Original Article

Biochemical studies on the effect of Crataegus aqueous extract on oxidative stress during ischemia/reperfusion induced myocardial injuries

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Abstract:

Background: myocardial ischemia-reperfusion (I/R) injury may occur in a variety of clinical settings and this remains a significant problem. Oxygen free radicals, produced on reperfusion have been shown to play a major role in myocardial I/R injury. Various therapeutic effects have been described for Crataegus. Additionally, it has been presented that Crataegus has protective effect against ischemia reperfusion induced myocardial injuries to various organs. Therefore, it seems possible that the administration of Crataegus might protect the heart against the ischemia reperfusion injury.

Objectives: I detected the chemical components and trace elements of the aqueous extract of Crataegus leaves and determine whether Crataegus extract prevents or decreases ischemia-reperfusion induced myocardial injuries in rats.

Methods: The chemical composition of the aqueous extracts of the Crataegus leaves was analysed and concentration of some trace elements was estimated .Thirty-six rats were divided into three groups as control, I/R group and Crataegus treatment group. Blood samples were taken from the rats for the biochemical parameters estimating, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) oxidative stress levels of nitric oxide (NO) and lipid peroxidation (LPO).

Results: The results showed that the extract contain: glycosides saponins, tannins ,phenolic compounds , proteins and flavonoids . The results also showed that there were high concentrations of K , Na , Ca with (171.2 , 19 , 18.3) ppm, respectively and low concentrations of Fe, Zn ,Cu, Mg with (5.1, 3.2 ,2.3 ,1.9) ppm, respectively ,very low concentrations Cr ,Cd , Pb with (0.9 ,0.7, Nil) ppm . The levels of heart enzymes and Oxidative stress in group 3 were significantly lower than those in the group 2.Our results suggest that Crataegus treatment protects the rat heart against ischemia-reperfusion induced myocardial injuries.

Conclusion: The present study confirms that the aqueous extract of Crataegus contains (glycosides, tannins, proteins, saponins, phenolic compounds and flavonoids) as well as our results suggest that Crataegus treatment protects the rat heart against ischemia-reperfusion induced myocardial injuries .

Key words: Crataegus, ischemia/reperfusion ,myocardial injuries.

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Introduction:

Ischemic heart diseases remain the leading cause of death in most developing countries as seen over the past quarter-century. Oxidative stress defines that, the level of Reactive Oxygen Species (ROS) exists in excess of antioxidant defenses. This inequity in the redox milieu results in a switch from ROS-stimulated ambient signaling processes to ROS-mediated pathophysiological consequences. Oxidative stress has been implicated in the installation and progression of several degenerative diseases via DNA mutation, protein oxidation and/or lipid peroxidation. In the vasculature, oxidant stress may result from either over production of ROS and/or a decrease in antioxidant capacity when either predominates in the vessel wall, the net result is ROS-mediated decrease in bioavailable nitric oxide and oxidative modification of lipids and proteins leading to impaired vasomotor reactivity, inflammation and dysregulated cell proliferation [1]. Cardiac ischemia is a condition in which blood flow and oxygen supply are insufficient the heart muscle. The main cause of cardiac ischemia is narrowed coronary arteries. When arteries are narrowed, there is less blood and oxygen supply to the heart muscles. Cardiac ischemia leads to coronary heart disease, angina pectoris, myocardial infarction, heart failure and ultimately heart attack [2]. Medicinal plants have been traditionally used in the treatment of several human diseases and their pharmacological and therapeutic properties have been attributed to different chemical constituents isolated from their crude extracts. Particularly the chemical constituent of antioxidant activity can be found at high concentration in plants and can be responsible for their preventing effects in various degenerative diseases, including, neurological and cardiovascular diseases as well as in cacer . Thus, the antioxidant properties of plants have full range of perspective applications in human health care [3]. Crataegus (Hawthorn) is one of the oldest medicinal plants and is described by many pharmacopoeias. Crataegus extract from leaves with flowers

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has been used to treat the early stages of congestive heart failure and angina pectoris[4]. Pharmacological studies also demonstrate that Hawthorn extract from fruit and flowerscan decrease the level of cholesterol in serum[5] and inhibit platelet conglomeration[6] .Evidences show that Crataegus extract from several parts of the plant including leaves has antioxidant effects in vitro or in vivo, Crataegus extract scavenges superoxide anion,hydroxyl radical, and hydrogen peroxides, and inhibits lipid peroxidation[7-8].In vivo experiments show that Crataegus extract from fruit increases the concentration of a-tocopherol and inhibits the oxidation of human low-density lipoprotein (LDL) [9]. Clinical studies indicate that its antioxidant activity is the mechanism of Hawthorn extraction's cardioprotective benefit[10].

Materials and Methods:

Leaves of Crataegus were collected from an area north of Baghdad, Iraq. Leaves were washed, cleaned with filter paper or soft clothes to remove all traces of dust and insects, then dried in shade 25-30ºC for one week, with continuous overturn to prevent mould ,weighed, ground in a mortar and pestle, placed in airtight bottles and stored in dissicator to be used for extraction . 50 g Air dried leaves were suspended in one liter of distilled water and left for 24 hrs at 35ºC with continuous stirring in a shaking incubator .Then the mixture was filtered by filter paper № 42 ,the filtrate was centrifuged for 10 min. at 2500 rpm ,and the extract evaporated to dryness at 40ºC in the incubator. The chemical components of the prepared watery extract were detected as shown in table .1.and the trace elements were determined [11] Experimental Design:Thirty-six male Wistar rats weighting 200-230g were used in this experimental study. All animals were maintained under standard conditions. Rats were deprived of food, but not water, for 24 h before surgery (ischemia and reperfusion). Animals were divided into three groups fed for 15 days with the same diet:, sham group (Group 1), I/R group (Group 2), and Crataegus (aqueous extract)treatment group (Group 3). All rats were anesthetized with 40-50 mg / kg of thiopental sodium. Crataegus was given to the rats in treatment group, before ischemia and before reperfusion at a dose of 1 mL/kg by intraperitoneal route. Surgical procedure: The ischemia-reperfusion injury was produced in rat heart based on Buerke's description with modifications [12-13] .Rats were placed on a warm board to control the body temperature at 37 °C for surgery. The chest was opened at the left fourth intercostal space. The pericardium was incised and the left atrium appendage was elevated to expose the left anterior descending (LAD) coronary artery. A 6-0 silk suture was passed around the LAD coronary artery, and the ends of suture were threaded through a small vinyl tube to form a snare. The thoracic cavity was covered with saline-soaked gauze to prevent the heart from drying. , ischemia was established by tightening the suture from both ends with fixed weight. The animals then underwent 45 min of ischemia, . Reperfusion was introduced by releasing the snare gently for a period of 60 min. The sham control animals were subjected to the entire surgical procedure described above, except the introduction of LAD ligation and release. At the end of reperfusion, the estimation of the biochemical parameters.

a)Heart enzymes : Plasma was used to measure AST, ALT and LDH as indicative parameters of heart function. The plasma activities of AST, ALT and LDH were estimated by commercially available kits using an autoanalyser (aeroset® Abbott Laboratories, Chicago, IL).

b)Oxidative stress indices: The left hemisphere was used to detect nitric oxide (NO) measured as total nitrite[14] and lipid peroxidation (LPO) as Thio Barbituric Acid Reactive Substances [15] . At the end of the experiment, heart tissue was immediately fixed in 10% buffered neutral formalin solution. The fixed tissues were embedded in paraffin and serial sections were cut. The sections were examined under light microscope after hematoxylin and eosin staining and photomicrograph.

Results:

The qualitative chemical analyses of the aqueous extracts are illustarted in Table.1,which indicate that leaves contents are glycosides, proteins, saponins, tannins and phenolic compounds and flavonoids. The obtained results were in agreement with preivious studies[16] .

Table 1: Phytochemical Composition for aqueous extract of Crataegus leaves.

Phytochemical	Reagents	Note	Result Watery extract
Glycosides	Iodine test Molish test Benedict test	Blue ppt. Violet ring Orange ppt.	Ve+ Ve+ Ve+
Proteins	Folin-Ciocalteau reagent	Blue color	Ve+
Saponins	Fast stirring Mercuric Chloride	Dense foam for long time White ppt.	Ve + Ve +
Phenolic compounds	Aqueous1٪ Ferric chloride	Green ppt.	Ve+
Tannins	Aqueous1٪ Ferric chloride Lead acetate1٪	Green ppt. Preface yellow ppt.	Ve+ Ve+
Flavonoids	aqueous1٪ Ferric chloride Ethanol hydroxide alcohol	Green ppt. Yellow ppt.	Ve+ Ve+

Key: +++ = Presence of bioactive compound in very high concentration

++ = High concentration

+ = Presence of bioactive compound

Figure 1 shows the concentrations of trace elements in Crataegus leaves. The results reveals the presence of a high concentrations of K , Na and Ca with 171.2 ,19 , 18.3 ppm, respectively and low content of Fe, Zn ,Cu and Mg with 5.1, 3.2 ,2.3 and 1.9 ppm, respectively . ther is very low concentrations of Cr and Cd.

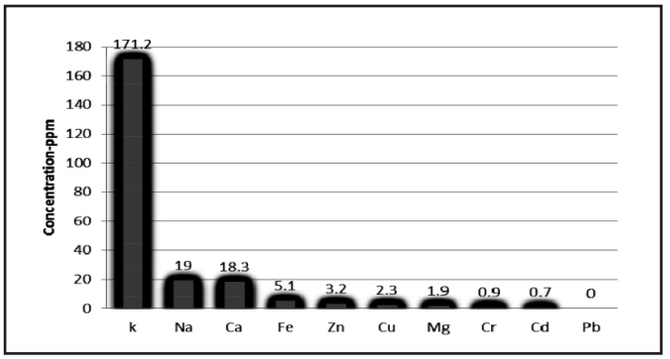

Figure 1: heavy metal content of Crataegus leaves.

As anticipated, I/R caused production of oxygen free radicals has been clarified in ischemic reperfused heart, leading to tissue damage as indicated by increased levels of ALT, AST, and LDH (in the I/R group) while ALT, AST, and LDH levels in the Crataegus treatment group were significantly lower than those in the I/R group (they were significantly higher in the I/R group than those in the control group). The results are depicted in Table 2.

Table 2. Clinical parameters in control, I/R and I/R + Crataegus, rats (n=12, mean± SD)

Clinical parameters	control	I/R	I/R + Crataegus	P
AST (U/L)	134 ± 18	995 ± 186	567±89	0.001
ALT (U/L)	84 ± 14	864 ± 208	574± 137	0.001
LDH (U/L)	524 ± 172	2854 ± 426	2461 ± 498	0.001

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase
Significances against controls: *P<0.001

Table 3 shows increased oxidative stress levels of NO and LPO while oxidative stress NO and LPO levels in the Crataegus treatment group were significantly lower than those in the I/R group. They were significantly higher in the I/R group than those in the control group.

Table 3: Oxidative stress in control, I/R and I/R + Crataegus, rats (n=12, mean± SD)

Oxidative stress	control	I/R	I/R + Crataegus	P
NO (µmol/ml) In Serum	14.02± 0.5	26.72 ± 1.4	18.07 ± 1.6	0.001
LPO (nmol/ml) In Serum	0.34±0.003	1.04 ± 0.03	0.71 ± 0.07	0.001

NO: nitric oxide , LPO: lipid peroxidation

As indicated in the histopathological evaluation, there were no pathological changes in heart tissue of the control group (Group 1). Heart specimens from rats after I/R exhibited focal necrosis and infiltration of leukocytes (Group 2). Crataegus treatment significantly decreased these pathological changes (Group 3). Histological tissue damage was milder in the Crataegus treatment group than that in the sham group.

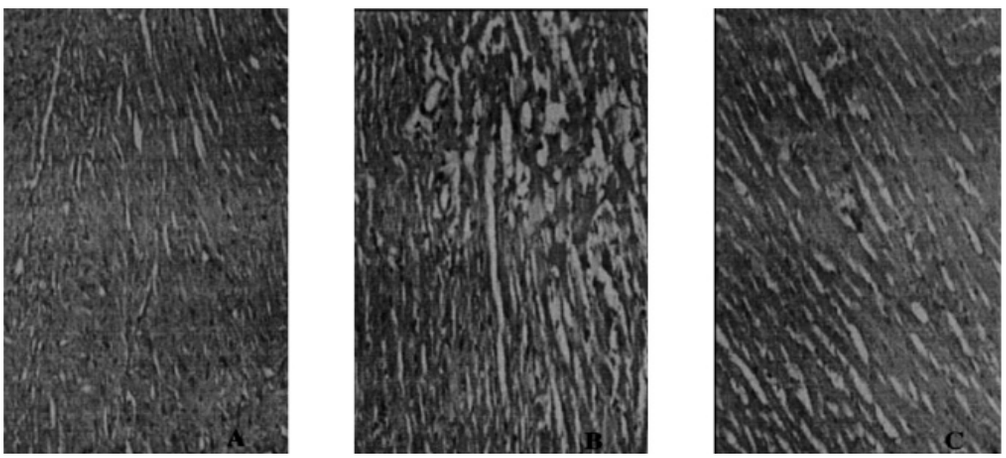

Figure 2 A: Normal heart tissue(Group 1) B: Histopathological findings 60 min after I/R(Group 2); C: Histopathological findings 60 min after I/R + Crataegus (Group 3).

Discussion:

The present results show that aqueous extract of Crataegus leaves could protect myocardial ischemic damage in a rat. through, reduced oxidative stress levels induced by myocardial ischemic. Aqueous extract of Crataegus leaves contains phytochemicals including glycosides, proteins, saponins, tannins , phenolic compounds and flavonoids which are known to exhibit medicinal as well as physiological activities. Flavonoid are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection . They have been found to be anti-microbial substances against wide array of microorganisms invitro. They are also effective antioxidants. [17] The presence of these phytochemicals in aqueous extract of Crataegus leaves could be contributory to its antioxidant activity observed in this investigation. In the present experiment the order of increasing relative abundance of these phytochemical in the aqueous extract of Crataegus leaves is glycosides, proteins, saponins, tannins, phenolic compounds and flavonoids. [18] The heavy metal levels observed in the aqueous extract of Crataegus are summarized in Table 2,In the aqueous extract, Potassium recorded the highest concentration in the leaves was 171.2ppm , Potassium is accumulated within human cells by the action of the Na, K- ATPase (sodium pump) and it is an activator of some enzymes; in particular co-enzyme for normal growth and muscle function .Potassium deficiency causes nervous disorder, diabetes, and poor muscular control resulting in paralysis. The high content of K in the leaves of Crataegus may be useful as a medicinal plant for the management and treatment of these disorders. [19]Sodium involves in the production of energy, transport of amino acids and glucose into the body cells. The concentration of Na in was 19ppm.The concentration of Ca was 18.3ppm .Calcium is essential for healthy bones, teeth and blood. The health of the muscles and nerves depends on calcium. It is required for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter acetylcholine, for the activation of enzymes such as the pancreatic lipase. Deficiency of calcium causes rickets, osteomalacia and scurvy [20]. The concentration of Fe was 5.1ppm iron is essential component of haemoglobin and the cytochromes. Serves in the expression of genes for receptors of ferritin, trans ferritin and metallothioneins.[21] Zinc recorded a concentration of 3.2ppm cofactor in more than 100 enzymatic reactions and essential component of nuclear DNA binding proteins; serves in the expression of genes for metallothioneins. Zinc deficiency causes a block in protein and nucleic acid synthesis. The immune system, the skin and the gastro-intestinal tract are the tissues of the body with the highest rate of protein synthesis that are affected by deficiency. [22]Copper is essential cofactor in several reactions concerning iron use, collagen synthesis, and suppression of free radicals. It Serves in the expression of genes for several enzymes[23]. The concentration of Cu in aqueous extract of Crataegus was 2.3ppm. The concentration of Mg was 1.9ppm .Magnesium improves insulin sensitivity, protect against diabetes and its complications and reduce blood pressure.In the leaves of Crataegus, the concentration of Cr ,Cd was less than one. Oxidative stress has been implicated in the pathology of many diseases such as inflammatory conditions, cancer, diabetes and aging [24].Free radicals induced by peroxidation have gained much importance because of their involvement in several pathological conditions such as atherosclerosis, ischemia, liver disorder, neural disorder, metal toxicity and pesticide toxicity [25].Reactive oxygen species (ROS) such as superoxide anions, hydroxyl radical and nitric oxide inactivate enzymes and damage important cellular component causing injury through lipid peroxidation and covalent binding[26]. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanism and thus prevent disease[27]. It is ubiquitously known that most antioxidants are flavonoids, like quercetin , rutin or myricetin [28]. In contrast, Crataegus leaves have been shown to possess antioxidant properties.

Crataegus can be reduced oxidative stress levels induced by myocardial ischemic. through increase coronary blood flow [29]. This may be due to relaxation of coronary arteries, which directly increases blood flow or through an increase in contraction and relaxation velocities, which increases the diastolic interval and thus allows more time for blood passage through the coronary arteries [30]. Crataegus's positive inotropic action may also be due to inhibition of oxidative stress levels of NO and LPO and myocardial Na+/K+ ATPase which is an integral membrane enzyme that maintains cardiac resting potential [31]. It also decreases blood pressure which results in an increase in exercise tolerance during the early stage of congestive heart failure [32]. Surprisingly, Crataegus has the ability to regulate both low and high blood pressure. With the bioflavanoids reportedly dilating both peripheral and coronary blood vessels leading to its use in angina [33]; the procyanidins content is claimed to support the vasorelaxant effects [34] . Crataegus's glycoside component has also been reported to increase vagal tone of the heart [35]. The principal activity, commonly proposed for its cardioactive effect is its ability to inhibit the enzyme phosphodiesterase which ultimately

results in an increase in intracellular cyclic nucleotides and a subsequent positive inotropic effect [36]. Crataegus has also been reported to have angiotensin converting enzyme inhibiting effect [37] .It may also have a cardio-protective effect due to its activity to decrease the oxygen demands of cardiac tissue [38]. Varying results have been observed regarding the effect of Hawthorn and its constituents on heart rate. In majority of in vitro studies, an increase in heart rate has been spotted while conversely, most in vivo studies report a decrease in heart rate [39] .

Conclusion:

The present study reveals that the aqueous extract of Crataegus (Hawthorn) contains glycosides, tannins, proteins, saponins, phenolic compounds flavonoids and some trace elements. Results suggest that Crataegus treatment protects the rat heart against Ischemia-reperfusion induced myocardial injuries.

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