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Original Research Article

Influence of hawthorn *(Crataegus Oxyacantha)* leaves extract administration on myocardial infarction induced by isoproterenol in rats

ABSTRACT

Aims: Investigate the influence of ethanolic hawthorn leaves extract (EHLE) oral administration (200mg / kg body weight /day) on isoproterenol (ISO) induced- myocardial infarction (MI) in rats and its bioactive constituents.

Methods: Healthy adult male albino rats (Sprague-Dawely strain) were divided into five groups (10 rats /group); MI was induced in rats by ISO at dose of {85mg/kg body weight/day subcutaneously (S.C.,)} on two consecutive days with 24hours interval.

Results: Pre-or pre-and post-treatment with EHLE significantly ($p \leq 0.05$) lowered the elevated serum cardiac enzyme marker activities namely, creatine kinase (CK), creatine kinase MB (CK- MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and specific MI markers level of galectin-3(Gal-3), cardiac troponin I (cTnI). Also, significantly ($p \leq 0.05$) ameliorated oxidant/ antioxidant status by decreasing oxidative stress biomarkers [tissue nitric oxide (NO), serum and tissue malondialdehyde (MDA)], and increasing antioxidant status [glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH)] in ISO-injected rats, these results was confirmed with microscopic examination of heart tissues.

Conclusion: Our results demonstrated that EHLE has a cardio-protective effect against ISO-induced MI in rats due to its high antioxidant properties, inhibition of lipid peroxidation and restoration of cardiac enzyme activities.

Keywords: Isoproterenol, Myocardial infarction, Biomarkers, Hawthorn, Oxidative stress, Antioxidant.

1.INTRODUCTION

Cardiovascular diseases (CVDs) remain the main cause of death throughout the world [1]. Myocardial infarction (MI) is the most lethal manifestation of CVDs cause morbidity and mortality in the world. It can be defined as sudden death to heart cells due to the insufficient oxygen supply of the myocardium which doesn't meet the oxygen demand of the myocardial tissue (anoxia); resulting in ischemia, if this ischemia is prolonged and isn't reversible, myocardial cell death and infarction occurs with tissue necrosis [2]. Myocardial infarction can result from chronic occlusion and accumulation of atherosclerotic or thrombotic lesions to one or more major branches of the coronary artery system. Also, the pathogenesis of MI is due to reactive oxygen species (ROS) [3]. During the reperfusion phase, ROS are produced following to ischemia; where ROS generated from ischemic tissue, often leading to biological chain reactions which contribute to several biochemical alterations such as oxidative damage in membrane unsaturated lipids, proteins, DNA, and consequently cell death [4].

The manifestations of myocardial infarction are varied and multiple such as chest pain, epigastric or arm discomfort, breathlessness, nausea and vomiting. Also, arrhythmia, sweating, weakness happen and sometimes loss of consciousness or even sudden death [5].

Isoproterenol (ISO) is a synthetic catecholamine and β- adrenergic agonist, which cause severe stress in the myocardium and necrotic lesions in the heart muscle [6].The biological mechanism of ISO for inducing MI as a result of disturbance in physiological balance between production of free radicals and anti- oxidative defense system by generating of high cytotoxic free radicals through the auto-oxidation of catecholamines [7], in addition to loss of integrity and function of myocardial membranes during induction of the disease [8], also results in alterations in enzyme activities , transport system and cause disturbances in cellular [9].

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Hawthorn plant (Crataegus Oxyacantha), also known as Haw, belongs to Rosaceae family, is part of a genus of spiny shrubs and trees native to temperate regions in different area of the world [10]. It prevents oxidative damage in ischemia by scavenging free radicals generated from ISO induction in which oxidative stress has long been known to contribute to the pathogenesis of MI [11]. Hawthorn therapeutic extract either derived from leaves, flowers or fruits of the plant have long been favored herbal remedy as cardio tonic agent through its active compounds such as flavonoids, phenols and oligomeric procyanidins [10,12].

Wang et al. (2013) illustrated that hawthorn extracts exert a wide range of cardiovascular pharmacological properties, including antioxidant activity, positive inotropic, anti-inflammatory, anti-cardiac remodeling, antiplatelet aggregation, vasodilating, endothelial protective, antiarrhythmic, lipid-lowering and decrease of arterial blood pressure effects, also has a protective effect against ischemia/reperfusion injury [10].

The present work aimed to investigate the influence of ethanolic hawthorn leaves extract (EHLE) on the functional bioavailability of the heart muscle, ameliorating isoproterenol induced MI in rats. As well as its effect on serum cardiac biomarkers, oxidative stress markers and antioxidant status.

2. MATERIAL AND METHODS

2.1. Plant material

Hawthorn leaves *(Crataegus Oxyacantha)* were purchased from traditional spices and herbs dealer shop from Cairo, Egypt. The dried hawthorn leaves were crushed to a fine powder in a mill, of which 25g were macerated/ soaked and mixed five times with 100 ml solvent (ethanol 75%). The extract was filtered over cotton cloth, then the filtrate was collected, and the solvent was removed by a rotary evaporator (WHEATON SP35) at 50℃ [13]. Every 25g of dried leaves gives 7g of the extract which stored at 4 ℃ until used. The extract was administered orally by gastric tube to the rats at dose 200mg/kg body three days weekly [14].

2.2. Chemicals

Isoproterenol (ISO) was purchased from Sigma-Aldrich Company for Chemicals, Egypt. Ethanol was purchased from Al-Gomhoria Company, (Cairo, Egypt). Biochemical kits were purchased from Science and Technology Center, (Mohandisieen, Egypt) and Bio diagnostic company, (Cairo, Egypt) and all other chemicals were of highest grade commercially available.

2.3. Animals

Healthy adult male albino rats (Sprague-Dawely strain) weighing185±15g were obtained from El Salam Farm, Giza, Egypt. The biological experiment was carried out in the Animal House of Biochemistry and Nutrition Department, Women's Faculty for Arts, Science and Education, Ain Shams University, where all rats (50 rats) were randomly housed individually in stainless steel cages with constant controlled environments; temperature 25℃ ± 5℃, air humidity 55%±10% and 12/12 hours light/dark cycle were held. All rats were offered the standard commercial pellet diet with drinking water *ad libitum* for one week before starting the experiment as adaptation period.

2.4. Induction of myocardial infarction (MI)

Myocardial infarction was induced by injecting rats with isoproterenol hydrochloride (ISO) at dose of 85mg/kg body weight/day subcutaneously (S.C.) on two consecutive days with 24 hours interval [15].

2.5. Experimental design

Fifty rats were divided into five groups; each group consisted of 10 rats; all rats were fed the standard commercial pellet diet and water *ad libitum* for six weeks as experimental period, the groups as following:

Group I: **Healthy untreated group (-ve control):** rats received distilled water by gastric tube three times weekly.

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Group II: **Healthy treated group:** rats administered 200mg/kg body weight / day of EHLE by gastric tube three times weekly.
Group III: **Myocardial infarction (MI) group:** rats were injected with ISO at dose of 85mg/kg body weight /day S.C., on two consecutive days with 24hours interval on 44th and 45th day.
Group IV: **MI treated group:** rats were injected with ISO at dose of 85mg/kg body weight /day S.C., on 1st and 2nd days with 24hours interval, and then received 200mg/kg body weight/day of EHLE by gastric tube three times weekly.
Group V: **Protected MI treated group:** rats received 200mg/kg body weight/day of EHLE by gastric tube three times weekly for 3 weeks then injected with the tested dose of ISO subcutaneously on 20th and 21st days with 24hs interval for producing MI condition then received the tested dose of EHLE by gastric tube three times weekly for another 3 weeks.

2.6. Handling of blood and heart samples

At the end of experimental period (6 weeks) all rats were scarified under ether anesthesia after 12 hours fasting. Blood samples were collected from hepatic portal vein and allowed to left for 15 minutes at temperature of 34ºC, and then centrifuged at 4000 rpm by EBA8 centrifuge for 20 min to separate serum. Serum was removed and kept in plastic vials at-20°C until used for biochemical analyses. The heart was separated, immediately rinsed and washed by saline solution then blotted on filter paper to remove water residue and weighed. Then part of the heart was immediately fixed in formalin 10% for microscopic examination, while the rest stored frozen at -20 °C until used for the tissue biochemical analysis.

2.7. Determination of bioactive constituents and anti-oxidant capacity of the tested ethanolic hawthorn leaves extract

Total phenolic content was determined using the Folin-Ciocalteu procedure [16] and expressed as mg gallic acid equivalents (GAEs) /g the extract, also phenols were separated and quantified using High Performance Liquid Chromatography (HPLC) Agilent 1200 series, in mg/100g of EHLE according to Goupy et al., (1999) [17]. Total flavonoids content was determined using aluminum chloride $AlCl_3$ assay [18] as mg of quercetin equivalent (QE)/g of the extract. Flavonoids were separated and quantified using HPLC Agilent 1200 series, in mg/100g of extract [19]. The stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical was used for the determination of free radical scavenging activity (antioxidant capacity) as described by Su and Silva, (2006) [20].

2.8. Biochemical Measurements

2.8.1 Serum cardiac muscle enzyme activities and specific MI biomarkers.

Serum creatine kinase (CK) , serum creatine kinase MB (CK- MB) and serum lactate dehydrogenase (LDH) Were determined according to Chemnitz et al., (1979) {21] ,Würzburg, et al., (1977) [22] and Weibhaar et al., (1975)[23] respectively, using Automated Biochemistry Analyzer SBA-733 plus, serum cardiac troponin I (cTnI) was determined by a two-site immunoenzymatic ("Sandwich") assay using Acess2 Immunoassay systems[24], serum galectin-3 (Gal-3) was determined by enzyme linked immunosorbent assay [25],serum aspartate aminotransferase (AST) was determined according to Reitman and Frankel, (1957) [26].

2.8.2. Oxidative stress biomarkers and antioxidant parameters.

Serum and tissue malondialdehyde (MDA) concentrations were determined according to Ohkawa et al., (1979) [27], nitric oxide (NO) content was determined in heart tissue homogenate according to the method described by Montgomery and Dymock, (1961) [28]. Tissue glutathione peroxidase (GPX) enzyme activity was determined according to Paglia and Valentine, (1967) [29], tissue superoxide dismutase enzyme activity (SOD) measured according to the method of Nishikimi et al., (1972)[30], catalase (CAT) enzyme activity in serum and heart tissue was determined according to Aebi, (1984) [31]and cardiac reduced glutathione (GSH) concentration was determined according to Beutler et al., (1963) [32].

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2.9. Microscopic Examination

The hearts were washed in ice cold saline. Then myocardial tissue was immediately fixed in 10% formalin solution according to Drury and Wallington, (1980) [33]. After fixation, tissues were embedded in paraffin wax and serial sections were cut by Cambridge rocking microtome of thickness 5 microns, and each section was stained with haematoxylin and eosin (H&E). The slides were examined under a light microscope and photographs were taken at 400X magnification, as described by Bancraft et al., (1996) [34].

2.10. Statistical analysis

Data were statistically analyzed by Statistical Package for Social Sience "SPSS" version 16.0 Micosoft Windows, SPSS Inc. Values were expressed as mean ± standard deviation (S.D) Statistical difference between groups were done by using one way analysis of variance ANOVA, the mean difference was significant at the (P≤ 0.05) level according to Levesque, (2007)[35].

3. RESULTS AND DISCUSSION

3.1 Bioactive constituents and anti-oxidant capacity of the tested ethanolic hawthorn leaves extract

The data presented in **table (1)** illustrate the mean values of total phenols as mg gallic acid equivalents (GAEs)/g of the extract, total flavonoids as mg quercetin equivalent (QE)/g of the extract, as well as total antioxidant capacity indicated by the 1, 1-diphenyl-2-picryl hydrazyl DPPH free radical scavenging activity by EHLE. Also **figures (1 and 2)** show the percentage of main phenolic and flavonoid compounds respectively in the tested EHLE as determined by HPLC method. Our findings indicated that the tested EHLE serves as good source of phenols, flavonoids and also high free radical scavenging activity which could have great effects on reducing the oxidative stress and the inflammation leading to decreasing the MI severity.

Polyphenols and flavonoids are important constituents and serve as powerful antioxidants due to the hydrogen-donating ability of their hydroxyl groups as well as their ability to donate electrons to arrest the production of free radicals as a result of oxidative stress [36]. Barros et al., (2011) [37] indicated that hawthorn extract is well known rich source for flavonoids which include: hyperoside, isoquercitrin, epicatechin, chlorogenic acid, quercetin, rutin and protocatechuic acid. Flavonoids are capable of inhibiting the formation of superoxide ions and hydroxy radicals, which are two strong peroxidation agents that are produced in the body under normal conditions and cause destruction of cells [38].

Rutin, Luteolin and ferulic acid are one of great importance flavonoids and phenols in EHLE. Rutin inhibits platelet aggregation [39]. Ferulic acid, can reduce the cytotoxicity of oxidized LDL cholesterol and lowers the risk of heart disease [40]. Numerous experimental data have revealed that luteolin possesses a wide range of pharmacological effects, including antioxidant [41], anti-inflammatory [42], anti-platelet and vasodilating activity [43].

Table 1. Bioactive components and total antioxidant capacity of the tested EHLE

Active components of ethanolic hawthorn leaves extract	Values / 1g of ethanolic extract
Total phenols	70.048 ± 0.5254 mg as gallic acid equivalents (GAEs).
Total flavonoids	154.417 ± 0.7406 mg as quercetin equivalent (QE).
Total antioxidant capacity	Each 0.05 ml of plant extract scavenged 5ml of DPPH by 89.55 ± 0.5683%

*Values are means of six replicates; values are mean ± SD, $P \leq 0.05$.

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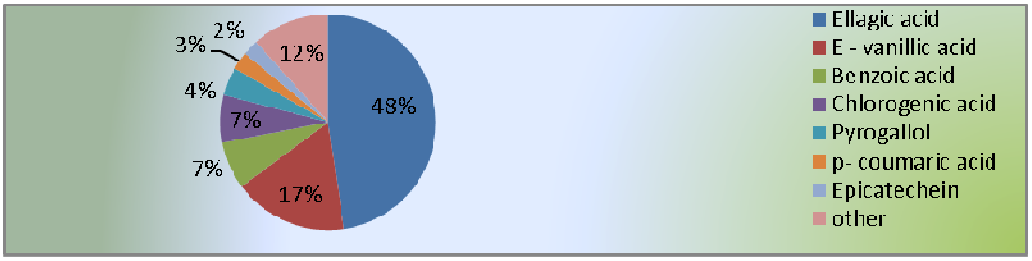

Fig. 1. Percentage of some main phenolic compounds derived from ethanolic hawthorn leaves extract

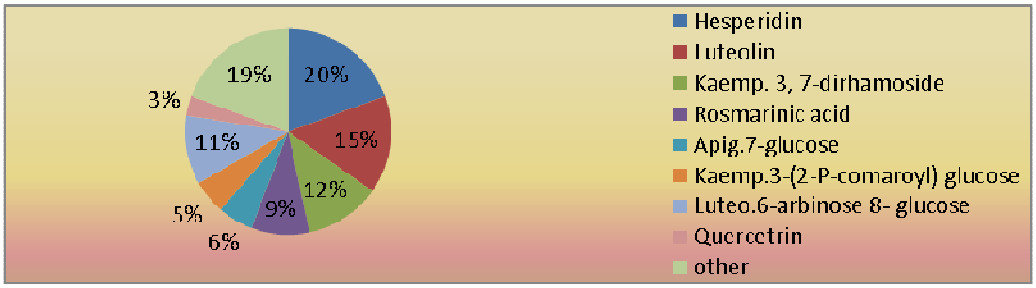

Fig. 2. Percentage of some main flavoind compounds derived from ethanolic hawthorn leaves extract

3.2. Serum cardiac muscle enzyme activities and specific MI biomarkers.

The data presented in **table (2a)** indicated that ISO injected rats have a statistical significant ($P \leq 0.05$) elevation in serum cardiac injury enzyme activities of LDH, CK, CK- MB and AST by 2.11, 1.44, 1.54 and 1.88 folds respectively as compared with healthy control rats. While oral administration of EHLE post MI induction resulted in a significant reduction in LDH, CK, CK- MB and AST enzyme activities by 29.14%, 11.7%, 13.84% and 34.178% respectively at ($P \leq 0.05$) as compared to MI untreated rat group. Meanwhile EHLE administration pre and post ISO injection showed more statistical significant improvement ($P \leq 0.05$) in the serum enzyme activities of LDH, CK and CK-MB and AST by 43.88%, 24.71%, 24.91% and 40.759% respectively in comparison with MI untreated rats.

From the data obtained in **table (2b),** it was obvious that, there was a significant ($P \leq 0.05$) elevation in the mean values of serum specific MI biomarkers of cTnI and Gal-3(0.012±0.0036 µg/L and 8.198±0.0055 ng/ml respectively) in ISO induced MI rat group when compared with healthy untreated one. While the rats treated with EHLE after MI induction showed a significant fall in serum cTnI and Gal-3 by 94.06% and 53.38% respectively and more significant decrease in rats treated with EHLE before and after MI induction by 96.18% and 54.63% for serum cTnI and Gal-3 respectively comparing with MI untreated rat group.

An insufficient supply of oxygen or nutrients to cardiac tissues or chemically induced cardiac damage may increase the permeability or even rupture the cardiac membrane, resulting in leakage of cytosolic enzymes, including CK, CK-MB, AST, LDH, cTnI (diagnostic markers of MI), into the bloodstream and a subsequent increase in their serum concentrations [44]. In the present study, ISO administration in rats caused a marked elevation in the activities of all the cardiac marker enzymes in the serum, in accordance with previously reported studies [45,46].

Swamy et al., (2011) found that there was a significant increase in activities of cardiac marker enzymes (CK-MB, LDH, and AST) in the serum during ISO administration, which used as markers of myocardial injury. Isoproterenol administration produces free radicals (via a beta adrenoceptor mechanism) that affect the cell metabolism, such that toxic free radicals are formed producing myocardial cell necrosis [47].

Lactate dehydrogenase LDH is an enzyme involved in anaerobic metabolism, reversibly converting pyruvate to lactate in redox reaction [48]. An increase in serum LDH activity is found following myocardial infarction beginning within 6 to12 hours and reaching a maximum at about 48 hours and it

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remains elevated for 4-14days before coming down to normal levels. The prolonged elevation makes it a good marker for those patients admitted to the hospital after several days of MI [49].
CK is an enzyme responsible for transferring a phosphate group from ATP to creatine. It is composed of M and B subunits that form CK-MM, CK-MB and CK-BB isoenzyme. Total CK is not cardiac specific as it raised in other diseases not from cardiac tissue but CK-MB is a sensitive as well as a specific marker for myocardial infarction. CK-MB begins to rise 4 - 6 hour after myocardial infarction, peak at 24 hours and return to normal within48-72 [50].

Troponin is a myocyte protein that regulates actin-myosin cross-bridge formation during the contraction-relaxation process in striated muscles. It consists of three different subunits: troponin T (TnT), the tropomyosin binding component, troponin C (TnC) the calcium binding component and troponin I (TnI) the inhibitory component. Both TnT and TnI have three different isoforms each of which is a structure unique to cardiac muscle but not in smooth muscle [51]. Fredericks *et al.,* (2001) showed that the content of cTnI and cTnT in skeletal muscle was below 0.6% that found in the heart, making troponin a specific biomarker in myocardial infarction [52]**.**

Galectin-3 is new biomarker involved in cardiac inflammation and fibrosis which could contribute and increased risk to MI and heart failure [53]; Galectin-3, a soluble beta-galactoside-binding lectin, is secreted by activated macrophages and modulates several physiological and pathological processes, including inflammation and fibrosis. This protein directly induces fibroblasts to proliferate and deposit type I collagen in the extracellular matrix [54].
Our results are in agreement with Sun et al., (2015) who found that there were significant increased serum levels of CK, CK-MB, LDH and cTnT expression in rat with AMI after isoproterenol induction [55], also Subashini and Rajadurai, (2011) found that subcutaneous injection of ISO (85 mg/kg) to male albino Wistar rats, exhibited a significant raise in the activities of cardiac marker such as cardiac troponin T, CK-MB, CK, LDH, AST in serum with subsequent decrease in the heart tissue. It is suggested that ethanolic hawthorn extract administration in the treatment and protection groups has successfully reduced the damage in the cardiac tissues by lowering CK-MB, CK, LDH, AST and cTnI levels in experimental rats [56]. Hypothetically, ethanolic hawthorn extract significantly helped in maintaining the integrity of the membrane cells, thus restricting the leakage of these enzymes. The severity of the myocardial necrosis in experimental rats was reduced with giving Crataegus extract, improved the recovery of cardiac contractile function, and reduced the size of the myocardial infarct which is in accordance with the findings from previous studies [57].

Table 2a. Effect of ethanolic hawthorn leaves extract administration on serum heart muscle enzyme activities in all experimental rat groups.

Parameter / Groups	Serum LDH (µkat/L)	Serum CK (µkat/L)	Serum CK-MB (µkat/L)	Serum AST (U/ml)
Healthy untreated group (-ve control)	4.809±0. 90[ad]	28.05±2.88[ad]	46.356±1.86[a]	73.581±3.97[a]
Healthy treated group	4.0375±0.74[a]	27.25±3.22[a]	44.719±2.19[a]	71.342±2.72[a]
MI group	10.165±1.34[b]	40.4±3.69[b]	71.45±3.23[b]	138.76±2.66[b]
MI treated group	7.203±0.88[c]	35.675±2.34[c]	61.562±3.11[c]	91.334±1.78[c]
Protected MI treated group	5.705±1.50[d]	30.416±1.79[d]	53.65±2.93[d]	82.202±4.90[d]

*Values are represented as mean ± SD., (n=10), there was no significance difference between means have the same letter in the same column, (P≤ 0.05).

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Table 2b. Effect of ethanolic hawthorn leaves extract administration on some specific serum biomarker of MI in all experimental rat groups

Parameters / Groups	Serum cardiac troponin I (µg/L)	Serum galectin-3 (ng/ml)
Healthy untreated group (-ve control)	0.012±0.0036[a]	8.198±0.0055[a]
Healthy treated group	0.012±0.0022[a]	8.195±0.006[a]
MI group	0.4715±0.096[b]	18.563±1.54[b]
MI treated group	0.028±0.0087[a]	8.653±0.44[a]
Protected MI treated group	0.018±0.0089[a]	8.422±0.25[a]

*Values are represented as mean ± SD., (n=10), there was no significance difference between means have the same letter in the same column, (P≤ 0.05).

3.3. Oxidative stress biomarkers and antioxidant parameters

According to the data summarized in **table (3 and 4)** ISO- induced MI in rats caused a significant (*P*≤ 0.05) elevation in oxidative stress biomarkers (serum and tissue MDA by 1.99 and 1.42 folds respectively and tissue NO by 4.0899 folds in comparison with healthy control group, with reduction in enzymatic antioxidant activities as cardiac glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) by (67.96%, 31.179% and 13.329%) respectively at (*P*≤ 0.05), In addition, diminished serum CAT by 27.599% as compared to normal rats, also reduction in cardiac non-enzymatic antioxidant GSH by79.674%. While the treatment with EHLE either pre-and/ or post MI induction caused a significant improvement in oxidative stress markers and antioxidant activities.

The upsurge in MDA contents due to ISO damages antioxidant protective mechanisms and rendered the myocardium more vulnerable to lipid peroxidation, hence oxidative degeneration of fatty acids in myocardial membrane [58]. Increased formation of degradation product of lipid peroxidation, MDA is an indication of the severity of the cellular injury to the heart induced by ISO, and this can be linked with altered membrane structure and enzyme inactivation [59]. In our study, diminished level of MDA following the hawthorn leaves extract treatment can be reasonably speculated to augmented actions of antioxidant defense in myocardium.

Nitric oxide is significantly increased in the myocardial infarction group this is due to that ISO induction causes an increased expression of iNOS. This increased iNOS produces large amount of nitric oxide which is known to react with super oxides leading to production of reactive nitrogen species (RNS) like peroxynitrite (ONOO-). A high concentration of nitric oxide has also been reported to induce apoptotic cell death in several cell types [60]. Increased NO activates COX-2, the mechanism responsible for the effect of nitric oxide on COX-2 activity involves nitrosylation of a cysteine residue in the active site of the COX-2 enzyme leading to the formation of nitrosothiols. These can cause structural changes in the enzyme leading to increased COX-2 catalytic efficiency. The expression of COX-2 together with that of iNOS leads to inflammation of myocardial tissue [61].

Our results are confirmed by Madhesh and Vaiyapuri, (2012), who found that ISO-induced acute and chronic models in rats showed significant increase in the levels of thiobarbituric acid reactive substances, lipid hydro peroxides in the heart and erythrocyte, and significant decrease in the activities of heart and erythrocytes SOD, CAT, GPX, GSH. While oral treatment with luteolin one at a daily dose of (0.3 mg/kg b.wt) in both acute and chronic models showed significant decrease in the levels of heart and erythrocyte lipid peroxidation by products and significant increase in the levels of antioxidant system[62].

In the current study, there was a significant decrease in heart tissue GSH concentration as well as all antioxidant enzymes activities in MI rats. These results are in agreement with Neri et al., 2015; Patel et al., (2010) & Goyal et al., (2015) [63-65]. Such may be due to the increased utilization of these enzymes for scavenging ROS and their inactivation by excessive ISO oxidants .Following the administration of ISO, a robust fall in the activities of endogenous antioxidant systems of the heart leads to the gradual loss of pro-oxidant/antioxidant balance that accumulates in cardiomyocytes and manifest as oxidative [66].

Free radical scavenger enzymes such as catalase are the first line of defense against oxidative injury, decomposing H_2O_2 and O_2 before their interactions to form the hydroxyl radical (OH). During AMI,

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superoxide radicals modulate the activity of catalase, resulting in reduced activity of this enzyme and accumulation of superoxide radicals, with consequent damage to the myocardium [67].

Ethanolic hawthorn leaves extract has shown involved in the scavenging of ROS and confer defense against lipid peroxidation in accordance to the previous observations demonstrated anti-oxidative mechanism against the free radical induced oxidative damages of body organs which proved the excellent anti- oxidant potential of EHLE as showed in the literature [68,69]

The treatment with hawthorn extract significantly prevented the exhaustion of GSH from heart as evidenced by restored GSH content in the heart of animals of treatment and protection groups when compared to ISO group, as well as made an improvement in the tissue SOD, CAT and GPx due to scavenging of ROS which demonstrated anti-oxidative mechanism against the free radical induced oxidative damages of body organs.

Table (3): Effect of ethanolic hawthorn leaves extract administration on oxidative stress biomarkers in all experimental rat groups.

Parameters / Groups	MDA		Tissue NO (µmol/g.tissue)
	Serum (nmol/ml)	Tissue (nmol/g.tissue)	
Healthy untreated group (-ve control)	9.791±0.43[a]	38.845±0.68[a]	13.726±0.93[a]
Healthy untreated group	9.641±.581[a]	38.058±1.30[a]	12.5865±1.53[a]
MI group	19.491±0.61[b]	55.197±0.86[b]	56.139±0.78[b]
MI treated group	14.037±1.32[c]	45.059±1.21[c]	32.444±3.21[c]
Protected MI group	11.781±0.83[d]	41.207±0.74[d]	19.972±1.53[d]

*Values are represented as mean ± SD., (n=10), there was no significance difference between means have the same letter in the same column, (P≤ 0.05).

Table (4): Effect of ethanolic hawthorn leaves extract administration on enzymatic and non-enzymatic antioxidant parameters in all experimental groups.

*Values are represented as mean ± SD., (n=10), there was no significance difference between means have the same letter in the same column, (P≤ 0.05).

Parameters / Groups	Tissue GPX (U/g. tissue) activity	Tissue SOD (U/g. tissue) activity	Catalase activity		Tissue GSH (mmol/g.tissue) concentration
			Serum (U/l)	Tissue (U/g.tissue)	
Healthycontrol group (-ve)	78.406±0.94[a]	515.43±2.83[a]	207.43±1.83[a]	9.723±0.03[a]	2.214±0.15[a]
Plant extract group	79.555±2.34[a]	517.75±3.61[a]	209.46± 1.33[a]	9.823±0.03[a]	2.293±.12[a]
MI group	25.120±1.02[b]	354.72±3.28[b]	150.18± 2.87[b]	8.427±0.19[b]	0.450±0.04[b]
Treatment group	50.391±1.55[c]	408.01±3.02[c]	171.9± 2.46[c]	9.019±0.28[c]	1.273±0.08[c]
Protection group	60.549±2.68[d]	439.37±2.47[d]	186.51± 2.82[d]	9.375±0.11[d]	1.772±0.14[d]

3.4. Microscopic examination.

The microscopic examination of heart tissue in MI group and the consequences of administration of hawthorn extract in other treated groups are shown in **figure (3)**. The microscopic examination of heart tissue of rats from healthy untreated group revealed normal cardiac myocytes, clear cell membrane integrity, normal myofibrillar structure with striations, appearance of branched and continuity with adjacent myofibrils. No evidence of inflammatory cell infiltration, edema or inflammation itself in **(A)**. Moreover, heart tissue from healthy treated group with EHLE showed no histopathological changes **(B).** Meanwhile, heart of rats from the MI group revealed marked co-agulative necrosis of cardiac myocytes associated with inflammatory cells infiltration and congestion of myocardial blood vessel showed in **(C).** Heart of rats from the MI treated group showed strands of fibroblasts

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proliferation between cardiac myocytes in **(D).** Heart tissues from protected MI treated group showed reduction in inflammation and rearrangement in myocardial muscles as in healthy normal rats where it revealed no histopathological changes in **(E).**

Our results matched those of Mudagal et al., (2011) who found that heart tissue from isoproterenol-treated rats showed moderate degree of myocardial degeneration, vacuolation and inflammatory cell infiltration as compared to control group [70]. Also our results matched Goyal et al., (2010) who showed marked myocardial necrosis after ISO induced MI. Crataegus Oxyacantha leaves extract (200mg / kg) showed less necrosis and proliferation between cardiac myocytes due to the antioxidant effects ,also the presence of polyphenolics compounds , flavonoids and the oligomeric proanthocyanidins all of these compounds have a role in reducing nitritive stress, lipid peroxides which disrupt the myocardial membrane and the inflammatory condition also reduced and the antioxidant status is improved[71].

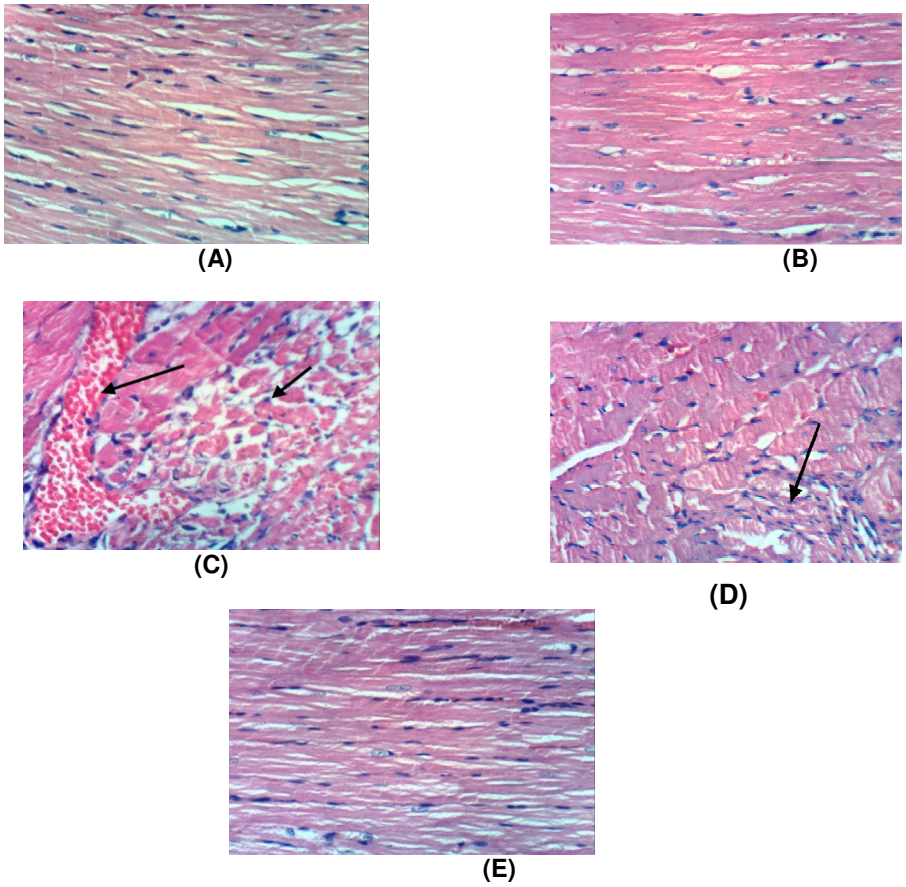

Figure. 3. Microscopic examination of heart tissue using (H & E X400)

4. CONCLUSION

Hawthorn leaves extract was found to be a good source of natural antioxidants, as confirmed by its high polyphenols, flavonoids, as well as its considerably high DPPH free radical-scavenging activities. Our *in vivo* study confirmed that EHLE significantly altered nearly all biochemical parameters associated with ISO-induced myocardial injury, as further supported by histopathological findings. EHLE may have anti-lipoperoxidative and antioxidant effects that confer these cardio-protective effects.

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ETHICAL APPROVAL

All authors hereby declare that "principles of animal care" were followed.

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