Biological activities of *Juglans regia* flowers

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**Abstract:** Antidepressant, anti-inflammatory, antihypoxic and antioxidant activities of methanol extract of *Juglans regia* L., Juglandaceae, flower were investigated. Antidepressant activity was examined by forced swimming test and tail suspension test in mice. Antihypoxic activity was investigated in haemic and circulatory models. The effects were pronounced in both models. It produced statistically significant anti-inflammatory activity in carrageenan induced edema at nearly all doses, when compared to control groups. IC50 for DPPH radical-scavenging activity was 674±27.6 μg mL⁻¹. Extract showed good Fe²⁺ chelating ability (IC50 43±1.5 μg mL⁻¹). It exhibited low antioxidant activity in linoleic acid peroxidation test. Its pharmacological effects may be attributed, in part, to the presence of phenols and flavonoids in the extract.

**Keywords:** antidepressant activity, antihypoxic activity antioxidant activity *Juglans regia*

**Introduction**

*Juglans regia* L. (Persian walnut) is a deciduous tree from Juglandaceae family. Its fruits are consumed as food, which are rich unsaturated fatty acids. Walnut leaf has been widely used in traditional medicine for the treatment of skin inflammations and ulcers and for its anti diarrheic, anti helminthic, antiseptic and astringent properties (Almeida et al., 2008). Anti-inflammatory, antinociceptive and anti diabetic activities of walnut leaves (Erdemoglu et al., 2003; Asgary et al., 2008), antioxidant effects of its seeds and radical scavenging of leaves have been previously reported (Almeida et al., 2008, Fukuda et al., 2003). However, there is no scientific report on biological activity of *J. regia* flower. In the present study, the antioxidant, antidepressant, anti-inflammatory and anhypoxic activities of methanol extract of *J. regia* flowers were evaluated. Its gallic acid, coumarin and quercetin contents were determined by HPLC/DAD. A possible relationship between the chemical composition and the biological and pharmacological potential was considered.

**Materials and Methods**

*Plant material and preparation of freeze-dried extract*

*Juglans regia* L., Juglandaceae, flower was collected from Dashtenaz area, Sari, Iran, in 2008 and identified by Dr. B. Eslami. A voucher specimen was deposited with the Sari school of pharmacy herbarium (No 629). Sample was dried at room temperature (r.t.) and ground before extraction. 100 g of sample was extracted by percolation with methanol (400 mL × 3) for 24 h. The resultant extracts were concentrated in a rotary evaporator until a crude solid extract was obtained (24%).

**Animals**

Male Swiss albino mice (20-24 g) or male Wistar rats (180-220 g), obtained from Institute Pasteur of Iran were used in this study. The animals were housed in standard cages with free access to food and water. Experimental protocols met the guidelines of animal experimentation approved by the commission of ethics in animal experimentation of Mazandaran University of Medical Sciences. All experiments were conducted between 10 am and 2 pm.

**Anti-inflammatory effect**

Carrageenan-induced hind paw edema model and toxicity test were carried out according...
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Different doses of extract were injected to separated groups of seven. After 48 h, the highest dose that did not induce any mortality was considered as the maximum non-fatal dose (Ebrahimzadeh et al., 2010c).

### Determination of total phenolic compounds and flavonoid content

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Ghasemi et al., 2009). Results were expressed as gallic acid equivalent from a calibration curve. Total flavonoids were estimated as previously described (Ghasemi et al., 2009). Total flavonoid contents were calculated as quercetin equivalent from a calibration curve.

### Antioxidant activity

#### DPPH radical scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the extracts (Dehpour et al., 2009). Vitamin C, BHA and quercetin were used as standard controls.

#### Determination of metal chelating activity

The ability of extract to chelate ferrous ions was estimated by our recently published papers (Ebrahimzadeh et al., 2008; Ebrahimzadeh et al., 2009). The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as \( \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100 \), where \( A_0 \) was the absorbance of the control and \( A_1 \) was the absorbance of the mixture, containing the extract or the absorbance of EDTA.

#### Assay of nitric oxide-scavenging activity

Sodium nitroprusside (10 mM) was mixed with different concentrations of extract dissolved in water and incubated at r.t. for 150 min. After the incubation period, 0.5 mL of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as positive control (Ebrahimzadeh et al., 2010b; Ebrahimzadeh et al., 2010d).

#### Determination of antioxidant activity by the FTC Method

The method was adopted from our recent paper (Nabavi et al., 2008). The absorbance of final red solution was measured at 500 nm and was measured again every 24 h until when the absorbance of the control reached the maximum value. The percent inhibition of peroxidation was calculated as: \( \text{(％) inhibition} = 100 - \left[ \frac{\text{absorbance}}{\text{absorbance}} \right] \times 100 \).
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The amount of some flavonoids detected in *Juglans regia* flower extract.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Quercetin</th>
<th>Gallic acid</th>
<th>Coumarin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Juglan regia</em></td>
<td>0.175</td>
<td>0.387</td>
<td>trace</td>
</tr>
</tbody>
</table>

Trace: concentration<0.10 μg/mg.

Phenols and polyphenolic compounds, such as flavonoids have been shown to possess significant antioxidant activities (Ebrahimzadeh et al., 2010a). Extract produced statistically significant inhibition of edema induced by carrageenan at nearly all doses when compared to the control groups (Table 2). The effect was dose-dependent. The highest activity showed at 1000 mg/kg *i.p.* that inhibited 77% of inflammation. The same activity was found for diclofenac at 100 mg/kg *i.p.* (73%) (*p*>0.05). The Carrageenin test is highly sensitive to non-steroidal anti-inflammatory drugs, and has long been accepted as a useful tool for investigating new drug therapies (Just et al., 1998). Extract produced statistically significant inhibition of edema at all doses when compared to the control groups (Table 2).

Anti-inflammatory activity of gallic acid (Kroes et al., 1992) and quercetin (Morikawa et al., 2003) were reported previously and we believe that the anti-inflammatory activity of extract might be due to the presence of this or these compounds. Extract exhibits no toxicity up to 4 g/kg body weight when injected *i.p.* in mice. A statistically significant antihypoxic activity of the extract was established in the experimental model of haemic and circulatory hypoxia in mice. The effects were found to be dose-dependent in both tests (Table 3). Administration of sodium fluoride increases the blood histamine content and decreases the oxygen carrying capacity (Sumina et al., 1978).

A significant protective effect on hypoxia has been reported by plants contains flavonoids (Karcher et al., 1984). Our results may be supported by other literature data that flavonoids increase cerebral blood flow and possess antihypoxic activity. The mechanism of this protective action may be in part due to the antioxidant activity of quercetin (Meli et al., 1990). The extract showed significant protective effect against hypoxia. In FST, extract at doses of 1000 and 1500 mg/kg significantly and dose dependently reduced the immobility period to 110.1±4.1 and 90.2±6.6 s, respectively as compared to control mice of 164.2±11.3 s (*p*<0.001), imipramine showed significant anti-immobility activity with immobility time of 88.0±7.3 s in comparison to its corresponding control group (164.2±11.3 s) (*p*<0.001). Extract at 1500 mg/kg showed the same activity as imipramine (*p*>0.05). In TST model, extract (500 and 1000 mg/kg) decreased significantly and dose dependently the immobility time.
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Table 2. Anti-inflammatory activity of *Juglans regia* flower on carrageenan induced paw edema in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg i.p.)</th>
<th>Initial paw thickness (cm)(b)</th>
<th>Paw thickness after 3 h (cm)(a)</th>
<th>a/b ratio$^a$</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>Vehicle</td>
<td>0.20±0.02</td>
<td>0.46±0.02</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>250</td>
<td>0.20±0.02</td>
<td>0.44±0.03</td>
<td>2.2$^{**}$</td>
<td>7.7</td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>500</td>
<td>0.30±0.02</td>
<td>0.48±0.02</td>
<td>1.7$^{***}$</td>
<td>30.7</td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>750</td>
<td>0.28±0.03</td>
<td>0.42±0.02</td>
<td>1.5$^{***}$</td>
<td>46.2</td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>1000</td>
<td>0.30±0.04</td>
<td>0.36±0.01</td>
<td>1.2$^{***}$</td>
<td>76.9</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1000</td>
<td>0.23±0.02</td>
<td>0.30±0.02</td>
<td>1.30$^{***}$</td>
<td>73.1</td>
</tr>
</tbody>
</table>

$^a$A ratio less than 1.5 was considered to be a significant inhibitory effect. Values are mean±SD (n=6), NS, not significant, **p<0.001 with respect to control.

Table 3. Anti-hypoxic activity of *Juglans regia* flower in the different tests.

<table>
<thead>
<tr>
<th>Group</th>
<th>Doses (mg/kg)</th>
<th>Sodium nitrite test (min)</th>
<th>Sodium fluoride test (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.50±0.30</td>
<td>9.36±0.32</td>
<td></td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>31.25</td>
<td>10.08±0.10***</td>
<td></td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>62.5</td>
<td>10.52±0.60****</td>
<td>10.23±0.11*</td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>125</td>
<td>12.03±0.39****</td>
<td>11.71±0.37***</td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>250</td>
<td>15.63±0.65***</td>
<td></td>
</tr>
</tbody>
</table>

Each group represents the mean±SD (n=10). *Not significant, **p<0.05, ***p<0.01 and ****p<0.001 vs. control.

Table 4. Effect of methanol extract of *Juglans regia* flower on the duration of immobility during forced swimming test and Tail suspension test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Doses (mg/kg)</th>
<th>Duration of immobility (s), FST</th>
<th>Duration of immobility (s), TST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>164.2±11.3</td>
<td>157.8±12</td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>250</td>
<td>154.67±12.9*</td>
<td>141.6±7.8*</td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>500</td>
<td>122.89±8.4**</td>
<td>114.3±15.1**</td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>1000</td>
<td>110.10±6.2**</td>
<td>79.38±5.4**</td>
</tr>
<tr>
<td><em>J. regia</em></td>
<td>1500</td>
<td>90.14±6.6**</td>
<td>-</td>
</tr>
<tr>
<td>Imipramine</td>
<td>15</td>
<td>88±7.3**</td>
<td>74.2±6.3**</td>
</tr>
</tbody>
</table>

Each group represents the mean±SD (n=10). *p<0.05 and **p<0.001 vs. control.

**Conclusion**

*J. regia* flower showed remarkable antihypoxic, anti-inflammatory, antioxidative and antidepressant activities in safe dose, which may be due to its high...
phenol and flavonoid contents, especially quercetin. It is therefore very promising for further pharmacological and biochemical experiments.

Figure 1. Antioxidant activities of *Juglans regia* flower extract in linoleic acid peroxidation test. Each value is expressed as mean of three measurements. Vitamin C and BHA were used as positive control.

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