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Evaluation of the efficacy of *Cordia myxa* fruit on Isoproterenol induced cardiotoxicity in male albino rats

Loay H. Ali[1] *, and Muna M. Alkarbolii[2]
[1]*BiologyDepartment, College of Education for Pure Sciences, University of Anbar, Iraq*
[2]*Anbar Health Directorate – Fallujah, Iraq*

Corresponding author: E-mail*: **hatemloay81@uoanbar.edu.iq

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ABSTRACT

The aim of this study was to assess the therapeutic effects of *Cordia myxa* (Bumber) against cardiotoxicity caused by isoproterenol (ISO) in male Wistar rats. Twenty eight adult Wistar male albino rats were equally divided into four groups, Group I (control group received saline at a dose of 0.1 ml/100 g, S.C.), Group II (group MI, ISO dissolved in sterile saline)was injected S.C under a dose of 0.1 ml/100 g/day for the last two consecutive days of the study to induce MI .Rats were killed 24 hours after the last injection, while Group III: rats oral dosage with C. myxa (250 mg/kg body weight) for 28 days, and Group IV (ISO+ *C. myxa*)On days 13 and 14, they were injected with Iso (dose of 0.1 ml / 100 g/day s.c) and then oral dosage with the alcoholic extract of the C. myxa fruit. . Cardioprotective effects of *Cordia myxa* were manifested by decrease in the elevated serum levels activities of cardiac enzymes (CK-MB, cTnI, AST and LDH) together with the improvement of heart bio- indicators of oxidative stress (MDA) and antioxidant defense system (GSH). Histopathological changes of heart were observed in second group (treated with ISO) and fourth group (ISO + *Cordia myxa* treated) as compared to control group. This study demonstrated the antioxidant effect of *Cordia myxa* and against ISO -induced cardiac injury.

تقييم فعالية ثمار البمبر *Cordia myxa* في علاج السمية القلبية الناتجة عن الأيزوبرينالين في ذكور الفئران البيضاء

لؤي حاتم علي[1] و منى ماهر الكربولي[2]
[1]قسم علوم الحياة – كلية التربية للعلوم الصرفة – جامعة الانبار – العراق
[2] مديرية صحة الانبار – الفلوجة – العراق

الخلاصة
هدفت الدراسة الى تقييم التأثيرات العلاجية لمستخلص ثمار البمبر *Cordia myxa* ضد السمية القلبية التي يسببها إيزوبروتيرينول (ISO) في ذكور الجرذان البيض. تم تقسيم ثمانية وعشرين من ذكور الجرذ الابيض البالغة بالتساوي إلى أربع مجموعات، في المجموعة الأولى (مجموعة السيطرة) التي تلقت محلول ملحي بجرعة (0.1 مل / 100 جم، تحت الجلد)، المجموعة الثانية) مجموعة (MI) تم حقن إيزوبروتيرينول المذاب في محلول ملحي معقم تحت الجلد (0.1 مل / 100 جم) لاخر يومين متتالين من نهاية التجربة لتحريض احتشاء عضلة القلب. قُتلت الفئران بعد 24 ساعة من الحقنة الأخيرة، بينما المجموعة الثالثة: جرعت فمويا بالبمبر (250 مجم / كجم من وزن الجسم) لمدة 28 يومًا، والمجموعة الرابعة : حقنت بريتونيا بايزوبروتيرينول في اليوم 13 و14 ثم بعدها جرعت فمويا بمستخلص الكحولي لثمار البمبر . اظهرت التأثيرات العلاجية لمستخلص البمبر على نسيج القلب من خلال انخفاض مستويات مصل الدم المرتفعة لأنشطة إنزيمات القلب CK-MB و cTnI و AST و (LDH جنبًا إلى جنب مع تحسين في المؤشرات الحيوية للإجهاد التأكسدي (MDA)ونظام الدفاع المضاد للأكسدة (GSH) لعضلة القلب ، وكما لوحظت تغيرات نسيجية مرضية في القلب في المجموعة الثانية) المعالجة بـ (ISO والمجموعة الرابعة) المعالجة بـ ISO + *Cordia myxa*) بالمقارنة مع مجموعة السيطرة. أظهرت هذه الدراسة التأثير المضاد للأكسدة لـ *Cordia myxa* ضد إصابة القلب الناجمة عنISO .

INTRODUCTION

Acute myocardial infarction or infarct (AMI) or infarct to the myocardium is, therefore, one of the most common sequelae of necrosis from ischemic injury to the myocardium. This has, therefore, become a critical health problem in several countries, particularly because of high rates of disability and mortality (Goyal *et al*., 2015). This paper has aimed at reviewing the experimental models of infarction in relation to the observed changes in blood pressure. So, myocardial infarction is an extremely dynamic process: oxygen supplied to the myocardium is only relatively inadequate as compared to its "demand" and emergent phenomena were characteristic of this ''stimulating" type of coronary heart disease (Arozal *et al*., 2022). An isoproterenol overdose in rats produces a beta-adrenergic agonist that duplicates many of the metabolic and morphological cardiac tissue abnormalities observed in actual human myocardial infarction (Mnafgui *et al*., 2016). The major causes of isoproterenol-induced myocardial ischemia are believed to be free radicals, reactive oxygen species lipid peroxidation, oxidative stress, and calcium overloading (Zhou *et al*., 2020). These influence the alteration in membrane permeability in apoptosis and necrosis, slowing of conduction between myocardial cells, electrical activity of heart and activity; and change in movement.

Fan declared that isoproterenol infarction model disturbances in redox cellular homeostasis initiate free radicals and reactive oxygen species, and free radical-induced lipid peroxidation represents maladies separate. Indeed the excessive free radical and ROS reactions through the free radicals initiate oxidative stress but may make a significant contribution to initiation of cardiac injury. The outcome of oxidative stress is peroxidation which further exacerbates membrane integrity. Now worse myocardial injury by excessive calcium through apoptotic as well as necrotic pathways would multiply membrane integrity. A stronger enzyme

protective system inhibits over about functionality as regarding vital enzymes like GPx and SOD will eventually increase cardiac damage (Pullaiah *et al*., 2021). Therefore, heightened performance may be related to the excess Ca in myocardium brought by apoptotic and necrotic processes. These processes above relate to the final pathological process that translates into altered electrical phenomena and hence its relation to arrhythmias as well as conduction abnormalities in MI (Fan, 2019).

Medicinal plants gained their importance owing to the possibilities of physical illnesses which they can handle (Ali, 2017). Due to its high efficiency, safety and low cost, the use of such agents in depressive therapies is increasing. One such plant of interest is *Cordia myxa* L. (bumber), claimed to possess cardio protective potential, belonging to the family Boraginaceae (Al-Snafi, 2016). The small-fruited *Cordia myxa* L. is sometimes called "Indian cherry", "lehsua," or "lasoda" and is a member of a genus in the family Boraginaceae and is indigenous to the arid and semi-arid regions of India (Meghwal *et al.*, 2014). Many species of Cordia are popular for cultivation purposes for both ornamental and commercial uses. This species occurs widely in Central and South America and tropical and subtropical Africa. The plant has been used for centuries because of its diuretic and anti-septic properties. Recent studies have suggested that it may help prevent cardiovascular damage caused by oxidative stress (Murthy *et al.*, 2019). Numerous bioactive substances, including flavonoids, tannins, and polyphenols, are present in Cordia myxa plant products and support cell-strengthening properties, and also produce calming effects this has been identified to trap free radicals thus reducing lipid peroxidation and increases levels of antioxidant enzymes in the body and these bioactive compounds protect cardiomyocytes from the effects of oxidative damage (Al-Gburi and Jasim, 2022). Our current research was conducted to study the effect of *Cordia myxa* L. seed on ISO-induced cardiotoxicity.

MATERIAL AND METHODS

Ethical Approve:

Permission to use the white rats in this experiment was sought from the Scientific Research Ethics Committee of Anbar University before the experiment and starting work to observe the internationally accepted ethical consideration which minimizes the suffering of these animals during the course of the experiment.

Animals used in Experimental

Twenty-eight male Swiss-white (Sprague Dawely) rats weighing between 220 and 290 g were used in the experimentation. They have been housed in the animal house of the Department of Biology, College of Education for Pure Sciences, Anbar University in special plastic cages with a mesh top. The animals have been maintained on standard laboratory food and tap water was given ad libitum up to test time, the environmental conditions regarding light and temperature specifically had been made constant and they were acclimatized two weeks prior to the start of the experiment.

***Cordia myxa* Fruit collection:** Botanists at the Desert Studies Center/Anbar University/Iraq confirmed the *Cordia myxa* L found in the Baghdad city fruits

Preparation of extract: After collecting the fruits of the bumber (*Cordia myxa*), they were carefully cleaned and washed several times with tap water and then left to dry, the dried fruits were crushed by an electric grinder to obtain a powder and kept at 4°C. The alcoholic extract was prepared by adding 500 grams of the fruits of the plant and placing them in 1 liter of solvent consisting of 700 ml of 95% ethanol and 300 ml of distilled water at room temperature for 72 hours, then the solution was shaken for 4 hours using a shaking water bath., The mixture was filtered using double filter paper and then the resulting extract was concentrated under pressure using a rotary evaporator at 40°C. The powder was taken and stored in dark bottles in the refrigerator for the purpose of preparing the required concentration in the experiment. (Ali *et al.*, 2015).

Drug: Sigma Substance Co., St. Louis, MO, USA, supplied us with isoproterenol, 2, 2′-diphyenyl-2-picrylhydrazyl, and 2, 2′-azinobis-(3-ethyl-benzothiazoline-6 sulfonic corrosive).

Experiment design: The male rats (weight of approximately 200-250gms) were divided into 4 groups each containing 7 rats: Group I; control group received saline at a dose of 0.1 ml/100 g, S.C for the last two days of the trial period, Group II (MI group); Subcutaneous injection of ISO dissolved in sterile solution at a dose of 0.1 ml/100 gm on the last two consecutive days of the experiment to induce myocardial infarction. MI rats were untreated throughout the experimental period. Group III; Each rat was given 250 mg/kg body weight of C. myxa fruit extract orally in 48 hours for 28 days. Group IV (C. myxa later); rats were first injected with ISO on the third and fourteen day with same details as the second group, and for 14 days they were treated orally through a gastric tube with the alcoholic extract of C. myxa (250 mg/kg).

Analysis of cardiac biomarker: In accordance with the instructions provided by the manufacturer, commercial detecting kits were used to measure serum of LDH, AST, troponin (cTnI), and CK-MB.

Lipid peroxides and antioxidants: Using a standard protocol and commercially available kits, serum GSH activity and MDA concentration were measured.

Histopathological analysis: After sacrifice, the heart tissues were collected immediately and washed with saline. These were fixed in 10% formalin. Paraffin blocks were prepared for those tissues. Sections of 5-7 μm thickness were cut from deparaffinized samples and stained with hematoxylin and eosin (H & E). The fixed sections were used to study the histopathological alterations with the help of a light microscope.

Statistical Analysis:

Mean standard deviation is used to represent all data. The data were analyzed with Graph Pad Prism 5.0 and SPSS 18.0 software. One-way ANOVA and the test of Tukey were used to compare the mean values of the groups using a normal distribution. A difference considered statistically significant was ($P \leq 0.05$).

RESULTS AND DISCUSSION

Cardiovascular serum markers (CK-MB, cTnI, LDH and AST) are summarized in Figures 1,2,3 and 4. Marker levels were significantly increased in MI rats (group II) subjected to ISO (group II) compared with group I (untreated) and III (treated) with *C. myxa*. Treatment with *C. myxa* (group IV) for 14 days and the ISO challenge test showed a significant reduction in the activity and levels of both cardiac markers compared to mice administered alone. 250 mg/kg body weight of *C. myxa* increases the activity of CK-MB, cTnI, LDH and AST close to the activity of the control group.

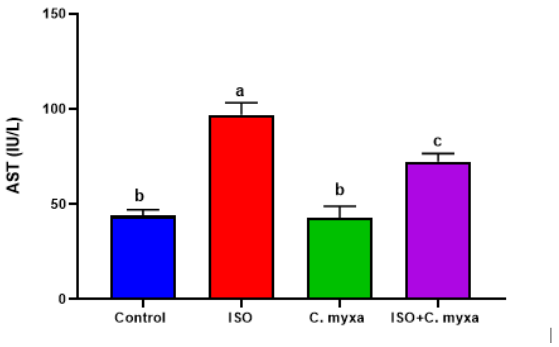


Figure 1- Effect of *C. myxa* fruit on the activity of AST in white rats treated with ISO

Number of animals = 7, different small letters indicate a significant difference at the level ($P \leq 0.05$)

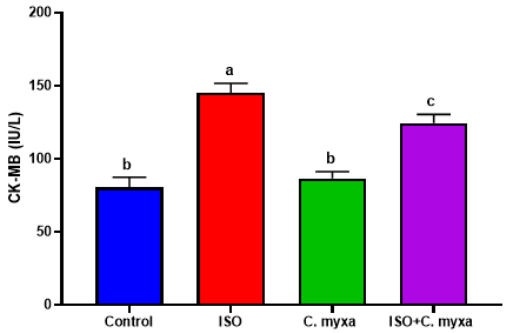


Figure 2- Effect of *C. myxa* fruit on the activity of CK-MB in white rats treated with ISO

Number of animals = 7, different small letters indicate a significant difference at the level ($P \leq 0.05$)

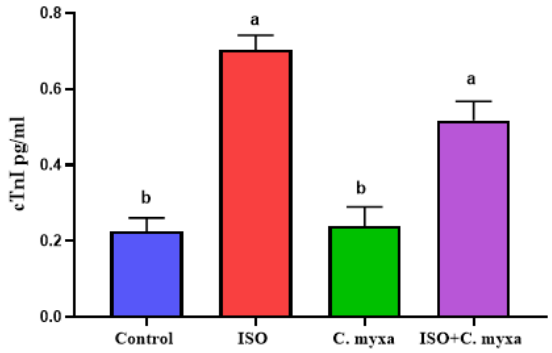


Figure 3- Effect of *C. myxa* fruit on the activity of cTnI in white rats treated with ISO

Number of animals = 7, different small letters indicate a significant difference at the level ($P \leq 0.05$)

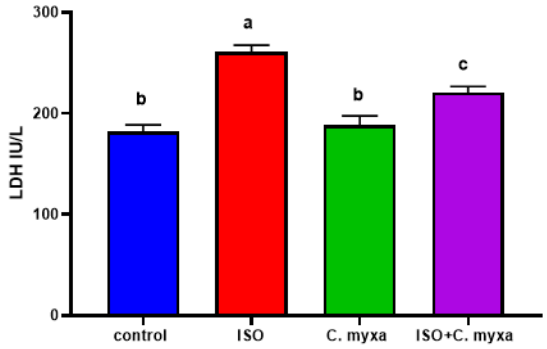


Figure 4- Effect of *C. myxa* fruit on the activity of AST in white rats treated with ISO.

Number of animals = 7, different small letters indicate a significant difference at the level ($P \leq 0.05$)

Many metabolic and morphologic anomalies in the myocardium of experimental animals with isoproterenol-induced MI are similar to those found in human MI (Shackebaei *et al*., 2022). As a catecholamine synthetic drug, ISO has drastic effects on heart disease due to the destruction of necrotic heart tissue and lack of blood supply (Kalkan *et al*., 2018). ISO condition is described by metabolism changes, intracellular high calcium, hypoxia, ischemia, and cardiovascular failure (Rong *et al*., 2023). Assessment of the activity of cytosolic enzymes like troponin T, LDH, CK-MB, and AST reflects changes in the integrity and permeability of the cardiac cell membrane. Elevated plasma levels of these enzymes indicate leakage into the blood due to increased permeability or cell membrane disruption (Rong *et al.*, 2023).

The biomarkers for cardiac damage usually encompass CK-MB, cTnI, AST, and LDH, Myocardial damage by insufficient transport of oxygen results in leakage of cardiac enzymes, for example, including CK-MB, cTnI, AST, and LDH(Ouyang *et al*., 2019). The length and height are important indicators of the disease. A prior study also reported increased activity of these enzymes in ISO-treated rats as an implication of ISO-induced necrosis (Sajid *et al*.,

2022). The term necrosis relates to the breaking down or loss of heart cells by releasing enzymes, and infiltration, or cleavage of heart muscle collectively these enzymes get into the bloodstream and elevate the level there (Rababa'h and Alzoubi, 2021). Cardiac damage induced by ISO was accompanied by an elevation in the serum cardiovascular markers (I and III) compared to the untreated group. *C. myxa* treatment reduced serum cardiovascular markers in MI-induced rats. Thus, the existence of active compounds likely exerts a cardioprotective effect by restraining enzyme leakage from the myocardium. (Keshani-Dokht *et al*., 2018). These current findings proved that *C. myxa* administration expressed significant therapeutic effects against ISO-induced experimental MI in rats. Further, a marked increase in serum cTnI and CK-MB levels in the samples confirmed MI development in rats.

These results are in corroboration with studies in ISO-treated rats that have been carried out to validate MI models. ISO is a semisynthetic catecholamine that causes MI when the oxidants and antioxidants in the heart are not balanced (Shaikh *et al*., 2019). Administration of ISO leads to heart failure that initiates further cardiovascular oxygen demand, ischemia progress and ATP catabolism. The authors are of the opinion that predominantly in the heart, cTnI and CK-MB are regarded as prognostic markers in MI because these are elevated owing to specific myocardial damage. The current histopathological findings are in line with the literature and confirm the myocardial damage caused by ISO. In the view of the foregoing, *C. myxa* could act directly on myocardial cells; this might explain low serum activities of these enzymes in MI rats. It decreased the extent of heart damage resulting from ISO-induced by diminishing leakage of these proteins from the myocardium. (Tak *et al*., 2024) This may be induced by the therapeutic efficacy of the alcoholic extract due to its possession of active antioxidants that participate directly in protecting cellular membranes against the impact of oxidative stress created by the liberation of free radicals during the metabolism of ISO (Al-Snafi, 2016). The defense system in this plant is referred to by phenolic compounds, with the most important of them being flavonoids acting in the preservation of the functional and tissue structure of cell membranes against necrosis and oxidation. It also prevents enzymes from leaking into the blood, which decreases their amount in serum. Moreover, flavonoids have a key role in free radical scavenging as well as in the inhibition of their activity with subsequent reduction in the level of oxidative damage (Al-Khafaji *et al*., 2021).

Figures 5 and 6 show the alteration of lipid peroxidation in plasma index in MI induced by ISO in rats. The level of serum MDA showed a highly significant increase in the ISO-treated group as compared to the control group ($P \leq 0.05$). Treatment with *C. myxa* (250 mg/kg body weight) resulted in a highly significant decrease in serum MDA levels in ISO-treated rats ($P \leq 0.05$). Figure 2 displays the antioxidant enzyme activity such as GSH in the serum of control and experimental rats of ISO myocardial infarction. GSH activity was depleted significantly in rats that received ISO when compared to normal control rats. Normalized enzymatic activity of GSH was found in these rats after treatment with *C. myxa* at a dose of 250 mg/kg body weight.

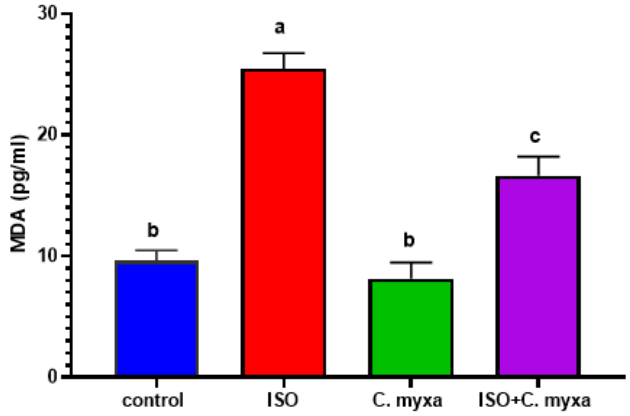


Figure -Effect of *C. myxa* fruit on the activity of MDA in white rats treated with ISO

Number of animals = 7, different small letters indicate a significant difference at the level ($P \leq 0.05$)

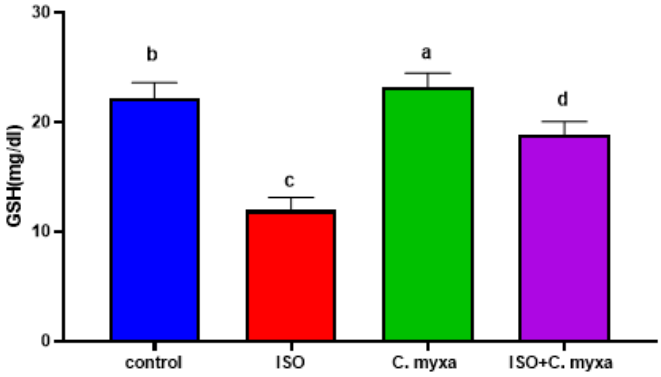


Figure (6): Effect of *C. myxa* fruit on the activity of GSH in white rats treated with ISO

Number of animals = 7, different small letters indicate a significant difference at the level ($P \leq 0.05$)

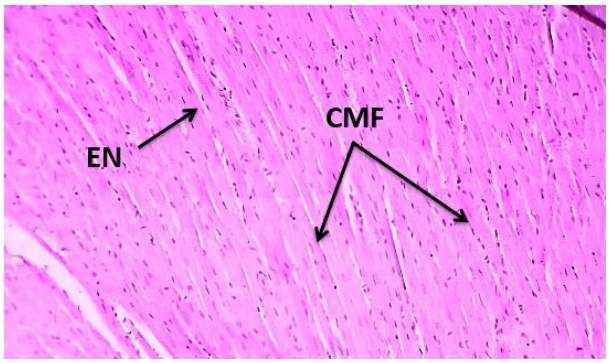
Increased oxidative stress during and after MI has been generally elucidated in text. ISO's activation of adrenergic receptors during this process leads to lipid oxidation, elicited by the vast production of reactive oxygen species in the myocardium. LPO initiated by ISO possibly contributes to the proliferation and mitochondria increase in oxidative damage within the heart, hence leading to MDA intoblood (Mert et al., 2018). The increase in MDA enhances both the activity of the antioxidant defense system and free radical generation.

Various cellular defense systems might be able to maintain free radical levels during oxidative damage. Such is the case with GSH (Neha et al., 2019). In this study, the levels of endogenous antioxidants were decreased by ISO. Like for instance GSH. An appreciable ISO administration elevated MDA within the heart tissues. The results go hand in hand with serum GSH activity being increased and MDA level in cardiomyocytes dropped after treatment with C. myxa. These results add recent evidence to inhibition by these antibodies of CBL (Abdel-Aleem et al., 2019). According to the recently conducted study with an ISO-induced MI

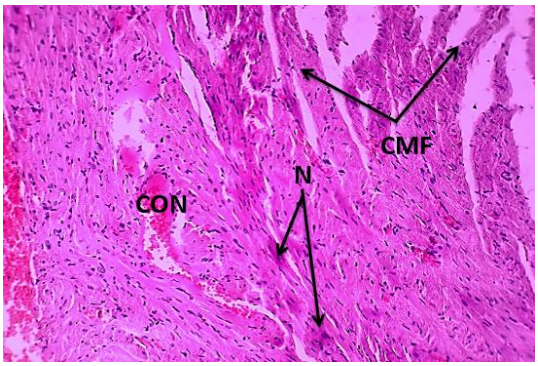
model, it was discovered that C. myxa ameliorates oxidative stress as well as LPO in rats. The free radical scavenging and antioxidant activities of C. myxa may arise from several stimulant compounds within the ROS scavenging system and antioxidant enzyme activities, free radical reactions inhibited by them, and protection of mitochondria from toxicant damage against mitochondria. (Al-Gburi & Jasim, 2022)

Hence, the antioxidant activity of C. myxa also showed a reduction in the activity of essential antioxidant enzymes, which could further have tempered the imposed oxidative stress. Such attenuation of imposed oxidative stress is groundless. In fact, the appropriateness of the rat model is a big question, just because with the help of this appropriateness one can carry out these controlled types of experiments, but it certainly creates difficulty in the direct application of the results from here to the human aspects. There are bound to be some physiological and metabolic differences between the rats and humans; hence, the applicability of these results has to be carefully considered, especially concerning the metabolism and response to drugs.

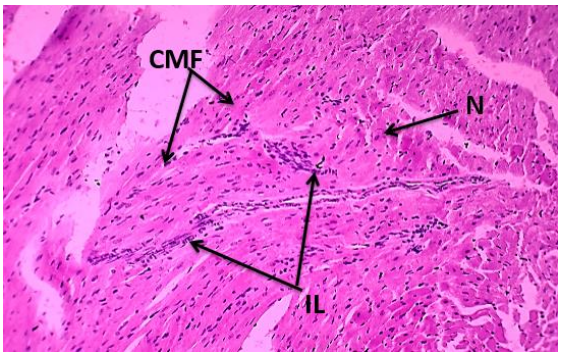
The results showed the presence of histological changes in the cardiac muscle fibers in the second group treated with the ISO, represented by the irregularity of the muscle fibers in the heart muscle with the presence of severe congestion (CON), in addition to the observation of necrosis (N) of muscle fibers for heart, In addition to the appearance of infiltration of inflammatory cells (IL) of white blood cells as shown in the pictures (2, 3). Under the light microscope, histological observations showed the appearance of the normal histological pattern of the cardiac muscle fibers (The normal tissue structure of the second group that received the extract was nearly identical to that of the control group (pictures 1,4). The mononuclear infiltration in ISO-treated rats with *C. myxa* (250 mg/kg body weight) was reduced, accompanied by mild congestion and necrosis. (Picture 5,6,7,8 and 9).



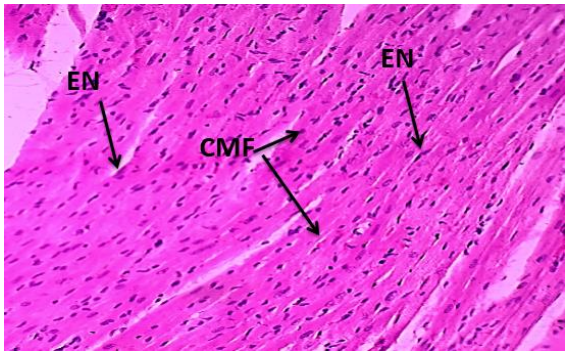
Picture (1): A cross-section of the heart of the control group showing regularity of cardiac muscle fibers (CDF), noting the elongated nuclei (EN) H&E X400.



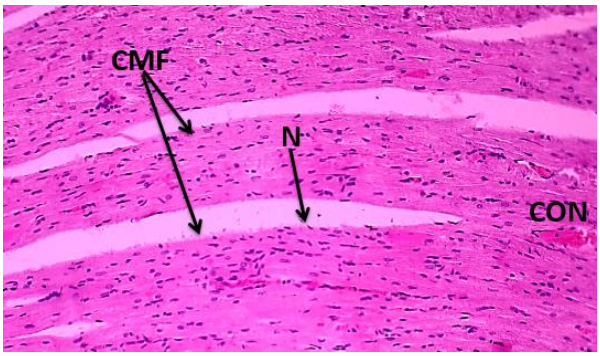
Picture (2): Cross-section of the heart of the ISO group showing irregular cardiac muscle fibers (CMF), with necrosis of myocytes (N) and congestion (CON) H&E X400.



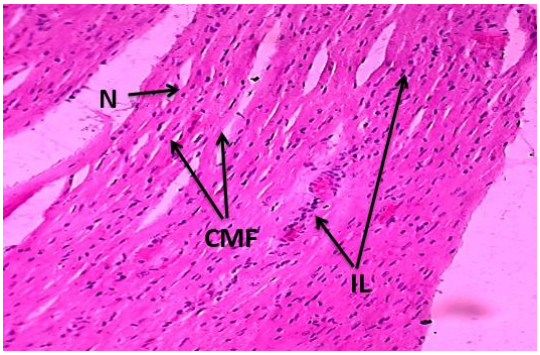
Picture (3): Cross-section of the heart of the ISO group showing irregular cardiac myofibrils (CMF), with inflammatory infiltrate (IL) and necrosis of myocytes (N) H&E X400.



Picture (4):): A cross-section of the heart of C. myxa group showing the regularity of cardiac muscle fibers (CMF), with nuclei (EN) with H&E X400



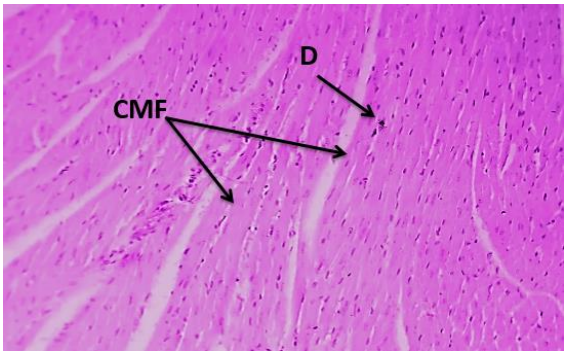
Picture (5): A cross-section of the heart of the ISO + C. myxa group showing regularity of cardiac muscle fibers (CMF), with necrosis of fibers (N) and noting congestion (CON) H&E X400.



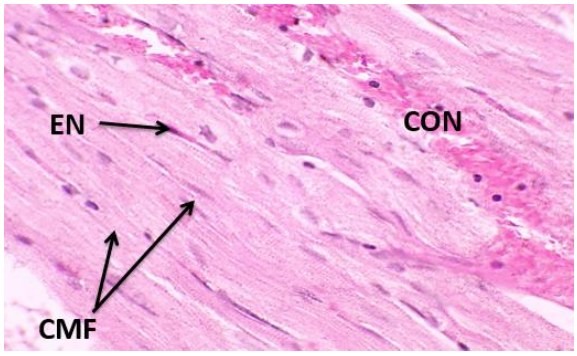
Picture (6): A cross-section of the heart of the ISO + C. myxa group showing irregular cardiac muscle fibers (CMF), with myocyte necrosis (N) and inflammatory infiltrate (IL) H&E X400

Among the ISO-exposed rodents, the histopathological findings in cardiovascular tissue were characteristic of heart muscle fiber degeneration and necrosis, mononuclear infiltration, and striking purification. Myocardial fiber damage, mononuclear infiltration, and mild necrosis were decreased in the ISO rats treated with 250 mg/kg body weight of *C. myxa* compared to the ISO-only rats. This also proves that the myocardial infarction heart treated with *C. myxa* was almost the same as the ISO-induced heart and did not have any necrosis. A previous study showed effective reduction of AST, ALT, lipid peroxidation, and increased GSH in CCl4injury in albino rats. The fact that the superoxide and hydroxyl radicals are scavenged initiates free radical reactions found in lipid peroxidation of the impurities by *C. myxa* to explain the protection of the heart from heart disease (Murad and Kareem, 2020). *C. myxa* is possibly inhibiting intracellular concentration of free radicals by inactivating them through one of the mechanisms by which it can directly bind to them (Al-Hamdani et al., 2019). *C. myxa* is possibly inhibiting intracellular concentration of free radicals by inactivating them through one of the mechanisms by which it can directly bind to them. (Wani et al.,

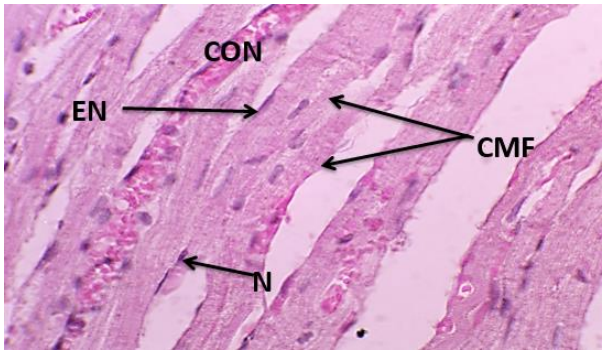
2023). In addition, Therapeutic properties in ISO-treated rats were confirmed by histopathology of *C. myxa*-pretreated myocardium. Hence, this study readily assessed antioxidant and healing effects after *C. myxa* treatment



Picture (7): Cross-section of the heart of the ISO + C. myxa group showing regularity of cardiac muscle fibers (CMF), with the degeneration of cells (D) H&E X400.



Picture (8):): A cross-section of the heart of the ISO + C. myxa group showing regularity of cardiac muscle fibers (CMF), with nuclei (EN) and noting congestion (CON), cell degeneration (N) H&E X400



Picture (9):): A cross-section of the heart of the ISO + C. myxa group showing regularity of cardiac muscle fibers (CMF), with nuclei (EN) and noting congestion (CON), cell degeneration (N) H&E X400

CONCLUSION

It could be concluded that the inclusion of postbiotics, which are produced from either *L. acidophilus* (0.70%) or *L. plantarum* (0.70%), or a combination of both (0.35% Lap + 0.35% Lpp), can result in an improvement in egg production percentage, egg number, SOD and CAT activity, and also lead to a reduction in CHOL and TAG concentration. Hence, postbiotics can serve as a substitute for antibiotics, without any impact on the health of the birds or their productive performance.

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