Influence of Aqueous Extracts of Seven Plants Used in Traditional Medicine on Osmotic Fragility of Human Erythrocytes

تأثير خلاصة سبع نباتات تقليدية شائعة الاستعمال على كريات الدم الحمراء

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Abstract

Background: Many plants contain chemical substances that might have a hemolytic or anti-hemolytic effect on human erythrocytes. **Aim**: To test the influence of the aqueous extract of seven commonly used plants in Jordan on the osmotic fragility of human erythrocytes. **Methods:** Osmotic fragility test was used to assess hemolytic activity of the crude extracts of the plants. The dried plants were extracted and dissolved in dimethyl sulphoxide. Blood samples were collected from healthy volunteers, dissolved and then divided into control and treated groups. The results of hemolysis were determined spectrophotometrically. Data were analyzed using SPSS 15. **Results:** The plants were classified into three groups: group I: has no effect and includes *Solanum tuberosum*, *Solanum melongena*, *Hibiscus sabdariffa*, and *Malva sylvestris*, group II: decreases hemolysis and includes *Colchicum ritchii*, and group III: increases hemolysis and includes *Ferula harmonis*, and *Silybum marianum*. Results from group II and III showed that hemolysis was independent of time, while the degree of

hemolysis was concentration- dependent. **Conclusion:** Some plants have anti hemolytic effect on human erythrocytes; others induce it, and a third group does not have any effect.

Key words: osmotic fragility, hemolysis, human erythrocytes, plant extracts, Jordan.

ملخص

خلفية: تحتوي العديد من النباتات مواد كيميائية قد يكون لها تأثير إنحلالي أو مضاد للإنحلال على كريات الدم الحمراء.

الهدف: اختبار تأثير المستخلص المائي لسبع نباتات تستخدم عادة في الأردن على مدى تحلل كريات الدم الحمراء.

طريقة العمل :تم إستخدم إختبار هشاشة كريات الدم الحمراء لتقييم النشاط الإنحلالي أو مضاد الأنحلال الذي تحدثه مستخلصات النباتات. تم تجفيف النباتات وإستخلاصها ثم إذابتها في DMSO. تم جمع عينات دم من متطوعين أصحاء ، ثم تقسيم العينات إلى مجموعة المراقبة ومجموعة المعالجة. نتائج إنحلال الدم تم قياسها بواسطة تحليل الطيف الضوئي. تم تحليل البيانات باستخدام البرنامج الاحصائي SPSS 15.

النتائج : تم تقسيم النباتات الى ثلاث مجموعات بناء على النتائج: المجموعة الأولى: ليس لها أي تأثير على إنحلال كريات الدم الحمراء وتشمل:

Solanum tuberosum, Solanum melongena, Hibiscus sabdariffa, Malva sylvestris ،

المجموعة الثانية: تقلل من الإنحلال في كريات الدم الحمراء وتشمل *Colchicum ritchii* والمجموعة الثالثة : تؤدي الى زيادة إنحلال كريات الدم الحمراء وتشمل Harmonis ferula ، Silybum marianum. اظهرت النتائج من المجموعة الثانية والثالثة ان انحلال كريات الدم الحمراء حصل بغض النظر عن الزمن ، في حين أن درجة انحلال كريات الدم الحمراء كانت تعتمد على التركيز.

الاستنتاج: بعض النباتات لها تأثير مضاد لانحلال كريات الدم الحمراء في الإنسان، وبعضها الآخر يسبب إنحلالا في كريات الدم الحمراء، ومجموعة ثالثة ليس لهاتأثيرعلى كريات الدم الحمراء.

Introduction

Plants constitute a major component of diet and traditional medicine in the Middle East area (Afifi and Abu-irmaileh 2000; Abu-Irmaileh and Afifi 2003; Aburjai et al., 2007; Al-Qura'n 2006; Otoom et al., 2006; Abu-Rabia A 2005; Mati and de Boer 2010). However, many plants are reported to have serious adverse effects, which include induction of hemolytic anemia (de Freitas et al., 2007). This might be of value for patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency which is common in Jordan (Talafih et al., 1996; Karadsheh et al., 2005). Patients and health care providers need to be provided with lists of commonly used plants that could worsen the hemolytic conditions of patients with hemolytic disorders. Therefore, many of the commonly used plants need to be evaluated for their potential hemolytic activity.

Examples of commonly used plants as food and in traditional medicine in Jordan include**:** potatoes, eggplants, chamomile and peppermint. Therefore, the objective of the present study was to evaluate the hemolytic activity of seven crude plant extracts on human erythrocytes. The selected plants were: potato, *Solanum tuberosum* L.; eggplant, *Solanum melongena* L.; red sorrel, *Hibiscus sabdariffa* L.; mallow, *Mava sylvestris* L.; autumn crocus, *Colchicum ritchii* L.; holy thistle, *Silybum marianum* L.; and zallouh, *Ferula harmonis* L.

Methodology

Plants material

The following plants were used in the present investigation: potato, *Solanum tuberosum* (ST); eggplant, *Solanum melongena* L. (SM); red sorrel, *Hibiscus sabdariffa* L. (HSL); mallow, *Malva sylvestris* L. (MSL); autumn crocus, *Colchicum ritchii* L. (CR); holy (Milk) thistle, *Silybum marianum* L. (SML);, and zallouh, *Ferula harmonis* L. (FH)]. Different parts of plants including calyx from Hibiscus sabdariffa, leaves from *Malva sylvestris*, *Solanum tuberosum*, *Solanum melongena*, bulbs from *Colchicum ritchii*, aerial parts of *Silybum marianum* and *Ferula*

harmonis were collected from various parts of Jordan or purchased from the local market.

Extraction procedure

1. Tested plants were air dried and finely powdered.
2. Plant materials were extracted in a Soxhlet apparatus with 1000 ml methanol for 6 hr.
3. The extract was filtered.
4. The filtrate was evaporated using vacuum at <50^{o}C on a rotary evaporator.
5. All crude extracts were stored in a closed container at 4^{o}C in the refrigerator.
6. The crude extracts were dissolved in 2 ml DMSO and 1 ml of physiological-buffered saline then filtered through millipore sterile filters.
7. The crude extracts were: CRe 100 mg, FH 140 mg, HSL 130 mg, SML 110 mg, ST 100 mg, SM 100 mg, and MSL 110 mg.

Blood Samples collection

Blood samples were collected from healthy volunteers after their consent in writing. The blood samples were washed with phosphate-buffered saline (PBS) three times before the experiments. All experiments were conducted at the same time of the day of collection. Blood samples were suspended in PBS and DMSO, and then divided into two groups, one group with plant extract, and the other without. Samples were then incubated for 40 minutes before osmotic fragility was measured.

Osmotic fragility Measurement:

The osmotic fragility method adopted in this study was that of Malamos and Fessas (Malamos and Fessas 1962). Osmotic fragility of cells was measured by suspending washed cells at 2% haematocrit in buffered physiological saline that had been diluted with water to give various osmolalities. The suspension was centrifuged after 1 min, and the hemoglobin in the supernatant was measured spectrophotometrically at

540 nm. The results were analyzed using statistical package for social sciences version 15 (SPSS), and plotted on osmotic fragility curve, taking the hemoglobin concentration of suspended cells in distilled water as 100% lysis.

Results & Discussion

The plants were divided into three groups based on the results obtained. The first group included the plant extracts from ST, SM, HSL and MSL whose osmotic fragility curves were located within the normal range of osmotic fragility test. Additionally, there was no difference in the concentration of saline that causes 50% lyses and that of the plants extracts of this group (Figures 1 A through 1D). The plants of the second group included the plant extract of CR. The osmotic fragility curve was significantly shifted to the left (decreased hypotonic lyses) when CR is added in two different extract volumes (20, 60 µl and more) (Figure 2). The plants of the third group included extracts of SML and FH plants which induced significant hemolysis of erythrocytes with 60 µl. Erythrocyte hemolysis induced by FH and SML extracts of at 60 µl was tested at different time intervals. Results showed that the hemolysis was similar at 20, 40 and 60 minutes of incubation (Figures 3A and 3B).

In this study, two of the tested herbal aqueous crude extracts (FH and SML) induced hemolysis while one herbal aqueous crude extract (CR) had shown an anti hemolytic activity. The mechanisms underlying the effects of FH and SML on hemolysis are not clear. However, it is known that many Ferula species are rich in sesquiterpene components (Ahmad et al., 2007; Lhuillier et al., 2005) which have shown cytotoxic activity (Suzuki et al., 2007). The Milk thistle (SML) extracts are also under intense study in the experimental therapeutics of cancer (Sagar 2007). Both FH and SML might induce hemolysis by a mechanism similar to that of *Livistona chinensis*, which have been reported to have cytotoxic activity through a cell membrane medicated mechanism (Singh and Rajini 2008).

One of the important active ingredients in *Colichicum* species is colchicine which had shown an anti-hemolytic activity against osmotic

fragility *in vitro.* The clinical use of colchicine is known to induce anemia by bone marrow related mechanism (Bhat et al., 2009). Therefore, colchicine is contraindicated in patients with G6PD (www.G6PDDeficiency.org). However; our results suggest that the in vitro activity of CR might be different from the systemic in vivo activity. The effect of CR on hemolysis might be explained by increasing the membrane surface, or by increasing the fluidity of the membrane, so membrane expansion of the erythrocyte would lead to an increase in the surface area/volume ratio. This property of colchicine could be attributed to the cyclic structure of the compound which makes it fit in the lipid bilayer.

The Electron spin resonance (ESR), spin-label studies have demonstrated that the fluidity of the lipid bilayer correlates with the rate of hemolysis (Bartucci et al., 2006). Cholesterol and phospholipids are supposed to form complexes that stabilize the membranes. The fluidity of the erythrocyte membrane is determined by a number of factors among which are cholesterol content, fatty acid composition and the protein matrix. The interaction of these factors seems to influence, to a varying degree, the physiological properties of the membranes which may change the osmotic sensitivity (Ehrström et al., 1981).

It is concluded that plant extracts of *Solanum tuberosum* (ST), *Solanum melongena* (SM), *Hibiscus sabdariffa* (HSL), and *Malva sylvestris* (MSL) did not show any effect on osmotic fragility. On the other hand, plant extract from *Colchicum ritchii* (CR) reduces hemolysis whereas plant extracts from *Ferula harmonis* (FH), and *Silybum marianum* (SML) increase hemolysis. Milk thistle has been used as an edible food by many individuals in the Middle East community; it has some recognized benefits such as liver tonic (Kroll et al., 2007). Research has reported that milk thistle has a protective effect on the liver and can be used to treat hepatitis, cirrhosis, and gallbladder disorders (Greenlee et al., 2007; Tamayo and Diamond 2007).

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