European Review for Medical and Pharmacological Sciences 2013; 17: 1881-1883

Antihemolytic activity and mineral contents of *Juglans regia L.* flowers

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Abstract. – OBJECTIVES: *Juglans (J.) regia L.* is known to possess many biological properties. In this study, antihemolytic activity of methanol extract of *Juglans regia L.* flower were investigated

MATERIALS AND METHODS: Antihemolytic activities of *Juglans regia L.* flowers were evaluated by various *in vitro* assays. In addition, scavenging of hydrogen peroxide and mineral contents of flowers were determined using atomic absorption spectroscopy.

RESULTS: Extract showed good antihemolytic activity against H_2O_2 and CuOOH induced hemolysis in comparison with control. Extract was capable of scavenging H_2O_2 in a concentration dependent manner. IC_{50} for H_2O_2 scavenging activity was 311±12.8 μg ml^{-1}. The amount of eight elements was determined and was in the order: Mn > Cu > Fe > Zn.

CONCLUSIONS: Our study indicate that *J. regia* flower has remarkable antihemolytic activity, which maybe result of its high phenol and flavonoid contents, especially quercetin.

Key Words:

Antihemolytic activity, Atomic absorption spectroscopy, *Juglans regia*, Mineral contents, Scavenging of hydrogen peroxide.

Introduction

Juglans (J.) 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 antidiarriec, antihelmintic, antiseptic and astringent properties[1]. Antiinflammatory, antinociceptive, antioxidant and antidiabetic activities of walnut leaves have been reported[2,3]. Recently, antioxidant, antidepressant, anti-inflammatory and anihypoxic activities of *J. regia* flowers were reported[4]. There is no scientific report on antihemolytic activity of *J. regia* flower. In the present study, antihemolytic activity, scavenging of hydrogen peroxide and mineral contents of *J. regia* flower were evaluated.

Materials and Methods

Plant Material and Preparation of Freeze-Dried Extract

J. regia. flower was collected from Sari, Iran. A voucher specimen was deposited with the Faculty of Pharmacy Herbarium (No 629). Sample was dried at r. t. and ground before extraction. 100 g of sample was extracted by percolation with methanol (400 ml × 3) for 24 hrs. The resultant extracts were concentrated in a rotary evaporator until a crude solid extract was obtained (24%).

Determination of Total Phenolic Compounds and Flavonoid Content

Total phenolic compound contents were determined by the Folin-Ciocalteau method[5]. Total flavonoids were estimated as previously described[5]. The absorbance of mixture was measured at 415 nm with a double beam spectrophotometer (UV-Visible EZ201, Perkin Elmer, Waltham, MA, USA). Total flavonoid contents were calculated as quercetin equivalent from a calibration curve.

Scavenging of Hydrogen Peroxide

The ability of scavenging was determined according to the method of Dehpour et al[6]. Extract in distilled water was added to a H_2O_2 solution (0.6 ml, 40 mM). The absorbance of H_2O_2 at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging by the extract and standard compounds was calculated as follows: % Scavenged (H_2O_2) = $[(A_o - A_1)/A_o] \times 100$ where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard.

Antihemolytic Activity of Extract Against H_2O_2 Induced Hemolysis

The inhibition of rat erythrocyte hemolysis was evaluated according to the procedure described by Ebrahimzadeh et al[7]. To 100 μl of 5% suspension

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of erythrocytes in PBS, 50 μl of extract with different concentrations was added. To this, 100 μl of 100 mM H_2O_2 was added. The reaction mixture was shaken gently while being incubated at 37ºC for 3h. The reaction mixture was diluted with 8 ml of PBS and centrifuged at 2000 × g for 10 min. The absorbance of the resulting supernatant was measured at 540 nm to determine the hemolysis. Likewise, the erythrocytes were treated with 100 μM H_2O_2 and without to obtain a complete hemolysis. The absorbance of the supernatant was measured at the same condition. The inhibitory effect of the extract was compared with vitamin C. To evaluate the hemolysis, erythrocytes were pre-incubated with 50 μl of extract for 1 h and the hemolysis was determined. Percentage of hemolysis was calculated by taking hemolysis caused by 100 μM H_2O_2 as 100%. The IC_{50} values were calculated from the plots as the antioxidant concentration required the inhibition of 50% hemolysis.

CuOOH-Induced Hemolysis

RBC (Red Blood Cells) were isolated from male Wistar rats and suspended in balanced phosphate buffered saline to obtain a 1% suspension. Aliquots (3.5 ml) were incubated at 37°C for 210 min in presence of 50 μM CuOOH and the cellular integrity determined turbidimetrically at 710 nm at 30 min intervals[8]. The extract was preincubated for 30 min with RBC before the addition of CuOOH (blanks were RBC added with ethanol, at a final concentration always less than 0.1% (v/v)). Percentages of hemolysis were determined setting as a 100% hemolysis the absorbance value determined in RBC suspensions sonicated for 5 sec at 50% power.

Determination of Metal Content

The properly dried and ground plant samples were ash-dried overnight at 400-420°C in a vitreosil crucible. 2 grams of ash were dissolved in a 1:3 mixture of HCl and HNO_3 [9] diluted to 50 ml with distilled water and used for analysis by means of an atomic absorption spectrometer Perkin Elmer AAS 100 (Wellesley, MA, USA).

Results

Total phenol compounds were reported as gallic acid equivalents by reference to standard curve (y = 0.0054 × + 0.0628, r^2 = 0.987). The total phenolic content was 71.7±3.2 mg gallic acid equivalent/g of extract. The total flavonoid contents was 61.7±2.7 mg quercetin equivalent/g of extract, by reference to standard curve (y = 0.0063 ×, r^2 = 0.999). Extract was capable of scavenging H_2O_2 in a concentration dependent manner. IC_{50} was 311±12.8 μg ml^{-1}. The IC_{50} values for vitamin C and BHA were 21.4±1.1 and 52.0±3.2 μg ml^{-1}, respectively. The effect of extract was tested and found that it did not show any harmful effect on erythrocytes. The extract inhibited the hemolysis of rat erythrocytes in a dose dependent manner with 92.4 % as maximum inhibition of erythrocyte hemolysis at 1.6 mg ml^{-1}. The extract showed 50% hemolysis inhibition at concentrations ranging from 25 to 400 μg ml^{-1} of extract (IC_{50} = 71±5 μg ml^{-1}) that was better than vitamin C (IC_{50} = 235±9 μg ml^{-1}). Extract delays the onset of the CuOOH induced hemolysis at 120 min hemolysis was inhibited by 48.6%, at 80 μg ml^{-1} compared with control group (Figure 1). Table I presents the elemental analysis by AAS technique. The concentration of various elements analyzed decreases in the order: Mn> Cu> Fe > Zn > Ni > Cd.

Discussion

Extract contained high level of total phenol and flavonoid contents. Phenols and polyphenolic compounds, such as flavonoids have been shown to possess significant antioxidant activities[7]. Scavenging of H_2O_2 by extract may be attributed to their phenolics, and other active components which can donate electrons to H_2O_2, thus neutralizing it to water[8]. Flavonoids interactions with cell membranes which generally serve as targets for lipid peroxidation (LP), constitute an important area of research[10]. Various model membrane systems like LDL and RBC membrane comprising physiologically important membrane protein components of-

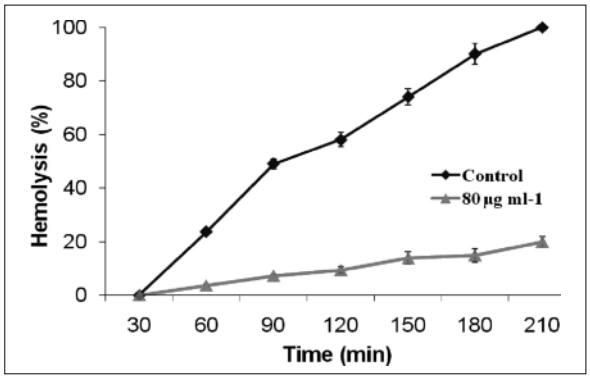

Figure 1. Protective effect of extract on red blood cell hemolysis induced by CuOOH. Values are the Mean±SD. (3 independent experiments).

Table I. Amounts of trace elements in the plants by AAS Analysis (μg/g).

Ni	Pb	Cd	Cr	Cu	Fe	Mn	Zn	Sample
2	ND	0.8	ND	8.08	5.6	17.2	5.2	J. regia

Values are averages of three independent measurements having a precision of approx ± 1%. ND: Not detected.

fer a physiologically relevant and a relatively simple system for studying LP[11]. RBC has been chosen as an in vitro model to study the oxidant/ antioxidant interaction since its membrane is rich in polyunsaturated fatty acids, which are extremely susceptible to peroxidation[10]. During recent years, a few interesting studies have been reported, indicating the protective effects of some plants extracts against oxidative damage in intact RBC membranes[5,7]. Hemolysis has a long history of use in measuring free radical damage and its inhibition by antioxidants but only few studies have been performed with erythrocytes in whole blood. In this study, we used a biological test based on free radical-induced erythrocytes lysis in rat blood. Lipid oxidation of rat blood erythrocyte membrane mediated by H_2O_2 induces membrane damage and subsequently hemolysis. The extract inhibited the hemolysis of rat erythrocytes in a dose dependent manner with 92.4% as maximum inhibition of erythrocyte hemolysis at 1600 μg ml^{-1}. The extract showed 50% hemolysis inhibition at concentrations that was lower than vitamin C. Extract delayed the onset of the CuOOH-induced hemolysis. At 120th min hemolysis was inhibited by 48.6 % at 80 μg ml^{-1} (Figure 1). Antihemolytic activity of quercetin and other flavonoid previously reported and potent activity of extract maybe result of high flavonoid content especially quercetin[8]. Table I presents the elemental analysis in ash by AAS technique. The concentration of various elements analyzed in the present work decreases in the order: Mn > Cu > Fe > Zn > Ni > Cd. The knowledge of the chemical form of the elements in plants of economic interest might be crucial because actions can be taken to reduce or minimize the toxic effects of the environment pollutant heavy metals[9].

Conclusions

Our study indicate that *J. regia* flower has remarkable antihemolytic activity, which maybe result of its high phenol and flavonoid contents, especially quercetin. It is therefore very promising for further pharmacological and biochemical experiments.

Acknowledgements

This research partially supported by a grant from Pharmaceutical Sciences Research Center, Mazandaran, Iran.

Conflict of Interest

None.

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