Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Study of the Effect of Prepared Isatin Spiro Compounds on Some Biochemical and Histological Parameters in Male Atherosclerosis Rats

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Abstract

OUR objective was to prepare some isatin derivatives. The compounds were purified using a silica gel column and identified by FT-IR and ^{1}HNMR. This study was designed to investigate the anti-atherosclerosis effect of the preparative Spiro isatin derivatives on lipid profile, cardiac markers, liver, and kidney function in atherosclerosis rats. The experiment was designed, and the total number of animals was 25 rats. We divided them into groups of 5 rats each, with a (negative) group receiving only distilled water and an (positive) group receiving cholesterol (500 mg/kg of body weight with coconut oil) orally to induce atherosclerosis for 25 days. The other groups received isatin compounds (0.571 mg/kg of body weight for 30 days) orally. Blood, cardiac, and aorta tissue samples were collected. The results indicated a significant decrease in the levels of MDA, cTnI, CK-MB, T.chol, T.G., LDL-c, and VLDL-c for all compounds prepared compared to the positive control. Compound C showed a significant decrease in the levels of urea and creatinine and a significant increase in their level of HDL-c compared to the control. Compound B showed a significant decrease in the levels of AST, ALT, and myoglobin and a significant increase in levels of HDL-c and GSH compared to the control. The results also indicated that compound A significantly reduced the levels of ALT, myoglobin, and creatinine and showed a significant increase in levels of GSH compared to the control. In tissue sections, compound C showed positive effects on cardiomyocytes and the aorta compared to the healthy control group.

Keywords: Atherosclerosis, Isatin, Cardiac marker, lipid profile.

Introduction

Atherosclerosis is one of the cardiovascular diseases, which is one of the most important causes of human death in the developed world. The American Heart Association reports that this disease kills one million people annually and is therefore considered higher than the death rate from cancer. Therefore, there is an urgent need to develop effective preventive and therapeutic strategies to change this reality [1]. Atherosclerosis arises as a result of initial damage to the lining of the arteries, which can be the result of several physiological, environmental, and functional factors. This causes damage effect encourages an inflammatory and destructive response [2] , Also it can cause the formation of activated macrophages, and these macrophages can produce enzymes capable of digesting protein and degrading the collagen that provides the protective fibrous covering its strength. This effect can weaken the fibrous covering, making it brittle and less resistant to tearing [3]. Recent research indicates a relationship between atherosclerosis and lipids on the one hand and inflammation on the other. According to the lipid oxidation hypothesis, cholesterol, which is usually bound to low-density lipoprotein found in the lining of blood vessels, has an increased uptake by macrophages [4]. Thus encourages the development of foam cells, which is considered an advantage for the development of plaques in atherosclerosis. foam cells and macrophages in the vascular endothelium are the main features of atherosclerosis and its progression. It works on the abnormal absorption of cholesterol found in low-density lipoprotein without esterification. This irregular absorption leads to the accumulation of free cholesterol in the form of fat droplets, which promotes the formation of foam cells [5,6]. and thus, leads to increased coagulation, inflammation, and programmed cell death [7]. Cytokines and their receptors play a role in directing and regulating the immune response, including white blood cell response. Which leads to increasing the possibility of developing atherosclerosis, that increases the risk and worsening of atherosclerosis. These factors increase, along with increased

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(Received 23 May 2024, accepted 26 June 2024)
DOI: 10.21608/EJVS.2024.291667.2114

intracellular mitochondrial dysfunction and elevated levels of active oxygen species [8].

The medicament that reduces fat is the basis for reducing preventing the risk of the heart of the heart and blood vessels, which has proven statins reduce the level of cholesterol in the blood, as well as reducing the risks and deaths in people with heart and blood vessels, and indicated that it occasionally accompanied by the sides effect. These statins act on plaque in atherosclerosis and lower fats. Atherosclerosis is largely characterized by thick fibrous covers and thus works to stabilize the arteries [9] .The statins also act as inhibitors of the 3-hydroxy-3-metylglutryl CoA reductase , which is currently used in the treatment of hypercholesteremia , is at a risk factor for cardiovascular disease. These drugs reduce stroke and peripheral arteries by reducing vascular inflammation and improving the function of the ventricular cells and the stabilization of plaque, in addition to inhibiting the aggregate of platelets [10].

Isatin (2,3-dioxindole) is a biologically active compound derived from the indole. It was initially founded by Erdman and Laurent in 1841[11,12] Interestingly, isatin is a naturally occurring small molecule within the human body and is extensively spread in Body fluids and various tissues of creatures [13-15]. At reasonable costs, isatin and its several alternative derivatives are easily obtained in the market. Synthesis and Reaction of isatins, which have an indole motif with γ-lactam and ketone, have been extensively studied. The results of the study have shown several interesting features of chemical reactions and processes [16]. Isatins can undergo a variety of chemical reactions, including chemo selective reductions, spiro-annulations, ring-expansions, oxidations, N-substitutions, nucleophilic additions onto the C-3 carbonyl group, and electrophilic aromatic substitution at positions C-5 and C-7 of the phenyl ring [16], Because of their special capacity to act as both nucleophiles and electrophiles, as well as their ease of accessibility, isatins are suitable for building blocks in the synthesis of organic compounds. Many heterocyclic structures having biological significance, such as quinolines, pyrrolidines, β-lactams, indoles, and 2-oxindoles, have been synthesized with the help of isatins. the creation of isatin derivatives with a variety of biological actions, including anticancer, antiviral, anticonvulsant, and antibacterial qualities [17,18]. Preparation of isatin derivatives having spiro component through a reaction involving isatin and amino acids under base and catalyst-free conditions is described. In this reaction, amino acids serve as nucleophiles, leading to the substitution at the γ-lactam nitrogen [19,20].

The extremely reactive C-3carbonyl group, which also functions as a prochiral center, is unquestionably the most intriguing use of isatins in organic synthesis. The C-3 carbonyl group of isatins' reactions, which are primarily nucleophilic It becomes 2-oxindole derivatives via additions or spiroannulation 2-Oxindoles, particularly those mixed with other cyclic frameworks via a process called spirofusion [21].

Over the past ten years, a thorough examination of this aspect of isatin chemistry has resulted in the active design and synthesis of many carbocyclic and heterocyclic molecules with a spiro-fused 2-oxindole ring that, on the one hand, include stereocenters [22].

In this research, we synthesized spiro-isatin derivatives, confirmed their structures by some physical properties and FT-IR and ^{1}HNMR spectroscopy, and studied their biological effects on induced atherosclerosis in male rats by studying the biochemical parameters and histological alterations in the heart and aorta.

Material and Methods

General

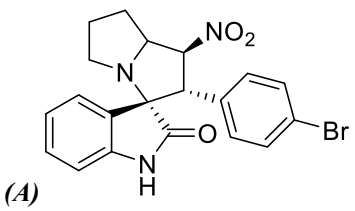
All chemicals were used from Sigma –Aldrich and BDH companies . Melting points (MP) were measured using the Electro Thermal IA1900. IR spectrometer with a 4000-400 cm^{-1} range on a (Bruker alpha II) Platinum-ATR. ^{1}H NMR spectra were measured using a Varian Agilent USA 400 MHz spectrometer at Laboret Center, Tehran university. All synthesized compounds were purified using column chromatography and silica gel (particle size 60-100 mesh).

Preparation of nitro styrene compounds

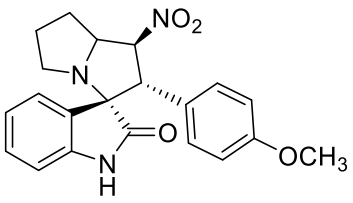
A mixture of (0.01 mol) of aldehyde, (0.02 mol) of nitro methane, and (0.02 mol) of ammonium acetate in (20 mL) of glacial acetic acid was heated to reflux for two hours. The reaction mixture was cooled to room temperature and poured into (100 mL) icy water. The solid desired product was calculated, and purified using silica gel column chromatography.

Preparation of spiro compounds

A mixture of (1 mmol) nitro styrene, (147 mg, 1 mmol) of isatin, and (115 mg, 1 mmol) of L-proline in (5 mL) of methanol was heated at 50 °C for two hours. The solvent was removed under reduced pressure. The solid dissolved in (10 mL) of ethyl acetate then washed with (5 mL)10% sodium bicarbonate, (5 mL) water, and (5 mL) of brine. The organic layer was dried over sodium sulfate, evaporated under reduce pressure then the solid was purified using column chromatography to obtain the compound.



(A)



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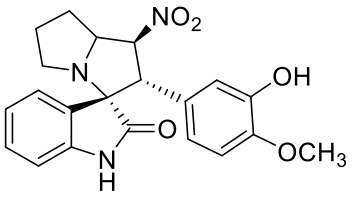


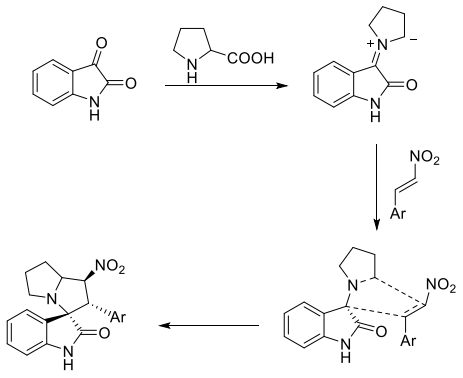
(C)

Scheme 1.Structure formula of spiro compounds (A,B C)

Compound (A) .$C_{20}H_{18}BrN_3O_3$, color: As a light yellow solid , melting point=137 °C, (yield 80%), FT-IR (ν cm^{-1}) : (3259,2963,2913, 1721,1518,1252,1144,1029). ^{1}H NMR (400 MHz, DMSO) (δ,ppm) 10.32 (s, 1H,NH), 7.89 (d, 1H, *J* = 7.5 Hz , Ar-H), 7.56 (dd, 1H *J* = 21.5, 8.5 Hz, Ar-H), 7.37 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.20 (dd, 1H, *J* = 15.6, 7.7 Hz, Ar-H), 7.12 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.02 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.65 (d, *J* = 7.6 Hz, 1H), 6.43 – 6.35 (m, 1H), 4.64 – 4.52 (m, 1H), 2.58 (dd, *J* = 17.3, 10.1 Hz, 1H), 2.25 (ddd, *J* = 22.2, 21.6, 8.5 Hz, 1H), 2.09 – 1.76 (m, 3H), 1.70 – 1.54 (m, 1H), 1.42 – 1.17 (m, 2H).

2 - compound (B) $C_{21}H_{21}N_3O_4$, color: As a light yellow solid, melting point= 178. °C, (yield 73%), FT-IR (ν cm^{-1}) : (3142,2967,2928,1699,1617,1546,1337,1260). ^{1}H NMR (400 MHz, DMSO) (δ,ppm) 10.28 (s, 1H), 7.25 – 7.15 (m, 2H), 7.07 (d, *J* = 8.6 Hz, 2H), 7.01 (t, *J* = 7.5 Hz, 1H), 6.72 (d, *J* = 8.7 Hz, 2H), 6.64 (d, *J* = 7.7 Hz, 1H), 4.60 (dd, *J* = 17.1, 8.1 Hz, 1H), 4.48 (d, *J* = 11.0 Hz, 1H), 2.58 (dd, *J* = 15.5, 8.5 Hz, 1H), 2.31 – 2.10 (m, 2H), 2.05 – 1.97 (m, 1H), 1.95 – 1.86 (m, 1H), 1.65 (ddd, *J* = 17.5, 11.1, 5.4 Hz, 1H), 1.35 (ddd, *J* = 15.1, 9.2, 3.7 Hz, 1H).

3 - compound (C) $C_{21}H_{21}N_3O_5$, color: As a light yellow solid, melting point= 197 °C, (yield 69 %), FT-IR (ν cm^{-1}) : (3155,2946,2892,1699,1543,1470). ^{1}H NMR (400 MHz, DMSO) (δ,ppm) 10.26 (s, 1H), 8.92 (s, 1H), 7.85 (d, *J* = 7.5 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 6.64 (d, *J* = 7.7 Hz, 1H), 6.59 – 6.52 (m, 2H), 6.33 – 6.25 (m, 1H), 4.60 (dd, *J* = 17.0, 8.1 Hz, 1H), 4.42 (d, *J* = 11.0 Hz, 1H), 4.12 (dd, *J* = 10.5, 5.2 Hz, 1H), 3.49 (s, 3H), 3.17 (d, *J* = 5.3 Hz, 1H), 2.64 (dd, *J* = 18.3, 11.2 Hz, 1H), 1.97 (ddd, *J* = 30.9, 12.5, 6.7 Hz, 2H), 1.70 – 1.58 (m, 1H), 1.44 – 1.26 (m, 2H).



Scheme (1) : Synthesis of spiro derivatives from isatin

Effect the spiro compounds in experimental animals

Experimental design:

Twenty-five (25) adult male Albino rats (4) months old, with weights ranging Between (200-250) g was used for the study. Atherosclerosis was induced using cholesterol dissolved in coconut oil at a concentration of (500 mg/Kg Body weight) [29] were dosed orally for 25 days, At the end of the 25th day, the infection was confirmed by histological investigation and blood tests , then animals were divided into (5) groups each group including (5) rats.

The five groups were used to study the compounds. 1st was healthy +ve control; 2nd was unhealthy -ve 3-5 groups were orally treated with a with a dose of 0.571 mg/kg body weight for 30 days by prepared spiro compounds.

The aortic tissue samples were removed at the end of the experimental and preserved at a physiological saline solution 0.9 % [30]. The injury was diagnosed by taking tissue samples from the heart and coronary artery for each group, as well as examining their lipid profile.

Biochemical analysis in serum

Determination of lipid profile in serum : Lipid profile in the serum was estimated using a ready-made analysis kit. Total cholesterol (T-Cho)., triglycerides (T.G) French company (Biolabo), by the enzymatic method [31]. HDL-C was measured by the enzymatic method using a ready-made assay kit [32]. VLDL-C and LDL-C in the blood serum were calculated according to the equation [33].

Glutathione GSH: The antioxidant glutathion in serum was measured using the modified method of Ellman's reagent containing [5.5'-dithiobis (2-nitrobenzoic acid)] (DTNB). DTNB, as a reagent, interacts with glutathione (GSH) and is reduced by the thiol group (-SH) to form a colored compound measured at 412 nm [34].

Malondialdehyde MDA: By measuring the amount of malondialdehyde produced as a byproduct of superoxidized lipids, it was used to estimate Lipid peroxidation in the serum. The process is based on the interaction of lipid peroxides (MDA) with thiobarbituric acid (TBA) in an acidic medium. The product's absorbance was measured at 532 nm [35].

Creatinine & Urea: Creatinine and urea were estimated using an enzymatic principle that used the Spanish biosystem analysis kit [36].

Transaminase enzymes in the serum: The activity of (AST and ALT) was measured by the enzymatic method using a ready-made assay kit from the French company BioLabo [37].

Troponin I (cTnI), creatine kinase(CK-MB) and myoglobin : Cardiovascular markers were estimated through Kit, which was processed by the United States company Lifesign MI [38].

Statistical analysis

Statistical analysis was performed using IMB SPSS Statistics version 27.0.1.0. The results were presented as mean (X) ± standard deviation (SD). was used to compare the values of the treatment groups and the control groups. Statistical significance was set at $P \leq 0.05$.

Results and Discussion.

Paralyzed dehydrates were treated at the beta site with nitromethane to obtain beta-nitro styrene compounds, then the last was entered into the preparation of the knots, and the compound was acquired. (A)*2'- (4-bromophenyl) -1'-nitro - 1',2',5',6',7',7a'-hexahydrospiro [indioline-3,3'-pyrolizin]-2-one*. The structure was confirmed by infrared, which gave absorption at 3259, 1721, and 1518 cm^{-1} and related to NH , C=O, and NO_2, respectively. The ^{1}H-NMR spectrum exhibited a single peak at 10.32 ppm, belonging to a proton NH, multiple peaks dating back to the two petrol rings at 7.02, multiple peaks at 6.38 ppm returning to 1H adjacent to the nitro group, and multiple peaks returning to the sum of the two role models at 1.33–2.25ppm.Also, prepare the boat. (B)*2'- (4-methoxyphenyl) -1'-nitro - 1',2',5',6',7',7a'hexahydrospiro[indioline-3,3'-pyrolizin]-2-one*.The structure was confirmed by infrared, which gave absorption at 3142, 1699, and 1546 cm^{-1} and related to NH, C=O, and NO_2, respectively. The ^{1}H-NMR spectrum showed a single peak at 10.28 ppm, belonging to the N-H proton, multiple peaks at 6.72–7.19 ppm, returning to the two-peasant rings, a dual peak at 6.64 ppm (H-NO_2), a single peak at 3.68 ppm for (3H-OCH_3), and multiple peaks returning to the sum of the two examples in the five-ring at 1.35–2.58 ppm . As the boat was prepared,(C)*2'- (3-hydroxy-4-methoxyphenyl) -1'-nitro -1',2',5',6',7',7a'-hexahydrospiro [indiline-3,3'-pyrolizin]-2-one*.The structure was confirmed by infrared, which gave absorption at 3155, 1699, and 1543 cm^{-1} and related to NH, C=O, and NO_2, respectively. The ^{1}H-NMR spectrum indicated a single peak at 8.92 ppm belonging to the N-H proton, multiple peaks at 6.55-7.85 ppm returning to the two-peasant rings, and a multiple peak at 6.29 ppm (H-NO_2), as well as a single peak at 3.49 ppm for (3H-OCH_3), and multiple peaks returning to the sum of the two-digits in the five-ring at 1.36–3.13 ppm .

Treatment protocol

The animals were treated with preparation spiro compounds depending on the dose of statins (40 mg), where animals were treated in this experiment with a concentration of 0.571 mg/kg body weight of prepared vehicles.

Histopathology

The changes of heart and aorta tissue in experimental Rat

Exile changes for the heart organs and aorta in experimental animals .The tissue examination of animals used to treat arteriosclerosis with cholesterol at a concentration of 500 mg/kg body weight dissolved in oil for 25 days compared to natural tissues appears in the figure. Textile changes in the aortic artery compared to the control group were caused by the presence of thickness in three layers of the aorta, many foam cells presented in the supplies containing cholesterol, the thrombosis of the smooth muscle fibers, and the deposition of cholesterol in the artery. IL-1 in infected foam cells narrows the arteries due to cholesterol accumulation and inflammation. The cause of these tissue changes is the metabolism disorder in fat, oxidative stress, and the secretion of LDL-C and its accumulation in the form of fatty drops with foaming cells stimulating in the cytoplasm, as shown in the fig.(1) [39].

The hypothetical examination of the anti-arteries is exhibited at a concentration of 500 mg/kg body weight , and for 25 days, tissue changes in the heart muscle occurred compared to the control group, Necrosis Zenker's is characterized by occurring in the cardiac muscle fibers with congestion in the blood vessels and infiltration of inflammatory cells, as shown in the figure. These changes may be caused by the increase in inflammation and oxidative stress represented by increasing the indicators of free radicals, programmed cell death, and the heart and artery surrounding with fatty tissue that leads to their hardening, fig. (2) [40-41].

Treating the animal which induced atherosclerosis suffering from atherosclerosis with the prepared compounds during four weeks of treatment daily led to the healing of large parts of the aortic tissue, which is close to the normal state compared to atherosclerotic animals , and the reason for these changes can be concluded to that of the compounds to repair damaged tissue by improving the mechanical properties of aortic tissue while strengthening collagen cross-links by increasing the activity of the endogenous lysyl oxidase enzyme. Lysyl oxidase 1 and lysyl oxidase 2, which promote tension balance and elasticity in internal tissues (through catabolic processes), Build by strengthening the bonds between collagen (responsible for tissue tension) and elastin (responsible for elasticity) with the formation of covalent bonds and their natural remodeling in fibroblasts, endothelial cells, and vascular smooth muscle cells [42]. Formation of free radicals on tissues [43], as shown in the fig.(3).

Biochemical analysis in serum

Determination of lipid profile in serum

Total cholesterol

The results in Table (1) have showed a significantly increased in the level of cholesterol ($p \leq 0.05$) in atherosclerotic animals group **(**106.88±8.62 mg/dL) compared to the healthy control **(**58.57±0.75 mg/dL), The reason for developing atherosclerosis is directly related to the accumulation of fats in the macrophage, especially cholesterol [44]. Cytokines of the inflammatory type IL-1B produced by the macrophage stimulate the production of molecules that adhere to the fatty substances in the blood vessel lining [45]. Compound (**A)** has showed a significant decrease in cholesterol levels compared to the atherosclerotic animals control group, and perhaps attributed to the inhibition of the enzyme HMG-CoA reductase.

Triglycerides (T.G.)

The results in Table (1) showed a significant increase in the level of TG ($p \leq 0.05$) in the atherosclerotic animals' control **(**161.96±14.20 mg/dL) compared to the healthy control **(**68.44±8.61 mg/dL)**.** The reason for this may be that triglyceride molecules are precursors to inflammatory mediators (prostaglandins and leukotrienes), with a decrease in lipoprotein lipase (LPL) responsible for lowering T.G. in LDL, the main carrier of it [46]. The prepared compounds showed a significant decrease in the level of T.G. compared to the atherosclerotic animals control group.

High-density lipoprotein (HDL-C)

The results confirmed an obviously decreased level of HDL-C ($p \leq 0.05$) in the atherosclerotic animal control group (41.02±14.88 mg/dL) compared to the healthy group (67.02±7.77 mg/dL) in Table 1**.** The reason may be attributed to decreased activity of the LP lipase enzyme and inhibition of the LCAT enzyme, which is essential for HDL [46]. It concludes to indicated a significant increase in the level of HDL in the treated groups with prepared compounds compared to the affected control group, which can play a role in activating the LCAT enzyme.

Low-density lipoprotein (LDL-C)

The results in Table (1) exhibited a significantly improved in the level of LDL ($p \leq 0.05$) in atherosclerotic animals group **(**34.53±15.98 mg/dL) compared to the healthy control **(**20.23±12.36 mg/dL), The reason is a decrease in its displacement in the blood serum by macrophages and hepatic cells or a malfunction in the synthesis of the particles found on its surface or receptors Apo-100 [47,48]. The prepared chemical compounds confirmed a significant decrease in their levels compared to the positive control group.

Very low-density lipoprotein (VLDL-C)

The results in Table (1) have appeared to show a substantial increase in VLDL levels ($p \leq 0.05$) in the

atherosclerotic animals control group **(**30.46±5.00 mg/dL) compared to the healthful control **(**12.16±3.73 mg/dL), as its depends on the concentration level of T.G., and the increase may be attributed by defect in some receptors, such as Apo-B [47]. When the animals were treated with the compounds, there was a substantial decrease in the VLDL levels. Perhaps this may contribute to the activation of the LP lipase enzyme responsible for breaking down the protein rich in T.G. Also, the activation of the liver lipase enzyme is responsible for breaking down the VLDL.

Creatinine and Urea

In the Table 2, the results showed a substantial increase in the levels ($p \leq 0.05$) of both creatinine and urea in the atherosclerotic animals control group (2.09±0.39 ; 56.90±8.21 mg/dL) compared to the healthy control (0.76±0.57 ; 42.00±5.24 mg/dL) Successively. This may be due to the high level of cholesterol [49], which is one of the most important factors affecting kidney function and narrowing of the kidney artery, in addition to kidney dysfunction and narrowing of the kidney artery. The relationship between arteriosclerosis and inflammation increases kidney enlargement directly, thereby affecting the functions, including tubular filtration. A diet high in fats and inflammation raises the level of cytokines, which increase the levels of urea and creatinine in the blood [50,51]. Compounds (A and C) showed a significant decline in the serum level of creatinine, but compound (C) showed a substantial decrease in the serum level of urea in the selected group.

Transaminase enzymes in the serum (ALT & AST)

The results exhibited in Table 2 show a significant increase in ALT and AST concentration ($p \leq 0.05$) in the atherosclerotic animal control group (352.5 ±47.16; 182.00±8.68 U/ml) compared to the healthy control group (220.25±44.28; 145.33±14.01 U/ml)**,** as atherosclerosis leads to increased levels of oxidative stress on the walls of blood vessels, resulting in plaque rupture [52]. Additionally, these enzymes are related to the risks of lipid metabolism [53]. Compounds (A and **C**) also appeared to decline in ALT levels compared to the diseased control group. While compound B showed a significant decrease in AST levels compared to the diseased control group.

Oxidants and antioxidants indicators

Malondialdehyde (MDA)

The results obtained in the table indicate a meaningful increase in MDA levels ($p \leq 0.05$) in the atherosclerotic animals control group **(**1.39±0.26 mmol/L) compared to the healthy control **(**0.48±0.28 mmol/L)**.** The reason for this increase is due to an increase in lipid peroxidation resulting from the oxidation of unsaturated fatty acids, which is associated with atherosclerosis. Additionally, there is a decrease in antioxidants in heart and artery diseases, especially glutathione (GSH) [54]. This concludes to decrease in MDA levels in the groups treated with the prepared compounds compared to the infected control group.

Glutathione (GSH)

The results in the table 2 exhibited a substantial decrease in GSH levels ($p \leq 0.05$) in the atherosclerotic animals control group **(**1.72±0.46 mmol/L) compared to the healthy control **(**1.72±0.46 mmol/L)**,** which is due to oxidative stress caused by reactive oxygen species that are among the causes of arteriosclerosis [55]. The results also indicated an obvious increase in the groups treated with compounds (A and B) contradictory to the infected control group.

Cardiac Marker

The results displayed in table 3 a substantial increase in the levels ($p \leq 0.05$) of cTnI, CK-MB, and myoglobin in the blood serum of the atherosclerotic animals control group **(**0.28±0.17 ; 87.68±8.70; 174.90±25.61 ng/ml) compared to the healthy control group **(**0.06±0.02 ; 45.90±3.87 ; 118.88±7.84 ng/ml) Successively in the current study. This is due to necrosis of heart muscle cells, which occurs when a part of the heart is deprived of oxygen due to blockage of the coronary arteries that supply the heart muscle with oxygenated blood. Without oxygen, the cells nourished by the artery begin to die [56]. Enzymes cTnI and CK-MB are present inside heart muscle cells in large amounts. Disruption of cell membranes because of a lack of oxygen leads to the release of these enzymes from the cellular cytosol into the systemic circulation. Based on the past study, there are used in a sensitive test for coronary artery disease. Myoglobin is a protein that binds oxygen and is released into the blood within 3 hours of a heart attack. A study suggested that myoglobin plays an important role in delivering oxygen to the mitochondria. When the animals were dosed by prepared organic compounds, the results displayed a significant decline in the levels of cTnI and CK-MB compared to atherosclerotic animals control group, the reason may be the inhibition of the enzyme CK-MB or the restoration of the walls of blood vessels by these compounds and the reduction of excretion of CK-MB and cTnI through the bloodstream, as well as the results indicated that compounds (A and B) reduced the level of Myoglobin significantly.

Conclusion

In conclusion, some new derivatives of isatin were synthesized, characterized, and evaluated for their effect in atherosclerosis rats as a model. They showed moderates effects on biochemical parameters of lipid profile and liver ,kidney function and cardiac markers which assessment in histological tissues compared to positive control group.

Acknowledgements

The author gratefully acknowledges support of the University of Mosul, College of Education for Pure Sciences, and Department of Chemistry for this research topic.

Ethical Approval

We hereby declare that the principles of Laboratory Animal Care NIH publication No. 85-23, revised 1985, were followed, as well as specific national laws where applicable. Experimental procedures concerning the use of animals were examined and approved by the Mosul University research and ethics committee.

TABLE 1. Lipid profile in serum of the atherosclerotic , healthy and the treated animals.

Groups n= 5	Lipid profile (mg/dl)				
	Cholesterol Mean±SD	Triglyceride Mean±SD	HDL-C Mean±SD	LDL-C Mean±SD	VLDL-C Mean±SD
Control +ve	106.88±8.62[a]	161.96±14.2[a]	41.02±14.88[a]	34.53±15.98[a]	30.46±5.00[a]
Control -ve	58.57±0.75[b]	68.44±8.61[b]	67.02±7.77b	20.23±12.36 [b]	12.16±3.73 [b]
Compound A	85.28±12.92c	96.57±4.13[c]	49.20±15.12 [a ,c]	19.88±2.06 [b,c]	20.05±1.80 [c]
Compound B	102.82±13.31[a,d]	65.02±8.56 [b,d]	64.25±14.65 [c,d]	23.14±4.60 [b,d]	15.42±5.03 [b,d]
Compound C	97.71±9.20[a,e]	113.97±15.67[e]	75.42±12.39 [e,b]	19.49±4.54 [e,b]	21.31±3.91 [e]

The difference vertically letters indicate a significant variation at $p \leq 0.05$.

TABLE 2. Some parameters in serum of the atherosclerotic , healthy and the treated animals.

Groups n =5	Parameters					
	Creatinine mg/dl Mean±SD	Urea mg/dl Mean±SD	AST U/ml Mean±SD	ALT U/ml Mean±SD	GSH mmol/L Mean±SD	MDA mmol/L Mean±SD
Control +ve	2.09±0.39[a]	56.90±8.21[a]	182.00±8.68a	352.5 ±47.16[a]	1.72±0.46[a]	1.39±0.26[a]
Control -ve	0.76±0.57[b]	42.00±5.24[b]	145.33±14.01[b]	220.25±44.28[b]	3.38±0.68[b]	0.48±0.28[b]
Compound A	2.38±1.02 [a,c]	48.10±2.92 [a,b,c]	186.50±16.03 [a,c]	246.50±30.49 [b,c,d]	3.99±0.70 [b,c]	0.95±0.44 [c,e]
Compound B	1.38±0.15 [b,d]	49.25±6.75 [a,b,d]	114.75±18.75[d]	297.00±25.15 [a,b,c,d]	4.11±0.95 [b,c,d]	0.91±0.20 [c,d,e]
Compound C	1.53±0.41 [e]	40.87±1.31 [b,e]	199.66±12.50 [a,c,e]	197.33±86.17 [b,c,e]	1.39±0.37 [a,e]	0.74±0.34 [b,e]

The difference vertically letters indicate a significant variation at $p \leq 0.05$.

TABLE 3. Cardiac markers in serum of the atherosclerotic , healthy and the treated animals.

Groups n =5	Cardiac marker ng/ml		
	cTnI Mean±SD	CK-MB Mean±SD	Myoglobin Mean±SD
Control +ve	0.28±0.17[a]	87.68±8.70[a]	174.90±25.61[a]
Control -ve	0.06±0.02[b]	45.90±3.87[b]	118.88±7.84[b]
Compound A	0.10±0.005 [c,d,e]	55.42±10.84 [c,d,e]	143.62±17.89 [c,d,e]
Compound B	0.12±0.02 [c,d,e]	56.37±14.09 [c,d,e]	144.52±16.97 [c,d,e]
Compound C	0.10±0.01 [c,d,e]	66.55±7.90 [c,d,e]	130.17±9.05 [c,d,e]

The difference vertically letters indicate a significant variation at $p \leq 0.05$.

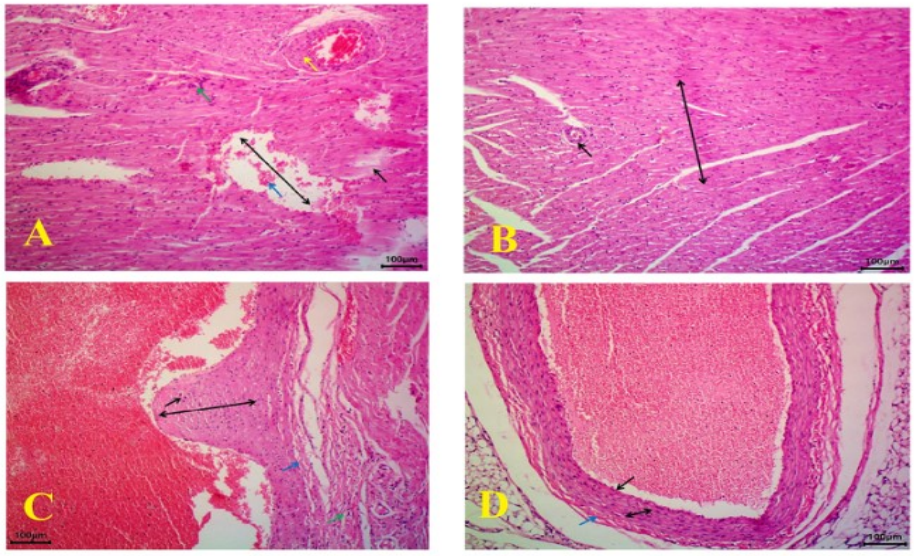


Fig. 1-A: A photomicrographs section of a rat heart from the positive control group shows breakage and irregularity of cardiac muscle fibers (↔) and necrosis (←), presence and hemorrhage (←), inflammatory cell infiltration (←) and thickening of the vessel wall (←). Hematoxylin and Eosine Pigment, 100X
Fig.1- B: A photomicrographs section of a rat heart from the negative control group shows the normal histological features of the heart tissue represented by heart cells and fibers (↔) and blood vessels (←). Hematoxylin and Eosine Pigment, 100X
Fig.1- C: A photomicrographs section of the aorta of a rat from the positive control group shows the emergence of lesions and plaques of atherosclerosis from the endothelial and middle tunic layers towards the cavity (↔), in which foam cells (←) break down smooth muscle fibers (←) and thicken tunica (←). Hematoxylin and Eosine Pigment, 100X
Fig.1- D: A photomicrographs section of the aorta of a rat from the negative control group (A) showing the normal histological features of the aortic layers represented by the endothelial tunica (←), the middle tunica (↔) and the tunica (←). Hematoxylin and Eosine Pigment, 100X

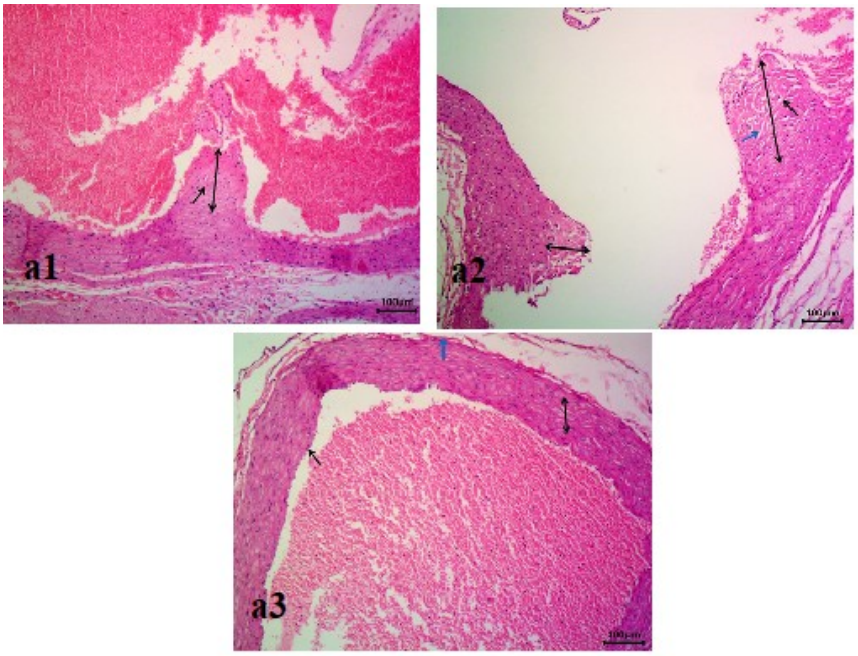


Fig.2: a1: A photomicrographs section of the aorta of a rat from the compound (B) group shows the protrusion of atherosclerotic plaque from the endothelial and middle layers of the tunica towards the cavity (↔) and the slight presence of individual foam cells (←). Hematoxylin and Eosine Pigment, 100X
Fig. a2: A photomicrographs section of the aorta of a rat from compound (A) group shows the protrusion of atherosclerotic plaques from the endothelial and middle layers of the tunica towards the cavity (↔) and the presence of foam cells (←) and foam streak strips in the middle tunica (←). Hematoxylin and Eosine Pigment, 100X
Fig. a3: A photomicrographs section of the aorta of a rat from compound (C) group shows the natural histological features of the aortic layers represented by the endothelial tunica (←), the middle tunica (↔) and the tunica (←). Hematoxylin and Eosine Pigment, 100X

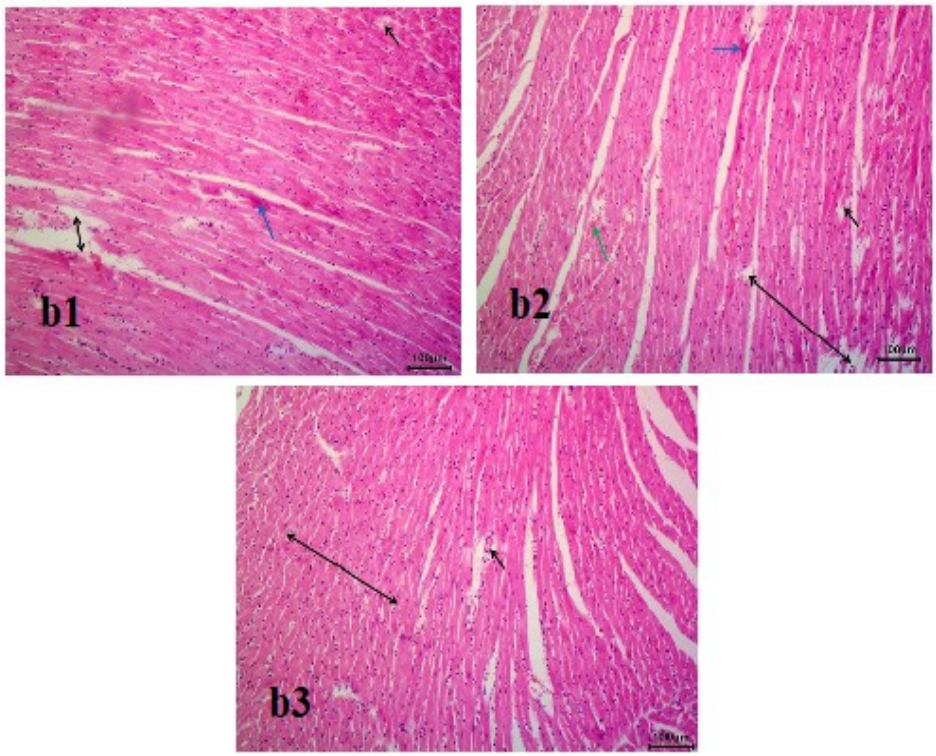


Fig. 3-b1: A : A photomicrographs section of a rat heart from the negative control group shows the normal histological features of the heart tissue represented by heart cells and fibers section of the heart of a rat from compound (A) group shows the breakdown and irregularity of cardiac muscle fibers (↔), necrosis (←) and vitreous degeneration (←). Hematoxylin and Eosine Pigment, 100X

Fig. 3-b2: A : A photomicrographs section of a rat heart from the negative control group shows the normal histological features of the heart tissue represented by heart cells and fibers section of a rat heart from compound (B) group showing the breakdown and irregularity of cardiac muscle fibers (↔), necrosis (←), vitreous degeneration (←) and hemorrhage (←). Hematoxylin and Eosine Pigment, 100X

Fig.3-b3: Photomicrographs section of a rat heart from compound (C) collection showing normal histological features of the heart tissue represented by cells and fibers of the cardiac myocardium (↔) and blood vessels (←). Hematoxylin and Eosin Dye, 100X

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دراسة تأثير مركبات سبيرو ايساتين المحضرة على بعض المتغيرات الكيموحيوية والنسيجية في ذكور الجرذان المصابة بتصلب الشرايين

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الملخص

كان هدفنا هو تحضير بعض مشتقات الإيزاتين. تم تنقية المركبات باستخدام عمود هلام السيليكا وتحديده بواسطة FT-IR و 1HNMR. تم تصميم هذه الدراسة للتحقيق في تأثير مضادات التصلب لمشتقات سبيرو الإيزاتين المحضرة على ملف تعريف الدهون ، ومؤشرات اصابة القلب ، والكبد ، ووظيفة الكلى في الجرذان بتصلب الشرايين . تم تصميم التجربة، وكان العدد الإجمالي الحيوانات 25 جرذ . تم تقسيمهم إلى مجموعات 5 جرذ لكل مجموعة، حيث تلقت مجموعة (السلبية) ماء مقطر فقط وتلقت مجموعة أخرى (إيجابية) الكوليسترول (500 ملغ/ كغ من وزن الجسم مع زيت جوز الهند) عن طريق الفم لتحفيز تصلب الشرايين لمدة 25 يومًا. بينما تلقت المجموعات الأخرى مركبات الإيساتين (0.571 ملغ/كغ من وزن الجسم لمدة 30 يومًا) عن طريق الفم . بعدها تم جمع عينات الدم و الأنسجة القلبية ، شريان الابهر .

أشارت النتائج إلى انخفاض كبير في مستويات MDA و cTnI و CK-MB و T.Chol و T.G و LDL-C و VLDL-C لجميع المركبات المحضرة مقارنة بالسيطرة الموجبة. أظهر المركب C انخفاضًا كبيرًا في مستويات اليوريا والكرياتينين وزيادة كبيرة في مستوى HDL-C مقارنة بالتحكم. أظهر المركب B انخفاضًا كبيرًا في مستويات AST و ALT و Myoglobin وزيادة كبيرة في مستويات HDL-C و GSH مقارنة بالتحكم. أشارت النتائج أيضًا إلى أن المركب A قلل بشكل كبير من مستويات ALT و Myoglobin و الكرياتينين وأظهرت زيادة كبيرة في مستويات GSH مقارنة مع السيطرة.

في الأنسجة ، أظهر المركب C تأثيرات إيجابية على خلايا عضلة القلب وشريان الابهر مقارنة بمجموعة السيطرة السليمة.

الكلمات الدالة: تصلب الشرايين ، الايزاتين ، مؤشرات اصابة القلب ، ملف الدهون.