK. Walnut (Juglans regia L): proximate composition, protein solubility, protein amino acid composition and protein in vitro digestibility. J. Sci. Food Agric., 2000, 80:1393–1401.
13. Venkatachakm M, and Sathe SK. Chemical composition of selected edible nut seeds. J. Agric. Food. Chem., 2006, 54:4705–4714.
14. Ravai M. Quality characteristics of califonia walnuts. Cereal Foods World; 2009, 37: 362-366.
15. Payne T. California walnuts and light food. Cereal Foods World ; 1985, 30: 215-218.
16. Souci SW, et al. Food composition and nutrition tables. Medpharm, CRC Press, Stuttgart. 1994.
17. Cosmulescu S, et al. Mineral composition of fruits in different walnut (Juglans regia L.) Cultivars. Not. Bot. Hort. Agrobot. Cluj., 2009, 37:156-160.
18. Girzu M, et al. Sedative effect of walnut leaf extract and juglone, an isolated constituents. Pharm. Biol., 1998, 36: 280-286.
19. Mouhajir F, et al. Multiple antiviral activities of endemic medicinal plants used by Berber people of Morocco. Pharm. Biol., 2001, 39: 364-374.
20. Vaidyaratnam PSV. Indian Medicinal Plants a Compendium of 500 species. Orient Longman Private Limited, Chennai 2005, 3: 264-65.
21. Fujita T, et al. Traditional medicine in Turkey VII. Folk medicine in Middle and West Black Sea regions. Econ. Bot., 1995, 49: 406-422.
22. Yesilada E. Biodiversity in Turkish Folk Medicine. In: Sener, B. (Ed.), Biodiversity: Biomolecular Aspects of Biodiversity and Innovative Utilization. Kluwer Academic/Plenum Publishers, London, 2002, pp. 119–135.
23. Kim HG, et al. Growth-inhibiting activity of active component isolated from Terminalia chebula fruits against intestinal bacteria. J. Food Prot., 2006, 69:2205-2209.

24. Jaradat NA. Medical plants utilized in Palestinian folk medicine for treatment of diabetes mellitus and cardiac diseases. J. Al-Aqsa Unv., 2005, 19:1-28
25. Kaileh Mb, et al. Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity J. Ethnopharmacol., 2007, 113: 510-516
26. Spaccarotella KJ, et al. The effect of walnut intake on factors related to prostate and vascular health in older men. Nutr 2008, . J.,7:13.
27. Gruenwald J. et al. PDR for Herbal Medicines, Medicinal Economic. 2001.
28. Robbers JE and Tyler VE. Tyler's Herbs of Choise: The therapeutic use of phytomedicinals, The Havvorth Herbal Press, New York; 1999.
29. Ali-Shtayeh MS and Abu Ghdeib SI. Antifungal activity of plant extracts against dermatophytes. Mycoses., 1999, 42: 665-772.
30. Blumenthal M. Herbal Medicine (Expanded Commision E Monographs), Integrative Medicine Communications, Newton Publisher, England. 2000.
31. Baytop T. Therapy with Medicinal Plants in Turkey (Past and Present), 2nd Ed., Nobel Medicine Publisher, Turkey. 1999.
32. Papoutsi Z, et al. Walnut extract (Juglans regia L.) and its component ellagic acid exhibit anti-inflammatory activity in human aorta endothelial cells and osteoblastic activity in the cell line KS483. British J. Nutr., 2008, 99:715-722.
33. Liu L, et al. Newalpha-tetralonylglucosides from the fruit of Juglans mandshurica. Chem.Pharm.Bull. Tokyo; 2004, 52:566-569.
34. Torres-Gonzalez. Protective effect of four Mexican plants against CCl4 –induced damage in the hyh7 human hepatoma cell. Annals hematology., 2011, 10:73-79.
35. Ibrar MFH, Sultan A. Ethnobotanical studies on plant resources of Ranyal Hill, District Shangla, Pakistan. Pak.J.Bot., 2007, 39:329-337.
36. Kunwar RM, and Adhikari N. Ethnomedicine of Dolpa district, Nepal: the plants, their vernacular names and uses. J. Ecol. App., 2005, 8:43-49
37. Tagarelli G, et al. Folk medicine used to heal malaria in Calabria (southern Italy). J. Ethnobiol. Ethnomed., 2010, 6:27 Teimori
38. Deshpande RR, et al. Antimicrobial Activity Of different extracts of Juglans Regia L. against Oral Microflora. Int. J. Pharm. Pharm. Sci., 2011, 3:200-201.
39. Poyrazolu EC, and Biyik H. Antimicrobial activity of the ethanol extracts of some plants natural growing in Aydin, Turkey. Afr. J. Microbiol. Res., 2010, 4: 2318-2323.
40. Oliveira I, et al. Total phenols, antioxidant potential and antimicrobial activity of walnut (Juglans regia L.) green husks. Food Chem. Toxicol., 2008, 46: 2326-2331.
41. Qa'dan F, et al. Characterization of antimicrobial polymeric procyanidins from Juglans regia leaf extract. Eur. J. Sci. Res., 2005a, 11:438-443.
42. Qa'dan F, et al. The Antimicrobial Activities of Psidium guajava and Juglans regia Leaf Extracts to acne-developing organisms. Am. J. Chin. Med., 2005b, 33: 197–204.
43. Citoglu GS and Altanlar N . Antimicrobial activity of some plants used in folk medicine. J. Fac. Pharm. Ankara., 2003, 32:159-163. Company, New York.
44. Shah T.I, et al. Prelimnary phytochemical evaluation and anti-bacterial potential of different leaf extracts of *Juglans regia*: A ubiquitous dry fruit from Kashmir-India. Int.J.Pharm.Sci.Rev.Res; 19(2), Mar-Apr 2003; 93-96.
45. Cruz-Vega DE, et al. Antimycobacterial activity of Juglans regia, Juglans mollis, Carya illinoensis and Bocconia frutescens. Phytother. Res., 2008, 22:557-559.
46. Nariman F, et al. Anti-Helicobacter pylori activities of six Iranian Plants. Helicobacter., 2004, 9:2.
47. Kong Y, et al. Natural product Juglone targets three key enzymes from Helicobacter pylori: inhibition assay with crystal structure characterization. Acta Pharmacologica Sinica., 2008, 29: 870-876.
48. Upadhyay V, et al. Antifungal activity and preliminary phytochemical analysis of stem bark extracts of Juglans regia linn. IJPBA., 2010c, 1:442-447
49. Ahmad S, et al. Fungistatic Action of Juglans. Antimicrob. Agents Chemother., 1973, 3:436-438.
50. Mei-zhi Z, et al. Study on Extraction Conditions of Active Antiviral Substance from Walnut Leaves. Chemistry and Industry of Forest Products. 02 [Abstract]. 2007.
51. Qamar W, and Sultana S. Polyphenols from Juglans regia L. (Walnut) kernel modulate cigarette smoke extract induced acute inflammation, oxidative stress and lung injury in Wistar rats. Hum. Exp. Toxicol., 2011, 30:499-506.
52. Carvalho M, et al. Human cancer cell antiproliferative and antioxidant activities of Juglans regia L. Food Chem. Toxicol., 2010, 48: 441-447.
53. Abbasi MA, et al. Investigation on the volatile constituents of Juglans regia and their in vitro antioxidant potential. Pakistan Acad. Sci., 2010, 47:137-141.
54. Rahimipanah M, et al. Antioxidant activity and phenolic contents of Persian walnut (Juglans regia L.) green husk extract. Afr. J. Food Sci. Technol., 2010, 1:105-111.
55. Zhang Z, et al. Antioxidant phenolic compounds from walnut kernels (Juglans regia L.). Food Chem., 2009b, 113: 160-165.
56. Almeida IF, et al. Walnut (Juglans regia) leaf extracts are strong scavengers of pro-oxidant reactive species. Food Chem., 2008, 106:1014-1020.
57. Fukuda T, et al. Antioxidative polyphenols from walnuts (Juglans regia L.) Phytochem., 2003, 63: 795-801.
58. Bullo M, et al. Effect of whole walnuts and walnut-skin extracts on oxidant status in mice. J. Nutr., 2010, 26: 823-828.
59. Zhang J, et al. Chemical constituents in green walnut husks of Juglans regia. Chinese Traditional and Herbal Drugs. 2009a, 06 [Abstract].
60. Fukuda T, et al. Effect of the walnut polyphenol fraction on oxidative stress in type 33 2 diabetes mice. Biofactors., 2004, 2: 251-253.
61. Jelodar G, et al. Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan induced diabetic rats. Afr. J. Trad. CAM., 2007, 43: 299-305.
62. Kale AA, et al. In vitro anthelmintic activity of stem bark of Juglans regia L. J. Chem. Pharm. Res., 2011, 3:298-302.
63. Upadhyay V, et al. Anthelmintic activity of the stem bark of Juglans regia Linn. Res J. Pharm. Phytochem. (RJPP) 2010a, 2: 465-467
64. Mei-zhi Z, et al. A Study on the Bioactivity of Secondary Metabolites from Walnut Green Gull University. Journal of Northwest Forestry University-01 [Abstract]. 2006.
65. Erdemoglu N, et al. Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine. J. Ethnopharmacol., 2003, 89: 123-129.

66. Mokhtari M, et al. Effect of alcohol extract from leave Juglans regia on antinociceptive induced by morphine in formalin test. Med. Sci. J. Islam. Azad. Uni., 2008, 18: 85-90.
67. Rath BP and Pradhan D. Antidepressant Activity of Juglans regia L. fruit extract. Int. J. Toxicol. Pharmacol. Res., 1992, 1: 24-26.
68. Ozer B, and Kivc MB. Antityrosinase activity of some plant extracts and formulations containing ellagic acid. Pharm. Biol., 2007, 5: 519-524.
69. Aitani M and Shimoda H. The Effect of Ascorbic Acid and Arbutin on B16 Melanoma cells. Japan Food Sci., 2005, 44: 58-63.
70. Hiroshi S, et al. Walnut polyphenols prevent liver damage induced by carbon tetrachloride and d-galactosamine: hepatoprotective hydrolyzable tannins in the kernel pellicles of walnut. J. Agric. Food Chem., 2008, 56: 4444-4449.
71. Hiroshi S, et al. Hepatoprotective constituents in endocarps of walnut. J. Pharm. Soc. Japan. 2006, 126:108-109.
72. Shimoda H, et al. Effect of polyphenol-rich extract from walnut on diet-induced hypertriglyceridemia in mice via enhancement of fatty acid oxidation in the liver. J. Agric. Food Chem., 2009, 57:1786-92.
73. Sugie S, et al. Inhibitory effects of plumbagin and juglone on azoxymethane- induced intestinal carcinogenesis in rats. Cancer Lett., 1998, 127:177–183.
74. Kamei H, et al. Inhibition of cell growth in culture by quinones. Cancer Biother Radiopharm., 1998, 13:185–8.
75. Segura-Aguilar J, et al. The cytotoxic effects of 5-OH-1, 4-naphthoquinone and 5, 8-diOH-1,4-naphthoquinone on doxorubicin-resistant human leukemia cells (HL-60). Leuk Res., 1992, 16: 631–637.
76. Ji Y, et al. Juglone induced apoptosis in human gastric cancer SGC-7901cells via the mitochondrial pathway. Exp. Toxicol. Pathol., 2011, 63: 69-78.
77. Kumudhavalli MV, et al. Phytochemical and pharmacological evaluation of the dried fruit of the plant Juglans regia linn. Oil Drug Invent. Today 2010, 2: 362-365.
78. Willis L, et al. Dose-dependent effects of walnuts on motor and cognitive function in aged rats. Br. J. Nutr., 2009, 101: 1140-1144.
79. Chauhan N et al. Walnut extract inhibits the fibrillization of amyloid beta-protein, and also defibrillizes its preformed fibrils. Cur. Alzheimer Res., 2004, 1:183-188.
80. Society for Neuroscience. "News Release: Diet of walnuts, blueberries improve cognition; may help maintain brain function", Society for Neuroscience, 5 November 2007.
81. Iwamoto M, et al. Walnuts lower serum cholesterol in Japanese men and women. J. Nutr., 2000, 130: 171-176.
82. Zibaeenezhad MJ, et al. Antihypertriglyceridemic effect of walnut oil. Angiology., 2003, 54: 4.
83. Ros E, et al. Walnut diet improves endothelial functions in hypercholesterolemic subject. Circulation; 2004, 109: 1609-1614.
84. Bellido C, et al. Butter and walnuts, but not olive oil, elicit postprandial activation of nuclear transcription factor κB in peripheral blood mononuclear cells from healthy men. Am. J. Clin. Nutr., 2004, 80: 1487-1491.
85. Oryza. Hepatoprotective and anti-oxidative extract for metabolic syndrome walnut polyphenol. ver.1.0. 2007.
86. Woods B, and Calnan CD. Toxic woods. Br. J. Dermatol., 1976, 94 (suppl, 13): 17.
87. Hausen B. Woods injurious to human health. Berlin: Publisher, Walter de Gruyter, 1981, pp 119–121.
88. Bonamonte D, et al. Hyperpigmentation and contact dermatitis due to Juglans regia. Contact Dermatitis., 2001, 44: 101.
89. Haque R, et al. Aqueous extract of walnut (Juglans regia L.) protects mice against cyclophosphamide-induced biochemical toxicity. Hum. Exp. Toxicol., 2003, 22:473-80.
90. Saad B, et al. Evaluation of medicinal plant hepatotoxicity in co-cultures of hepatocytes and monocytes. eCAM., 3:93-98. Salicornia Brachiata, Ind. Crops Prod., 2006, 23:177.
91. Hosseinzadeh H, et al. Antinociceptive, anti-Inflammatory and acute toxicity effects of juglans regia L. Leaves in mice. Iran Red Crescent Med. J., 2011, 13:27-33.
92. Hosamani, KM, and Sattigeri RM. Industrial utilization of Rivea Ornata seed oil: A moderate source of vernolic acid. Ind. Crops Prod., 2000, 12: 93.
93. Eganathan P, et al. Oil Analysis in Seeds of Salicornia Brachiata, Ind. Crops Prod., 2006, 23:177.
94. Brwon D. Encyclopedia of herbs and their uses. Dorling Kindersley publishers, London. 1995.
95. Facciola S. A source book of edible plants. kampong Publisher,USA. 1990.