Effect of Flavonoids Extracted from Hawthorn (*crataegus oxyacantha)* on some hematological parameters of female mice

A. I. O. Al-Abdaly
College of Veterinary Medicine / University of Baghdad

Abstract

This experiment was designed to study the effect of flavonoid extracted from Hawthorn (*crataegus oxyacantha)* on some hematological parameters in female mice. The experiment was performed on fifteen female mice which were divided randomly into three equal groups (5/group) and were treated daily as follows for four weeks: the first group was received distilled water and served as control, mice of the second group (T_1) was administrated 9 mg /kg B.W. flavonoids and the third group (T_2) was administrated 18 mg /kg B.W. flavonoids for four weeks. Blood samples were collected at the end of the experiment to study the following parameters:-RBCs count, PCV ratio, Hb concentration, differential leukocytes count, MCV, MCH and MCHC. The results showed that animals treated with flavonoids extracted from Hawthorn (T_1 and T_2) have not significant differences ($P>0.05$) in RBC count, MCV and MCH, as compared to control. There was significant decrease in packed cell volume in both treated groups as compared to control but there was significant increment ($P<0.05$) in Hb concentration of third group (T_2) as compared to second (T_1) and control groups. The results also showed marked increased of the MCHC percentage in both second and third treated group as compared to control. The effect of flavonoids extracted from hawthorn showed significant decrease of neutrophil percentage and significant increase of lymphocyte percentage in treated groups as compared to control, while there was no significant increase in the percentage of monocytes and eosinophils.

تأثير الفلافونويدات المستخلصة من ثمار الزعرور *crataegus oxyacantha* على بعض المعايير الدموية في إناث الفئران

أنوار إبراهيم عبيد العبدلي
كلية الطب البيطري/ جامعة بغداد

الخلاصة

صممت هذه التجربة لدراسة تأثير الفلافونويدات المستخلصة من الزعرور على بعض المعايير الدموية في إناث الفئران. تم استخدام خمس عشرة فأرة قسمت بصورة عشوائية إلى ثلاث مجاميع متساوية (5/ مجموعة) عدت المجموعة الأولى كمجموعة سيطرة، وجرعت المجموعة الثانية (T_1) بجرعة 9 ملغم/ كغم من وزن الجسم والمجموعة الثالثة (T_2) بجرعة 18 ملغم/ كغم من وزن الجسم من الفلافونويدات المستخلصة من ثمار الزعرور عن طريق الفم يوميا لمدة أربع أسابيع. تم جمع نماذج الدم لدراسة المعايير التالية: العد الكلي لكريات الدم الحمراء، حجم الخلايا المرصوصة، تركيز الهيموكلوبين، العد التفريقي للخلايا الدموية البيضاء، حجم الكرية الحمراء، كمية خضاب كريات الدم، تركيز خضاب كرية الدم. لم تظهر النتائج أي فروقات معنوية ($P>0.05$) في معدلات أعداد كريات الدم الحمراء، وحجم الكرية الحمراء، وكمية خضاب كريات الدم في المجموعتين المعاملتين مقارنة بمجموعة السيطرة. كما أشارت النتائج إلى وجود انخفاض معنوي ($P<0.05$) في النسبة المئوية لحجم الخلايا المرصوصة في المجموعتين المعاملتين بفلافونويدات الزعرور مقارنة بالسيطرة. بينما لوحظ وجود زيادة معنوية ($P<0.05$) في

معدلات تركيز الهيموكلوبين في المجموعة الثالثة مقارنة بالمجموعة الثانية ومجموعة السيطرة وزيادة معنوية في تركيز خضاب كرية الدم في المجموعتين الثانية (T_1) والثالثة (T_2) مقارنة بمجموعة السيطرة. أظهرت الدراسة تأثير واضح للفلافونويدات المستخلصة من ثمار الزعرور تمثلت بانخفاض معنوي في النسبة المئوية للخلايا العدلة وارتفاع معنوي في النسبة المئوية للخلايا اللمفاوية للمجموعتين الثانية والثالثة (T_2,T_1) مقارنة بمجموعة السيطرة. لم تظهر النتائج أي فروقات معنوية في النسبة المئوية لكل من الخلايا وحيدة النواة والخلايا الحمضة بين مجاميع التجربة الثلاثة.

Introduction

Crataegus oxyacantha is the biological name for the plant commonly known as "Hawthorn". It belongs to the Rosaceae family. Hawthorn (crataegus) is an odouerless, thorny, deciduous tree that can grow up to 10 meters high (1), at altitudes of 180-300 meters (2). The leaves are lobed with white flowering tops that end in a red berry fruit (1). Active ingredients found in hawthorn include, flavonoids (such as vitexin, rutin, quercetin, and hyperoside), oligomericproanthocyanidins (OPCs, such as epicatechin, procyanidin, and particularly procyanidin B-2), flavone-C, triterpene acids (such ursolic acid, oleanolic acid, and crataegolic acid), and phenolic acids (such as caffeic acid, chlorogenic acid, and related phenolcarboxylic acids), Saponins and Tannins,Vitamin C, Cratetegin (most prevelant in flowers, leaves, berries), (3,4,5,6). The recommended daily dose of hawthorn is 160-900 mg of a native water-ethanol extract of the leaves or flowers (equivalent to 30-169 mg of epicatechin or 3.5-19.8 mg of flavonoids). (7, 8, 9, 10, 11, 12). Hawthorn has recently been shown antioxidant properties (13, 14, 15). Hawthorn also exhibits anti-inflammatory property by preventing synthesis and release of inflammatory promoters such as histamines, serine proteases, prostaglandins, leukotrienes etc., as well as, inhibiting enzymatic cleavage by enzyme secreted by leukocytes during inflammation (16), it has mild to moderate sedative effect has been demonstrated in humans and animal studies with hawthorn constituents and OPC'S are reported to be partially responsible for this effect (17,18). Today, hawthorn is used primarily for various cardiovascular conditions. The cardiovascular effects are believed to be the result of positive inotropic activity, alsoexert considerable collagen-stabilizing effects, enhancing integrity of blood vessels wall and improve coronary blood flow, and positive effects on oxygen utilization. Flavonoids are postulated to account for these effects. (3, 8, 12, 19, 20). The aim of this study is to examine the effect of flavonoids of *crataegus oxyacantha* on some hematological parameters.

Materials and Methods

The method of Harbone (1984) (21), was used for the extraction of flavonoids .to 100 gm of hawthorn berry after removing of the seed and mixing with mixture in one liter conical flask, 200 ml of 2N HCL was added and covered by aluminum foil then mixed and boiled in a water bath at 100ºC for 45 minutes (to complete hydrolysis) with a gentle mixing every 15 minutes intervals then the mixture was cold to 25-27 ºC and filtered under vacuum using whatman No.2 Filter paper. The filtrate was transferred into separatory funnel and the caratinoids, chlorophyll and waxes were separated from the filtrate by 100 ml petroleum ether using 25 ml at each interval. Then, flavonoid was extracted from the filtrate residues by 100 ml ethyl acetate in which 25 ml was used at each interval. Finally, flavonoids were dried under vacuum using rotary evaporator at 40±2 ºC; purified flavonoid was weighted and kept in a dark glass container at -20ºC till use. fifteen adult female mice were randomly divided into three groups (5 mice/ group) and treated as follows for four weeks: animals in group one had free access to food and water and served as control; group two (T_1) animals in this group were subjected to oral

intubation of 9 mg/ kg B. W. flavonoids extracted from hawthorn,while animals in group three received orally 18 mg/ Kg B. W. flavonoids. Blood samples were collected by heart puncture technique for measuring the following parameters:-Total red blood cells count (RBCs), packed cell volume (PCV%) according to (22,23,24), estimation of hemoglobin concentration (26), Mean corpuscular volume (MCV).
($mcv = \frac{pcv\%}{total\ RBC count} \times 10$), Mean corpuscular hemoglobin (MCH)
($MCH = \frac{Hb\ concentration(g/dl}{total\ RBC\ count} \times 10$), Mean corpuscular hemoglobin concentration (MCHC) ($MCHC\% = \frac{Hb\ concentration\ (g/dl)}{pcv\%} \times 100$) (23, 24, 25), Differential leukocyte count according to (25, 26). Differences between experimental groups were evaluated by using one way analysis of variance (ANOVA). Specific group differences were determined using least significant differences (LSD). For all analysis of P value 0.05 were considered to be significant (27).

Results

The yield of crude flavonoids extracted from *crataegus oxyacantha* samples revealed that out of each 100 gm Hawhorn berry, approximately 0.2 gm of crude flavonoids was obtained. The effect of flavonoids extracted from *crataegus oxyacantha* on blood picture of female mice was shown in tables (1, 2, 3). Data pertaining to red blood cells count, packed cell volume and hemoglobin concentration of control and flavonoids treated groups are depicted in Table (1). The results revealed that non significant differences (P>0.05) in total red blood cells count but there was significant decrease in packed cell volume in two treated groups as compared to control. On the other hand there was significant increase (P<0.05) in hemoglobin concentration in group treated with 18mg/kg B. W. of hawthorn favonoids as compared to (T_1) and control groups.

Table (1) Effect of flavonoids of *crataegus oxyacantha* on Red blood cells count, packed cell volume and hemoglobin concentration in female mice

Group	Red blood cells /mm3	Packed cells volume (%)	Hemoglobin concentration (gm / dL)
Control	7.78±0.65 A	45.4±1.20 B	14.21±0.34 A
$T_1$9mgflavonoids /kgB.W.	6.56±0.21 A	38.6±0.67 A	14.84±0.35 A
$T_2$18mgFlavonoids /kg B.W.	7.13±0.62 A	41.0±0.44 A	16.09±0.20 B

Values expressed as means ± SE. n= 5 / group.
Capital letters denote between groups differences, P<0.05 vs. control.

The effect of oral intubation of flavonoids extracted from hawthorn on MCV, MCH, and MCHC in adult female mice was explained in table (2) showed significant increase (P<0.05) in the Mean corpuscular hemoglobin concentration (MCHC) in the groups T_1 treated with 9 mg/kg B.W. and T_2 treated with 18 mg/kg B.W. of hawthorn flavonoids respectively as compared with control. On the other hand there were non significant differences (P>0.05) in the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) between the groups.

Table (2) effect of different doses of *cratagus oxycantha* flavonoids on MCV, MCH, MCHC, of female mice

Group	MCV (vito liter)	MCH (pico gram)	MCHC %
Control	61.14± 8.45 A	18.95±2.04 A	31.67± 1.47 A
T_1 9 mg/kg B.W. of Flavonoids	59.07± 2.48 A	22.71± 0.98 A	39.26± 0.60 B
T_2 18 mg/ kg B.W. of Flavonoids	59.06± 4.46 A	23.25± 1.97 A	38.45± 0.41 B

Values expressed as means ± SE. n= 5/ group.
Capital letters denote differences between groups, P<0.05 vs. control.

The effect of two doses of flavonoids of crataegus oxyacantha on percentage of differential leukocytes count were observed in table (3). Significant suppression in the percentage of neutrophils in, two treated groups (T1, T2) As compared to control, meanwhile treatment of animals with flavonoids cause significant increase (P<0.05) of lymphocytes percentage of T1and T2 comparing to control. Finally there were non significant differences (P>0.05) in the percentage of monocytes andesinophils between treated groups and control.

Table (3) Effect of *crataegus oxycantha* flavonoids on differential leukocytes count in female mice

Groups	Neutrophil %	Lymphocyte%	Monocyt%	Eosinophil%	Basophil%
Control	20.57±2.31 B	55.87±2.40 A	22.81±3.33 A	0.63±0.28 A	0.00
T_1 9 mg/kg B.W. Of Flavonoids	8.23±1.02 A	75.47±1.22 B	15.41±1.87 A	0.11±0.08 A	0.00
T_2 18 mg/ kg B.W. of Flavonoids	5.88±0.87 A	78.21±3.59 B	15.44±3.12 A	0.45±0.34 A	0.00

Values expressed as means ± SE. n= 5 / group.
Capital letters denote between groups differences, P<0.05 vs. control.

Discussion

Crataegus oxyacantha has significant activity because of the high content of flavonoid compounds, particularly the OPCs; it increases coronary blood flow, enhancing oxygen flow and utilization by the heart. Crataegus extracts also have a positive inotropic effect on the contraction amplitude of myocytes. Due its flavonoid content, hawthorn exerts considerable collagen stabilizing effects, enhancing integrity of the blood vessels (28, 29, 30). The results obtained in the present study clearly show that, the flavonoids extracted from *crataegus oxyacantha* were effectively improving some parameters of blood homeostasis in female mice. There were no significant effect in total erythrocytes count, MCV, and MCH, significant decrease in hematocrit ratio and significant increase in hemoglobin concentration and MCHC percentage. Hawthorn, as had been mentioned by many authors, can significantly reduce the amount of fibrinogen, decreased blood viscosity, plasma and serum viscosity, RBC aggregation index and lower RBC hematocrit, indicate that Hawthorn can improve blood stasis state (31, 32). The hawthorn fruit and methanolic extract of this herb showed significant increased of hemoglobin (33, 34). Consequently the MCHC will be increased (24, 35). This investigation also pointed to the role of flavonoids extracted from *crataegus oxyacantha* on differential leukocytes count especially neutrophils and lymphocytes, the results showed significant decreased in neutrophils percentage and significant increased in lymphocytes percentage. It has been found that howthorn exert as anti-inflammatory effect by preventing synthesis and release of inflammatory promoters such as histamines, serine proteases, prostaglandins, leukotrienes (16, 17, 36). Besides,

hawthorn inhibited enzymatic cleavage byMyeloperoxidase, which present in neutrophils and has been successfully used to confirm inflammatory cell activationduring inflammation (37, 38, and39). Lymphocytes participate in specific immune responses (35), The increased lymphocytes due to the hawthorn, particularly its flavonoids constituents with antioxidative activity, reduced the oxidative stress and genotoxicity induced by toxic compounds, protection lymphocyte from genetic damage (40) in addition to flavonoids, hawthorn is rich in minerals and contain a small amount of active principle oligomeric procyanidine (1-epicatechol) which improved immunity and increased lymphocytes (41). It is concluded from this study that flavonoids extracted from hawthorn fruits maintained blood homeostasis, improved immunity and possessed anti-inflammatory effect.

References

1. Wink, M. & Van Wyk, B. E. (2004). Medicinal Plants of the World, 1st ed., Briza Publlications, Pretoria, South Africa, P.115.
2. Varma Pnand Vaid, I. (2002). Encyclopaedia of Homoeopathic pharmacopoeia, Vol 1, B Jain publishers, New Delhi, PP.883-884.
3. Gabor, M. (1972). Pharmacological effects of flavonoids on blood vessels. Angiologica, 9:355-374.
4. Popping, S.; Rose, H. & Ionescu, I. (1995). Effect of a hawthorn extract on contraction and energy turnover of isolated ratcardiomyocytes. Arzneimittelforschung,45:1157-1161.
5. Schussler, M.; Holzl, J. & Fricke, U. (1995). Myocardial effects of flavonoids from Crataegus species. Arzneimittelforschung, 45:842-845.
6. Rakotoarison, D. A.; Gressier, B. & Trotin, F. (1997). Antioxidant activities of polyphenolic extracts from flowers, in vitro callusand cell suspension cultures of Crataegusmonogyna. Pharmazie, 52:60-64.
7. Iwamoto, M.; Sato, T. & Ishizaki, T. (1981). The clinical effect of Crataegutt in heart disease of ischemic or hypertensive origin. A multicenter double-blind study [in German]. Planta Med. 42:1-16.
8. Weikl, A. (1996). Crataegus Special Extract WS 1442. Assessment of objective effectiveness in patients with heart failure (NYHA II). Fortschr Med., 114:291-296.
9. Schmidt, U. (1994). Efficacy of the hawthorn (Crataegus) preparation LI 132 in 78 patients with chronic congestive heart failure defined as NYHA functional class II. Phytomed; 1:17-24.
10. O'Conolly, M. (1987). Treatment of elderly, multimorbid patients with stenocardiac complaints with Crataegutt Novo. Double-blind placebo-controlled crossover study. Therapiewoche: 37:3587-3600.
11. Leuchtgens, H. (1993). Crataegus Special Extract WS 1442 in NYHA II heart failure. A placebo controlled randomized double-blind study. Fortschr Med., 111:352-354.
12. Rigelsky, J. M. & Sweet, B. V. (2002). Hawthorn: pharmacology and therapeutic uses. Am. J. Health Syst. Pharm. Mar., 1; 59(5):417-422.
13. Rakotoarison, D. A.; Gressier, B. & Trotin, F. (1997). Antioxidant activities of polyphenolic extracts from flowers, in vitro callus and cell suspension cultures of Crataegusmonogyna. Pharmazie., 52:60-64.
14. Cui, T.; Nakamura, K.; Tian, S.; Kayahara, H. & Tian, Y. L. (2006). Polyphenolic content and physiological activities of Chinese hawthorn extracts. Dec; 70(12):2948-2956. Epub.

15. Hosseinimehr, S. J.; Azadbakht, M.; Mousavi, S. M.; Mahmoudzadeh, A. & Akhlaghpoor, S. (2007). Radioprotective effects of hawthorn fruit extract against gamma irradiation in mouse bone marrow cells. J. Radiat. Res. (Tokyo). Jan; 48(1):63-68.
16. Dalli, E.; Milara, J.; Cortijo, J.; Morcillo, E. J.; Cosín-Sales, J. & Sotillo, J. F. (2008). Hawthorn extract inhibits human isolated neutrophil functions. Pharmacol Res., 57(6):445-450.
17. Della Loggia, R. (1983). Depressive effect of *Crataegus oxyacantha* L. on central nervous system in mice. Pharm., 51: 319-324.
18. Rewerski, V.; Piechocki, T.; Rylski, M. & Lewak, S. (1971). Some pharmacological properties of *Crataegeus oxyacantha* compound and isolated oligomeric procyanidins. ArzneimForsch Drug Res., 21: 886-888.
19. Jayalakshmi, R.; Thirupurasundari, C. J. & Devaraj, S. N. (2006). Pretreatment with alcoholic extract of crataegusoxyacantha (AEC) activates mitachondrial protection during isoproterenol- induced myocardial infarction in rats. Mol. Cell Biochem., 292:59-67.
20. Swaminathan, J. K.; Khan, M. & Mohan, I. K. (2010). Cardioprotective properties of crataegusoxycantha extract against ischemia-reperfusion injury. Phytomedicine., Feb. P. 17.
21. Harborne, J. B. (1984). Method of extraction and Isolation, Phytochemical methods, 2nd ed., London, New York, champanand Hall.
22. Schalm, O. W.; Jain, N. C. & Carroll, E. J. (1975). Veterinary Hematology. 3rd. ed. United states America, Philadelphia.
23. Campbell, T. W. (1988). Avian Hematology and cytology. 1st. ed. Iowa state University press/ Ames.
24. Prakasam Reddy, L.; GowriSankar Reddy, L. & Vidya Reddy, L. (2008). Practical physiology. 2ed. ed. paras medical publisher Hyderabad, New Delhi.
25. Varley, H.; Growenlock, A. & Bell, M. (1980). Practical clinical biochemistry.5th ed. London. William Heinemann. Medical Ltd.
26. Coles, E. H. (1986). Veterinary clinical pathology. 4th ed. W.B. Saunders company.
27. Steel, R. G. & Torrie, J. H. (1980). Principles and procedures of statistics. 2nded. Mac Graw Hill Book. Co. New York. USA.
28. Kim, S. H. & Kang, K. W. (2000). Procyanidins in crataegus extract evoke endothelium-dependent vasorelaxation in rat aorta. Life Sci., 67(2):121-131.
29. David, H. (1995). Alternative and Complementary Therapies., 1(3): 191-192.
30. Rechciski, T. & Kurpesa, M. I. I. (2005). Oligomericprocyanidins from hawthorn extract as supplementary therapy in patients with left ventriclesystolicdysfunction.: PrzeglLek., 62(4):243-244.
31. Jiao, L. (2008). Hawthorn leaves flavonoids on acute blood stasis model of the impact of hemorheology in rats. J. Chinese medicine and Clin., 8 (1): 49-50.
32. Weikl, A.; Assmus, K. D. & Neukum-Schmidt, A. (1996). Crategeus special extract WS 1442: Assessment of objective effectiveness in patients with heart failure. Fortschr Med., 114: 291-296.
33. Chang, W. T.; Dao, J. & Shao, Z. H. (2005). Protective effect of hawthorn fruit on murine experimental colitis. Am. J. Chin. Med., 33(2):167-180 (ISSN: 0192-415X) Resuscitation Center, Department of Medicine Section of Emergency Medici.ne, University of Chicago, IL 60637, USA.

34. Kanyonga, M. P.; Faouzi, M. Y. A.; Zellou, A.; Essassi, M. & Cherrah, Y. (2011). Effects of methanolic extract of Crataegusoxyacantha on bloodhomeostasis in rat. J. Chem. Pharm. Res., 3(3):713-717 ISSN No: 0975-7384 CODEN (USA): JCPRC5.
35. Michael, R.; Prinsky, L. & Brochard, J. M. (2006). Applied Phsiology in intensive care Medicine. 2nd ed. Springer – veriog Berlin Heidelberg.
36. Chinnasamy, E. & Sivasithambaram, N. D. (2010). Immunomodulatory effect of Hawthorn extract in an experimental stroke model. J. Neuroinflammation., 7: 97.
37. Liao, S. L.; Kao, T. K.; Chen, W. Y.; Lin, Y. S.; Chen, S. Y.; Raung, S. L.; Wu, C. W.; Lu, H. C. & Chen, C. J. (2004). Tetramethylpyrazine reduces ischemic brain injury in rats. Neurosci. Lett., 372:40-45.
38. Krasowska, A. & Konat, G. W. (2004). Vulnerability of brain tissue to inflammatory oxidant, hypochlorous acid. Brain Res., 997:176-184.
39. Elango, C.; Jayachandaran, K. S. & Niranjali Devaraj, S. (2009). Hawthorn extractreduces infarct volume and improves neurological score by reducing oxidative stress in rat brain following middle cerebral artery occlusion. Int. J. Dev. Neurosci., 27:799-803.
40. Hosseinimehr, S. J.; Azadbakht, M.; Tanha, M.; Mahmodzadeh, A. & Mohammadifar, S. (2010). Protective effect of hawthorn extract against genotoxicity induced by methyl methanesulfonate in human lymphocytes. Toxicol Ind. Health. (Article in Press).
41. Hosseinimehr, S. J.; Mahmoudzadeh, A.; Azadbakht, M. & Akhlaghpoor, S. (2009). Radioprotective effects of Hawthorn against genotoxicity induced by gamma irradiation in human blood lymphocytes. Radiat Environ Biophys., 48(1):95-98.