Influence of 70% Alcoholic Extract of Hawthorn (*Crataegus oxycantha)* on Some Physiological Parameters in Adult Male Rats Exposed to Hydrogen Peroxide

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Abstract

This study was designed to investigate possible preventive role 70%alcoholic extract of fresh fruit of hawthorn (*Crataegus oxycantha)* (300 mg/kg) which extracted from fresh fruits against the deleterious effect of 1% hydrogen peroxide (H_2O_2) on some physiological parameters in male rats. Fifteen adult male rats were divided randomly into three equal groups (5/group) and were treated daily as follows for one month: control group ©which were given distilled water, first treated group was given 1% H_2O_2 In drinking water (G1), the second treated group (G2) was given orally 70% alcoholic extract of hawthorn (300 mg/kg) with 1% H_2O_2 in drinking water. Blood samples were collected after 30 days for measuring the following parameters: total WBC count, the percentage of WBC differential count (%), platelet count, total protein, albumin and globulin, Neutrophil/Lymphocyte (N/L) index, Albumin/Globulin (A/G) index and phagocytic Index. The result showed a significant increase in total WBC count, platelet count, Lymphocyte percentage, phagocytic index, total protein and globulin concentration in alcoholic extract of hawthorn with H_2O_2 group (G2) as compared with H_2O_2 group (G1). The result also pointed a significant increase in monocyte, neutrophil percentage, N/L index and A/G index of H_2O_2 group (G1) as compared with (G2) group H_2O_2 with hawthorn. The result suggests that the adverse effect of 1% H_2O_2 can be prevented with 70% alcoholic extract of hawthorn in male rats.

Key words: Hawthorn (*Crataegus oxycantha)*, WBC, Platelet, H_2O_2, Phagocytic index, total protein, globulin.
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تأثير 70% من المستخلص الكحولي للزعرور(*Crataegus oxycantha*) في بعض المعايير الفسلجية في ذكور الجرذان المعرضة لبيروكسيد الهيدروجين

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الخلاصة

صممت هذه الدراسة لبيان الدور الوقائي المحتمل للمستخلص الكحولي 70% لثمار الزعرور الطازجة (100ملغم/كغم). خمس عشرة من ذكور الجرذان البالغة قسمت عشوائيا إلى ثلاثة مجاميع متساوية (5/المجموعة) وتمت معالجتها يوميا ولمدة 30 يوما وكالاتي: مجموعة سيطرة أعطيت ماء مقطر والمجموعة الأولى أعطيت 1% بيروكسيد الهيدروجين في ماء الشرب (G1)، والمجموعة الثانية (G2) أعطيت 70% المستخلص الكحولي لثمار الزعرور (100ملغم) مع 1% بيروكسيد الهيدروجين في ماء الشرب. جمعت عينات الدم بعد 30 يوم لقياس المعايير الآتية: العد الكلي لكريات الدم البيض، النسبة المئوية للعد التفريقي لكريات الدم البيض، عد الصفيحات الدموية، البروتين الكلي، الألبومين والكلوبيولين، مقياس العدلات/ اللمفاوية، مقياس الألبومين/ الكلوبيولين (A/G) ومقياس البلعمة. قد بينت النتائج زيادة معنوية في العدد الكلي كريات الدم البيض، عدد الصفيحات الدموية، والنسبة

المئوية للخلايا اللمفاوية، مقياس البلعمة، وتركيز البروتين الكلي والكلوبيولين في المجموعة المعالجة بالمستخلص الكحولي للزعرور مع بيروكسيد الهيدروجين (G2) كمقارنة مع مجموعة بيروكسيد الهيدروجين (G1). وأشارت النتائج إلى زيادة معنوية في الخلايا الأحادية، ونسبة العدلات، ومعيار العدلات إلى الخلايا اللمفاوية وكذلك الألبومين إلى الكلوبيولين للمجموعة الأولى (G1) مقارنة بمجموعة بيروكسيد الهيدروجين ومستخلص الزعرور (G2). استنتج من هذه الدراسة ان التأثير الضار لبيروكسيد الهيدروجين ممكن معالجته 70% مستخلص الزعرور الكحولي في ذكور الجرذان.

الكلمات المفتاحية: الزعرور (*Crataegus oxycantha*)، كريات الدم البيض، الصفيحات الدموية، بيروكسيد الهيدروجين، مقياس البلعمة، البروتين الكلي، الكلوبيولين.

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs has been isolated from natural sources, many based on their use in traditional medicine. Plant extracts are attractive sources of new drugs and have been shown to produce promising results in the treatment of various diseases (1). *Crataegus oxycantha*, commonly known as Hawthorn, is one of the most widely used herbal plant with divers pharmacological actions. It possess antioxidant and collagen stabilizing action. Hawthorn, is used to treat a wide variety of inflammatory conditions, but mainly, it used in treatment of hypertension, ischemic heart disease (2). It's also rich in antioxidants, that may aid to prevent damages induced by free radicals, like heart disease(3). Another important effect of hawthorn extract. Its enhanced of immune system function. The chemical compounds in hawthorn extract may encourage the production of white blood cells and alleviate pro-inflammatory immune responses (4). Hence, the aim of the present study is to determinate the effect of alcoholic extract of hawthorn on some physiological parameters of H_2O_2 treated rats.

Materials and Methods

The fresh fruits of hawthorn were extracted with 70% ethanol according to(5). Twenty mature (200-220 gm) adult albino wistar male rats were randomly divided into four equal groups (5/group), they had free access to food and water. The control group, rats were received tap water. Animals of the first group received 1% H_2O_2 in drinking water. Second group received (300 mg/kg) B.W of 70% alcoholic extract of hawthorn. At the end of the experiment blood samples were collected by heart puncture technique for measuring the following parameters: Total WBC, the percentage of WBC differential count (Lymphocytes, Neutrophil, Monocyte, Basophil, and Eosiophil), platelet count as described by (6), phagocytic activity (%) was measured as described by (7) while Neutrophil/Lymphocyte (N/L) and index was measured according to(8). These parameters were measured immediately after collection of blood samples. Besides, serum sample were used for measuring total protein and albumin enzymatically by using kit of linear chemicals. Also globulin concentration estimated indirectly, by measuring of albumin in serum and then it was subtracted from the result of total protein (9). Differences between experimental groups, were evaluated using one-way analysis of variance (ANOVA), specific groups differences were determined using least significant differences (LSD) for all analysis, $P<0.05$ was considered according to (10).

Results

Table (1), showed significant increase ($p<0.05$) in total leukocyte count following intubation of hawthorn extract with H_2O_2 comparing with H_2O_2 and control groups.

Table (1) Effect of 70% alcoholic extract (300 mg/kg) of hawthorn (*Crataegus oxycantha)* on the total WBC count ($\times 10^9$ cell /l) in 1% H_2O_2 treated male rats

Groups	Total WBC
Control (C)	5382 ± 161.4 B
H_2O_2 (G1)	4720 ± 62.6 C
H_2O_2 with hawthorn (G2)	6310 ± 51.1 A

Values expressed as means ± SE. (n=5/group)
Capital letters denote between groups differences,p≤0.05

Table (2) pointed, to significant increase in percentage lymphocyte at the level of ($p<0.05$) in H_2O_2 with hawthorn group as compared with H_2O_2 and control groups. The results, revealed that exposure of rats to H_2O_2 for 30 days showed significant increase at the level of ($p<0.05$) in neutrophil and monocyte percentage in H_2O_2 group comparing to H_2O_2 with hawthorn and control groups.

Table (2) Effect of 70% alcoholic extract (300 mg/kg) of hawthorn (*Crataegus oxycantha)* on WBC differential count (%) in 1% H_2O_2 treated male rats

Groups	Lymphocyte	Neutrophil	Monocyte	Basophil	Eosinophil
Control (C)	63.6 ± 1.3 B	30.2 ± 0.6 B	4.0 ± 0.8 B	0.4 ± 0.4	0.4 ± 0.4
H_2O_2 (G1)	25.0 ± 0.7 C	38.8 ± 1.2 A	34.0 ± 0.5 A	0.4 ± 0.4	0.4 ± 0.4
H_2O_2 with Hawthorn (G2)	81.8 ± 1.1 A	15.0 ± 0.3 C	3.0 ± 0.3 B	0.1 ± 0.1	0.8 ± 0.8

Values expressed as means ± SE .(n=/5 group)
Capital litters denote between groups differences, p≤ 0.05.

Table (3), clarified a significant increase ($p<0.05$) in phagocytic activity in a treated groups (G1&G2) as comparing with control group, especially H_2O_2 with hawthorn group. Besides significant depression in N/L index was observed in G2 treated group as shown in table (4) comparing to H_2O_2 and control groups.

Table (3) Effect of 70% alcoholic extract (300 mg/kg) of hawthorn (*Crataegus oxycantha)* on phagocytic index in 1% H_2O_2 treated male rats

Groups	Phagocytic index
Control (C)	67.6 ± 0.5 C
H_2O_2 (G1)	75.6 ± 0.5 B
H_2O_2 with hawthorn (G2)	88.8 ± 1.2 A

Values expressed as means ± SE. (n=5/group)
Capital letters denote between groups differences, p≤0.05

Table (4) Effect of 70% alcoholic extract (300 mg/kg) of hawthorn (*Crataegus oxycantha)*on N/L index in 1% H_2O_2 treated male rats

Groups	N/L index
Control (C)	0.447 ± 0.010 B
H_2O_2 (G1)	1.560 ± 0.050 A
H_2O_2 with hawthorn (G2)	0.184 ± 0.005 C

Values expressed as means ± SE. (n=5/group)
Capital letters denote between groups differences,p≤0.05

Table (5) demonstrated that platelet count significantly decreased ($p<0.05$) in H_2O_2 group as compared with other groups, but it return to the normal level after treatment with hawthorn extract.

Table (5) Effect of 70% alcoholic extract (300 mg/kg) of hawthorn (*Crataegus oxycantha)* on platelets ($\times 10^6$/L) in 1% H_2O_2 treated male rats

Groups	Platelet count
Control (c)	823.0 ± 2.40 B
H_2O_2 (G1)	218.8 ± 16.90 C
H_2O_2 with hawthorn (G2)	878.4 ± 2.54 A

Values expressed as means ± SE. (n=5/group)
Capital letters denote between groups differences,p≤0.05

Table (6) refers that hawthorn extract with H_2O_2 causes a significant increase in total protein and globulin as compared with other groups. Meanwhile, A/G index increased significantly in H_2O_2 group as compared with G2 group only.

Table (6) Effect of 70% alcoholic extract (300 mg/kg) of hawthorn (*Crataegus oxycantha)* on total proteins, albumin, globulin and Albumin/Globulin (A/G) index in 1% H_2O_2 treated male rats

Groups	Total proteins	Albumin	Globulin	A/G
control (c)	6.50 ± 0.050 B	3.60 ± 0.13	2.90 ± 0.60 B	1.56 ± 0.40 A
H_2O_2 (G1)	5.30 ± 0.080 C	3.56 ± 0.11	1.74 ± 0.20 C	1.54 ± 0.20 A
H_2O_2 with Hawthorn (G2)	7.40 ± 0.024 A	3.31 ± 0.09	4.10 ± 0.24 A	0.84 ± 0.05 B

Values expressed as means ± SE. (n=5/group)
Capital letters denote between groups differences,p≤0.05

Discussion

The effects of Hawthorn (*Crataegus oxycantha*) extract and 1% H_2O_2 on physiological parameters of male rats was illustrated in tables (1, 2, 3, 4, 5 and 6). Antioxidant, is a substance fights Reactive Oxygen Species (ROS) and protect the cells from their damaging effects (11). The results obtained in the present study, clearly showed that hawthorn treatment was effective in influencing blood homeostasis in rat. Many studies, refer that hawthorn extract may enhance immune system function (12, 13). The chemical compounds in hawthorn extract may encourage the production of white blood cells, which attack and destroy viruses, bacteria and fungi that causes disease(14). Elango and Devargi (15), conducted a study on neuro protective effect for hawthorn (*Crataegus oxycantha)* ethanolic extract. They focused on the immune modulatory effect in male rats. In their study, treatment with hawthorn lowered pro-inflammatory cytokines such as (IL-1B, TNF-α, IL-6), ICAM-1 (Intra Cellular Adhesion Molecule). Furthermore, the extract boosted levels of Pro-inflammatory Immune response cytokines and cells in the brain such as interleukin-10 (IL-10), and Forkhead box p3 (Foxp3) positive T regulatory cells, that may have contributed to the suppression of activated inflammatory cells. In parallel, through this action mimizes apoptotic cell death. Hawthorn extract, contains high levels of flavonoids compounds that have Protective effects against oxidative stress (16). Hawthorn, contains phenolic and flavonoid compounds which have antioxidant and radical scavenging activities (17). Previous studies, have showed that treatment with *Crataegus oxycantha* depressed neutrophil activation and recruitment leading to allivation tissue damage induced by oxidative stress, and an increase in ROS, and this aggress with our results. During

inflammation, neutrophils produce abundant hypochlorous acid from hydrogen peroxide and chloride ion during the neutrophil's respiration (abundance of reactive oxygen species). Hypochlorous acid is highly cytotoxic and has been demonstrated to damage tissue during inflammation (18). Furthermore, hawthorn exhibits immunostimulant activity by inducing phagocytic rates and phagocytic index (19). There is substantial evidence demonstrating, that many genes and signal transduction pathways influenced by H_2O_2 and antioxidants. Accordingly, treatment with hawthorn was associated with increase in mRNA and protein level such as Nrf2-dependent genes as glutathione-S-transferases, NAD(P)H: Quinon oxidoreductase 1 (NQO1) and hem oxygenase-1-in hepatocytes so, Hawthorn have antioxidant, detoxifying effect in hepatocytes and this may explain the hepatoprotective and chemo protective properties of these phytochemicals(20). Excess H_2O_2 are known to effect the expression of a number of genes transcription factors such as NF-KB factor and activated it in monocyte and lymphocyte then cell death(21). In addition, oxidative stress by H_2O_2 leading to production of several hormones of stress such as cortisol, epinephrine and nor-epinephrine which they causes increase in neutrophil and N/L index in blood (22). However, this type of stress also leading to increase in monocyte percentage and many mediators such as monocyte-chemo attractant protein 1 (MCP1) and macrophage inflammatory molecule 1α (MIP1α). Therefore, neutrophil and monocytes increased in circulation and phagocytosis increased in tissue, to prevent extensive damage to the host (23). For these reasons the chemoprevention ability of Hawthorn extract has ascribed to their chemical contains that posses a potent antioxidant activity which relief DNA damage in the cells. Free radicals, can impart important changes in the platelet glycoproteins, this protein become more susceptible to proteolysis, because oxidative stress can causes desialylation of platelet glycoproteins, then the life span of platelet decreased (24). As an antioxidant, hawthorn extract had an inhibitory effect on desialylation of platelet glycoproteins(25). Concomitantly, a powerful antioxidant hawthorn, may diminish oxidant stress by stabilizing the inflammation pathway (NF-KB) (Nuclear factor-kappa B) and prevent the activation of this pathway. Thus, reduce inflammatory response and decrease cell injuries which is the first events in the development of disease (26). Blood proteins affect by Oxidative. Albumin known as physiological antioxidant, it's the most abundant protein in serum. Recent evidence indicates that albumin may provide antioxidant protection by functioning as serum peroxidase in the presence of reduced glutathione, which is an intracellular antioxidant (27). Moreover, oxidative stress induces oxidative degradation of protein in vitro. Therefore, globulin also modified then decreased after attack by H_2O_2 (28). Recent studies, have been described the affinities between hawthorn extract and plasma proteins, such as globulin and protect it from the oxidative stress that could be produced after exposure of H_2O_2. The medicinal properties of hawthorn as discussed in this study highylighted significant pharmacological activities of this species. Pharmacological actions experimented on animals conclude its potential effect as immunostimulants antioxidant. Presence of bioflavonoids, polysaccharides and other active compounds might be responsible for these pharmacological activities (29). Last but not least, this study emphasize the potential genus Hawthorn to be employed in new therapeutic drugs against the oxidative stress and boost the immune system.

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