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Effect of Leek Extract on Histological and Physiological Changes Resulting from Cardiotoxicity Induced by Doxorubicin Drug in the Albino Rat

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Abstract

Doxorubicin (Doxor) drug is cytotoxic agent and belongs to anthracyclines sub-group of chemical complex and is used in cancer chemotherapy. The present experiment was conceived to that tested the role of leek (Allium porrum) extract regarding the protection of cardiac tissue from doxorubicin meditated injury in experimental animals. Thirty-two male white rats were assessed and randomly assigned to four equal groups: The first group was intraperitoneally injected with a normal saline solution (0.9%) twice a day for one week. The second group: were intraperitoneal injected with doxorubicin at dosing of 2.5 mg/kg/ 48 h. The third group: this group was administered a concentrated alcoholic leek extract at a dose of (400 mg/kg in the initial trial. Next after 4 hours of dosing it was intraperitoneally injected with doxorubicin at the as aforesaid concentration level for 8 weeks. Lastly, the last four animals were initially treated intraperitoneally with doxorubicin in a similar quantity for 4 consecutive weeks. Subsequently, the intraperitoneal injection was stopped and orally administered leek extract at the same volume as above was given for 4 weeks. After the end of the experiment period, blood samples were collected and made histological sections of the heart tissue of all experimental animals. The results showed an increase in the levels of each(cTn-1, LDH and AST) with a decrease in the levels of each(GSH and TAC) in the second group compared to the healthy control group, while it was noted in the third and fourth groups a decrease in the level of each cTn-1, LDH and AST with an increase in the level of antioxidants GSH and TAC compared to the second group, while the histological aspect showed the presence of massive histological changes in the second group with the observation of the presence of simple histological changes in the third and fourth groups. We conclude from the results of our study the effectiveness of the leek plant in protecting the body tissues, including the heart muscle, from the toxic effects resulting from giving the drug doxorubicin.

Keywords: cardiocytes , Inflammation , Necrosis , Antioxidant enzymes

تأثير مستخلص أوراق الكراث على التغيرات الوظيفية والنسجية الناتجة من التسمم القلبي المستحث بواسطة عقار الدكسوروبيسين في الجرذان البيض.

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الملخص

يعد عقار الدكسوروبيسين عامل تسمم خلوي ويعود الى تحت صنف الانثراسايكلين Anthracycline من المعقد الكيميائي والذي يستخدم في العلاج الكيميائي للسرطان. صممت هذه الدراسة لاختبار دور مستخلص الكراث Leek ودوره الوقائي لحمية انسجة القلب من الضرر الناتج من الدكسوروبيسين في الحيوانات المختبرية. 32 ذكر من الجرذ الأبيض استخدمت في الدراسة وقسمت عشوائيا الى أربعة مجاميع متساوية : المجموعة الأولى حقنت بريتونيا بالمحلول الملحي (0.9) مرتين يوميا ولمدة أسبوع واحد ، المجموعة الثانية حقنت بريتونيا بالدكسوروبيسين تركيز 2.5 ملغم / كغم / 48 ساعة، المجموعة الثالثة جرعت فمويا بالمستخلص الكحولي للكراث تركيز 400 ملغم / كغم وبعد اربع ساعات حقنت بريتونيا بالدكسوروبيسين وعند نفس التركيز أعلاه وأخيرا ولمدة 8 اسابيع المجموعة الرابعة حقنت أولا عن طريق الغشاء البريتوني بالدكسوروبيسين ولمدة 4 اسابيع وبعدها تم تجريع الحيوانات فمويا بالمستخلص الكحولي للرشاد ولمدة 4 اسابيع .وبعد انتهاء فترة التجربة تم جمع الدم وعمل مقاطع نسجية من القلب لكل حيوانات التجربة . أظهرت النتائج زيادة في مستوى كل من (CTn-1 , LDH و AST) وانخفاض في مستويات كل من GSH و TAC في المجموعة الثانية مقارنة مع مجموعة السيطرة السليمة، بينما بينت النتائج في المجموعتين الثالثة والرابعة انخفاض في cTn-1 ، LDH و AST مع زيادة في مستوى كل من GSH و TAC مقارنة مع المجموعة الثانية ، بينما وضحت المقاطع النسجية حصول تغيرات نسجية هائلة في المجموعة الثانية في حين كانت هناك ملاحظات نسجية بسيطة في المجموعة الثالثة والرابعة . نستنتج من نتائج دراستنا فعالية نبات الرشاد في حماية انسجة الجسم ومنها نسيج عضلة القلب من التاثيرات السمية الناتجة من عقار الدكسوروبيسين.

1. Introduction

The Anthracyclines are used as anticancer agents that have thi potential of causing cardiotoxicity, which is a known side effect of chemotherapeutic products. [1]. Anthracycline-derived Doxorubicin (Doxor), one of the most common anticancer drugs, is found to be handy and efficient in treating various tumors including solid tumors and malignant lymphomas[2]. However, its limited application as a therapeutic substance is due to the fact that it is cardiotoxic. Upon administering Doxor following coumulative dosing, there was development of congestive heart failure, cardiomyopathy as well as changes in the electrocardiogram at nine week [3].However, the exact pathways through which Doxor causes cardiomyopathy have not been fully elucidated, and much hypotheses has been advanced. These include change of transport of Ca2+ across sarcolemma, changes in lysosomes, release of vasoactive amines, inhibition of nucleic acid and protein synthesis, fluctuations in enzyme activity at mitochondrial membranes, and shift in myocardial electrolytes equilibrium. The exact reasons for Doxorubicin empowered cardiomyopathy have not been elucidated.[4] These are alterations in the sarcolemma Ca2+ transport, lysosomal alterations, release of vasoactive amines, inhibition of nucleic acids as well as protein synthesis, membrane-bound enzymes in the mitochondria, and myocardial electrolyte imbalance [5]. However, cumulative evidence has recently highlighted that oxygen-free radicals may be involved in the development of Doxor -induced cardiomyopathy.

Significant sources of phytosanitary medicines, or active ingredients utilized in drug manufacturing, have grown from medicinal plants. The secondary metabolic products that medicinal plants create are natural substances with important biological and pharmacological properties as well as chemical agent's myocardial electrolyte imbalance[6]. Hardy biennial plants belonging to the Amaryllis family (Amaryllidaceae/Liliaceae) include *Allium porrum*. Native to the Middle East and eastern Mediterranean, the leek is an old crop. The flavor of leeks is subtle, sweet, and oniony. Leek stalks can be cooked whole as a vegetable and are frequently used in European soups and stews, particularly as a compliment to potatoes. For Brazilians, it is one of the daily edible green veggies[7]. It is widely grown and consumed in Brazil or used as a source of food. It forms the staple of different European cuisine and is grown and consumed in different part of the western world [8]. Traditional herbal medicine also tries to focus on other advantages of this plant used in the treatment for boosting immunity, reducing inflammation and fighting cancer diseases. [9]. *A. ampeloprasum* L. is used often by patients because of it's therapeutic values associated with richness in sulphur compounds. Two sulfur compounds are known to be the most important and well-known bioactive compounds among all the ALLICIN and ajoene. Allicin is health enhancing since it is an anti-inflammatory, an anti –thrombotic, an antibacterial, an anti cancer and an anti –atherosclerotic.[10]. *A. ampeloprasum* L. includes a wide range of beneficial bioactive compounds important for the human diet including sulfur-free polyphenols like anthocyanins, flavonols, tannins, flavonoids, phenolic acids, phyto and sterols, carotenoids and saponin.[11]. Polyphenol antioxidants seems to have a major function in the shield of

biological structures in oxidative actions, which limits the formation of chronic diseases. Current research shows that leeks lower blood triglycerides in hypercholesterolemia; prevent colorectal, stomach, and prostate cancer; and also help in neural tube disorders and other problems.[12].

In this work, we used an experimental rat model to examine the protective role that *Allium porrum* has against Doxor-induced cardiotoxicity.

2. Materials and Methods

2.1 Selection of animals

The College of Education for Pure Sciences at the University of Anbar is where the current investigation was conducted. In this investigation, thirty-two adult male albino rats, weighing an average of 220-255 grams each, were used. The rats were 10–12 weeks old. The biology department's Animal House provided the animals, which were housed in typical laboratory settings with unrestricted access to food and water. Prior to starting the experiment, the Institutional Animal Ethics Committee granted ethical clearance for the use of animals. The animals received a regular ration of food and were always provided with clean, fresh water to drink. Before the experiment started, the animals were given a period of 14 days to become used to their surroundings.

2.2 Preparation of plant extract

The leek (*Allium porrum*) leaves were dehydrated to 45°C and ground into a powder using a blender. Then, 500ml of 80% ethanol was combined with about 100g of the powder, and the mixture was placed in a Soxhelt system for a full day. Subsequently, the extracts were filtered through filter paper and dried in a rotary evaporator at room temperature. The extract powder was then employed through many dilutions[13].

2.3 Chemicals

In a colorless vial (EIMC United Pharmaceuticals Company, 10 mg Doxorubicin hydrochloride/ vial), Doxorubicin/Adricin (Doxor) was obtained as a reddish-colored, transparent solution dissolved in 0.9% sodium chloride saline.

2.4 Biochemical study

Next, commercially available kits (Biosystems Spain) were used to estimate the cardiac biomarkers: serum lactate dehydrogenase (LDH), cardiac troponin-1 (cTn-1), and aspartate transaminase (AST). The kits were used in accordance with the manufacturer's instructions. Additionally, assay kits for measuring glutathione (GSH), total antioxidant (TAC), and malondialdehyde (MDA) were bought commercially from Sigma-Aldrich in Germany.

2.5 Experimental design

The animals were divided into four groups of eight each, at random, as follows:

One week of intraperitoneal injections of 0.9% sodium chloride saline solution were given to the rats in the control group. For four weeks, each rat in the second Doxor group (the induced group) received daily two intraperitoneal injections of 2.5 mg/kg of Doxor. Third

Group: Preventive I was given leek extract (400 mg/kg/day) initially, and then Doxor intraperitoneal injected four hours later. Group 4 (therapeutic): Doxor injections intraperitoneally alone for two weeks, followed by oral administration of leek extract (400 mg/kg/day) for the same duration. The experiment had a duration of four weeks, and the Doxor substance concentration in the third and fourth groups was comparable to that of the second group.

Rats were given ether anesthesia at the conclusion of the four-week experiment, and blood samples were taken straight from the heart. The samples were then placed into dry centrifuge tubes and spun for 20 minutes at 3000 rpm. Following separation, the sera were stored at -10°C for biochemical analysis. Rats who were sacrificed had their hearts taken for histological analysis.

2.6 Statistical analysis

The means ± SD were used to express the biological data. ANOVA was used for evaluating multiple comparisons. $P < 0.05$ was the threshold for statistical significance. Software used for all analyses was SPSS version 16.0.

3. Results

The results of the study, shown in Figures (1, 2 and 3), showed a significant increase in the values of both (cTn-1, LDH and AST) in the second group that was injected intraperitoneal with Doxor. compared to the healthy control group, while in the third and fourth groups that were dosed with leek extract with Doxor, either before or after treatment, a significant decrease was observed in the values shown above(cTn-1, LDH and AST) compared to the second group that was injected with Doxor.

While the results of our study showed a significant decline in concentration of the studied antioxidants, namely glutathione (GSH) and total antioxidant (TAC) , in the second group that was injected intraperitoneally with Doxor compared to the control group of animals, while the results in the third and fourth groups that were dosed with leek extract either before or after giving Doxor gave a significant increase in the level of the above antioxidants when compared to the animals of the second group. The results were the opposite when measuring oxidative damage, as our results showed a significant increase in the level of lipid peroxidation, namely malondialdehyde (MDA), in the second group that was injected intraperitoneally with Doxor compared to the total healthy control animals, while the above index decreased in the third and fourth groups that received leek extract either before or after Doxor compared to the second group.(fig. 4,5 and 6)

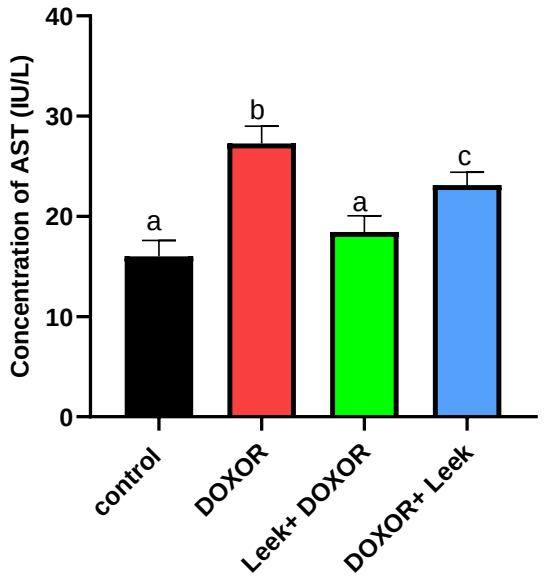

Figure-1 Effect of Leek extract on AST enzyme activity in male rats with cardiotoxicity. Different letters indicate significant differences at P≤0.05 in different groups (n = 8)

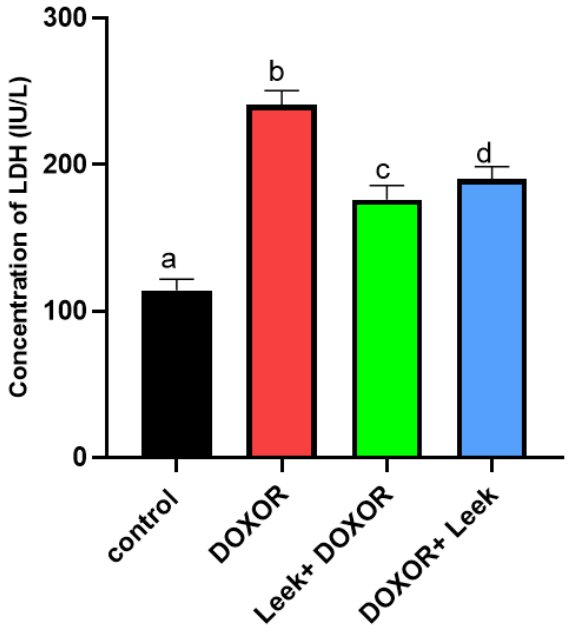

Figure -2 Effect of Leek extract on LDH activity in male rats with cardiotoxicity. Different letters indicate significant differences at P≤0.05 in different groups (n = 8)

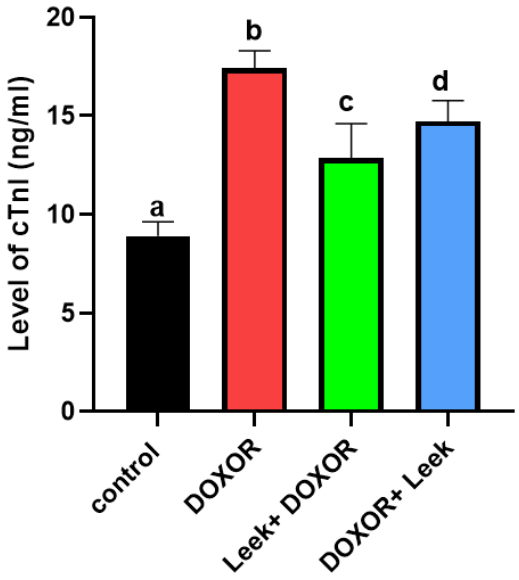

Figure -3 Effect of Leek extract on cTn1 activity in male rats with cardiotoxicity. Different letters indicate significant differences at P≤0.05 in different groups (n = 8)

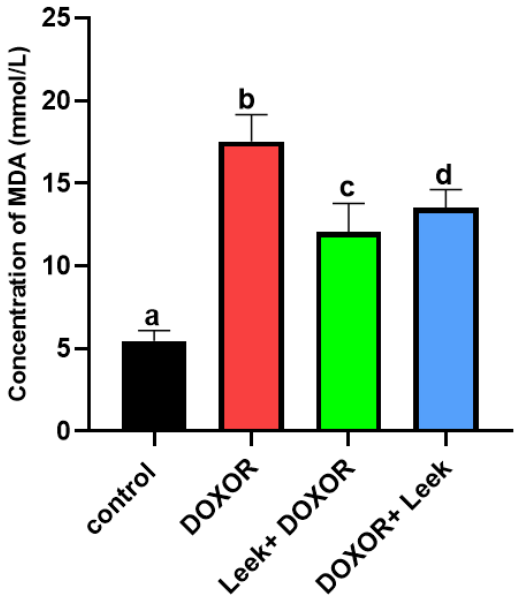

Figure -4 Effect of Leek extract on MDA level in male rats with cardiotoxicity. Different letters indicate significant differences at P≤0.05 in different groups (n = 8)

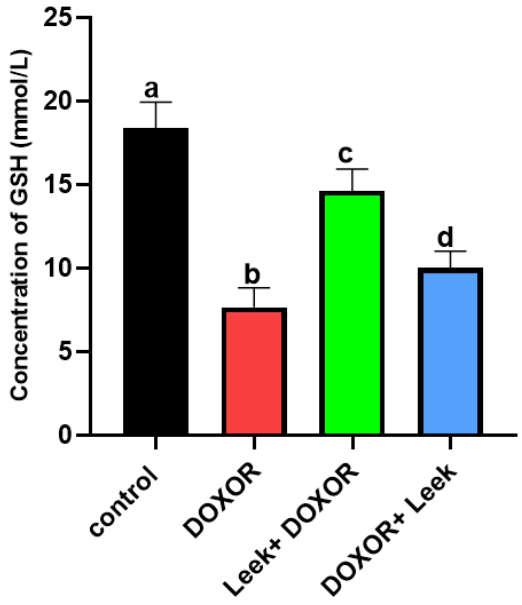

Figure -5 Effect of Leek extract on GSH level in male rats with cardiotoxicity. Different letters indicate significant differences at P≤0.05 in different groups (n = 8)

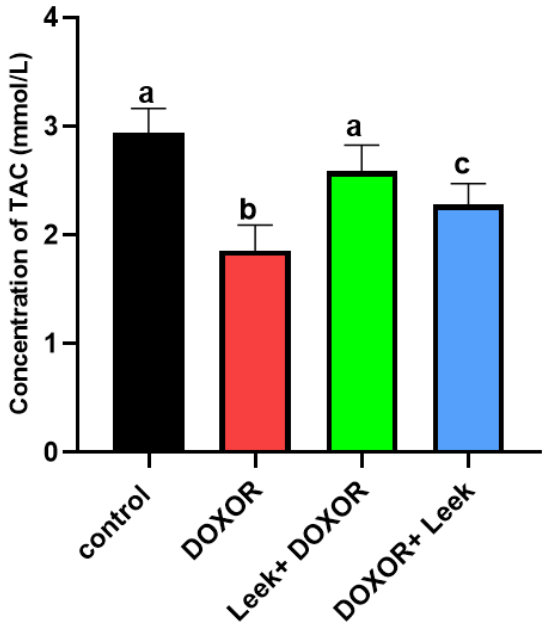

Figure -6 Effect of Leek extract on TAC level in male rats with cardiotoxicity. Different letters indicate significant differences at P≤0.05 in different groups (n = 8)

3.1 Histopathological observation

The histological changes were identical to the biochemical changes that accompanied our current study, as the results of the histological study of the heart muscle

of the control group showed the normal histological pattern of the heart muscle, as it was noted that the muscle fibers were regular, long and parallel, with the presence of centrally located vesicular nuclei, as in Figure 1, while the second group, which was treated with Doxor, showed histological changes represented by the irregularity of most of the muscle fibers and the loss of some of them, with the presence of degeneration of some heart cells and the presence of bleeding between the fibers. In addition, the results also showed the deposition of amyloid in some muscle fibers, as well as the appearance of inflammatory infiltration of white cells with severe blood congestion and the presence of necrosis of some cells, as in Figure (2,3,4). While in the third and fourth groups that were given the garden cress extract, whether before or after Doxor, the histological sections showed a clear improvement in the structural composition of the heart muscle, with the presence of simple changes represented by the presence of simple inflammatory infiltration with congestion. Hemorrhage and minor necrosis in the third group (Figure 5,6), while the fourth group gave better results as it showed the normal pattern of muscle fibers and their regularity similar to the control group with some minor changes such as necrosis and hemorrhage (Figure 7,8)

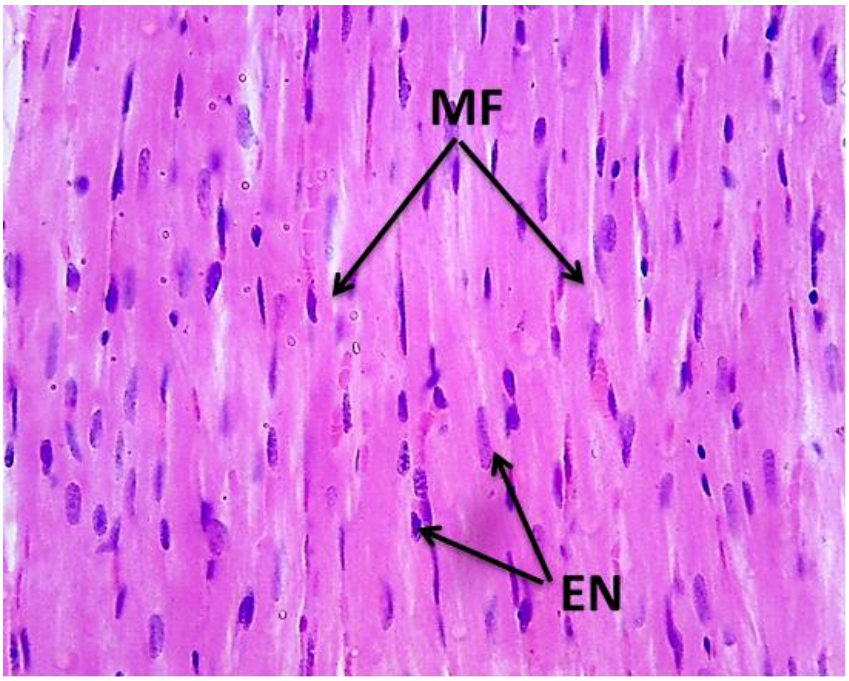

Image -1 A cross-section of the myocardium of the control group shows regularity of the muscle fibers (MF), with the presence of vesicular nuclei (EN) stained with eosin-hematoxylin H&E X400

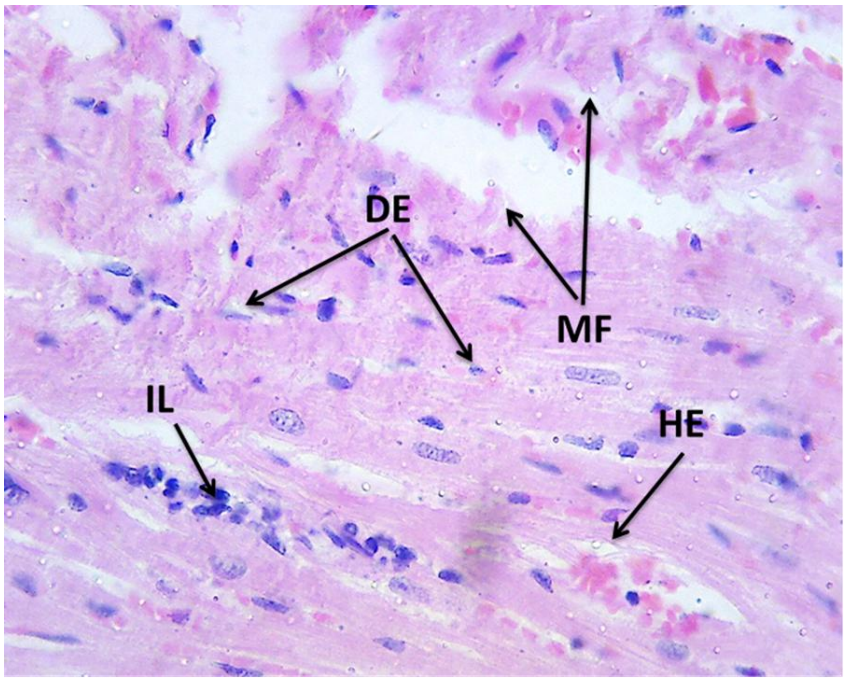

Image -2 A cross-section of the myocardium of the doxorubicin group shows loss of the normal organization of muscle fibers (MF), inflammatory infiltration of lymphocytes (IL), hemorrhage(HE), and cell degeneration(DE). H&E X400

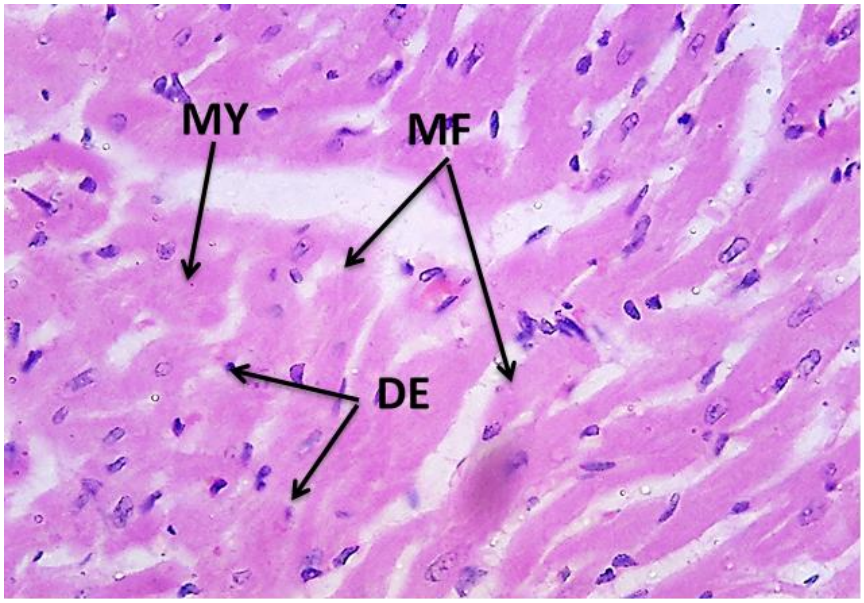

Image -3 A cross-section of the heart muscle of the group treated with doxorubicin, showing irregularity of the muscle fibers(MF), degeneration of some heart cells(N), and deposition of amyloid(MY). H&E X400

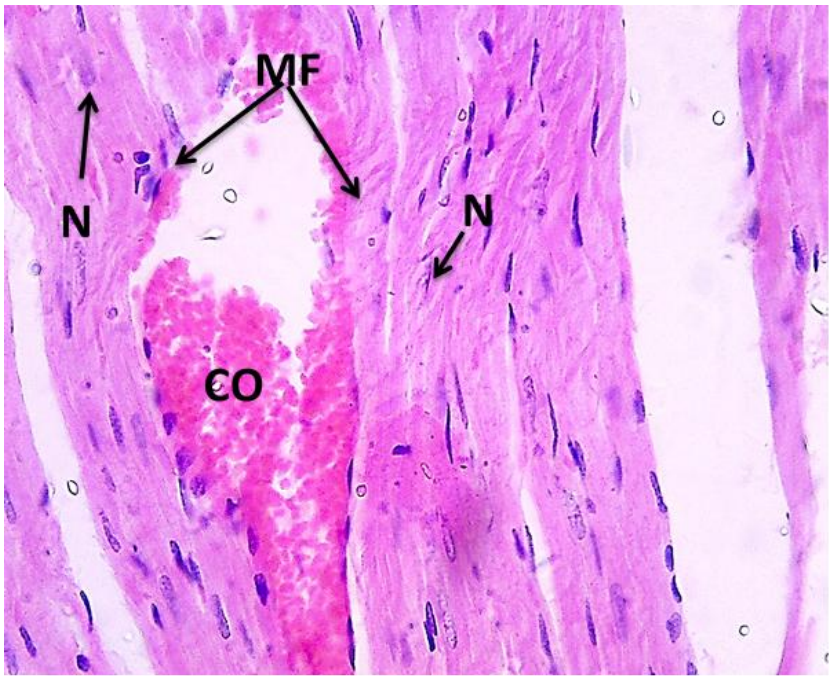

Image -4 A cross-section of the myocardium of the doxorubicin group shows severe blood congestion(CON), irregularity of the muscle fibers(MF), and necrosis of some cells(N). H&E X400

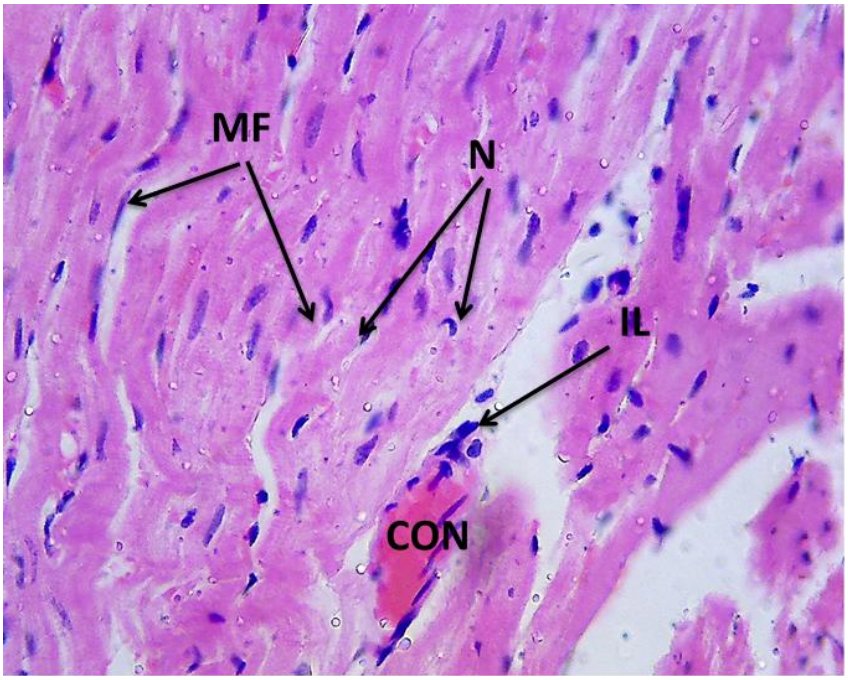

Image -5 A cross-section of the myocardium of the preventive group shows the normal pattern of muscle fibers(MF), slight inflammatory infiltration(IL), slight blood congestion(CON), and light Necrosis(N) of some cells. H&E X400

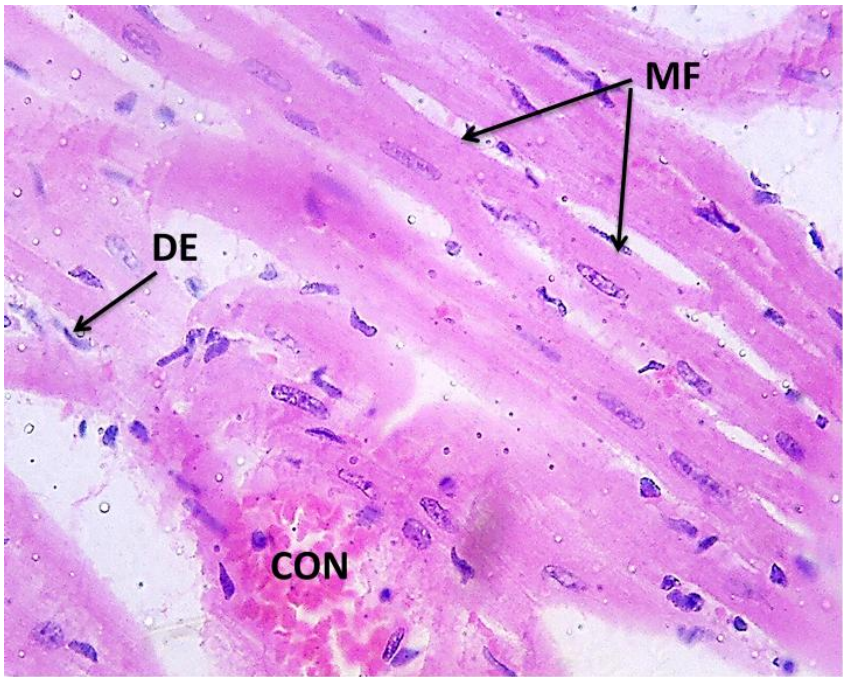

Image -6 A cross-section of the myocardium of the preventive group shows the normal arrangement of some muscle fibers(MF), congestion(CON), and mild degeneration(DE). H&E X400

Image -7 A cross-section of the myocardium of the treatment group shows the normal pattern of most muscle fibers(MF), with slight degeneration(DE). H&E X400

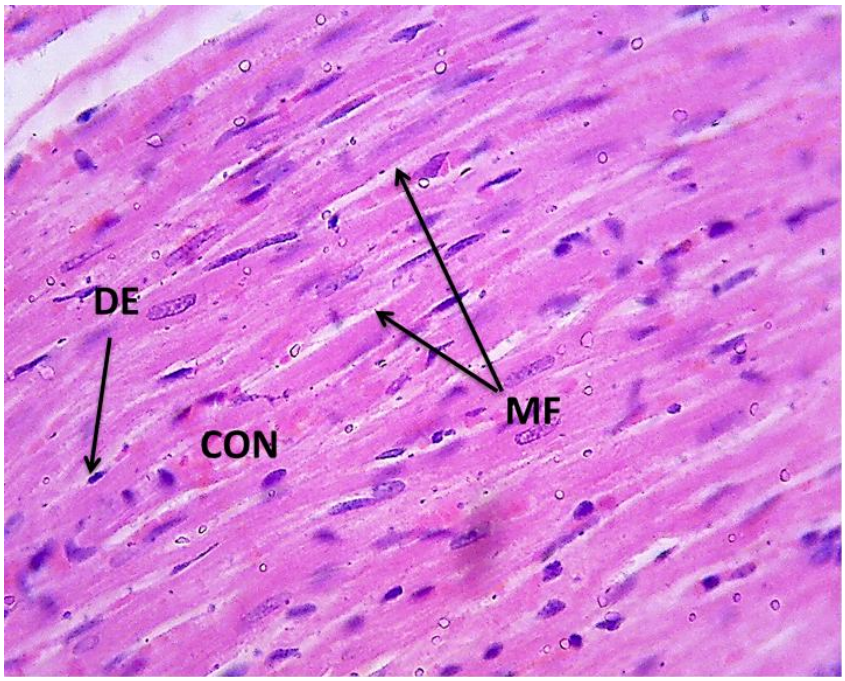

Image -8 A cross-section of the myocardium of the treatment group shows the normal pattern of muscle fibers(MF), slight blood congestion(CON), and slight degeneration(DE) of some cells. H&E X400

4. Discussion

Doxor is a chemotherapeutic drug that is frequently used to treat hematologic malignancies, sarcomas, and carcinomas. It is a member of the anthracycline cytotoxic antibiotic class. In addition to its beneficial effects on tumor cells, Doxor can be harmful to healthy tissues and have major adverse effects[14].

After receiving Doxor for an extended period of time, the experimental investigation shows significant metabolic alterations as well as oxidative damage in the serum[15]. A well-known cardiotoxic substance, doxorubicin has the capacity to destroy cardiac cells. This resulted in the entry of lactate dehydrogenase (LDH), aspartic transaminase (AST), and troponin-1 into the bloodstream, serving as diagnostic markers of damage to the tissue of the myocardium.[16]. The levels of these cellular enzymes in the bloodstream reflect a change in the permeability or integrity of the plasma membrane. The present study discovered that the concentrations of these diagnostic marker enzymes (cTn1, LDH, and AST) were notably increased in rats administered doxor. Moreover, in line with an earlier publication, elevated amounts of these enzymes indicate the extent of myocardial injury induced by Doxor.[17, 18].

A. porrum has been used in medicine. Vegetable components rich in allium have been associated with various health benefits, such as enhanced immune function, lower blood sugar levels, protection against radiation, immunity to microbial infections, and anti-cancer characteristics[19]. The rise in blood marker enzymes caused by doxor was markedly decreased with the administration of A. porrum. This reduction in enzyme levels

shows that *A. porrum* restricts the leakage of these enzymes, which explains A. porrum's capacity to stabilize membranes and preserve the normal structural and architectural integrity of cardiac myocytes.[20]. Additionally, the antioxidant characteristics of Leek might lessen cellular damage and high levels of AST, LDH, and cTn-1. Prior studies have shown the antioxidant properties of kaempferol, commonly found in leeks, alongside d-limonene, ferulic acid, and organosulfur compounds.[21]. It has also been demonstrated to inhibit blood elevations of AST, LDH, and cTn-1. A comparison of cardiac tissue from Doxor-injected rats with that of control subjects showed a notable decrease in the activities of antioxidant enzymes (GSH, TAC), aligning with results from an earlier investigation. The heightened generation of reactive oxygen species, including superoxide and hydrogen peroxide, leads to a reduction in the activity of these enzymes, which is subsequently succeeded by their inhibition [22]. Pre-treatment with A. porrum reduces cardiac damage induced by free radicals and enhances GSH and TAC activity while neutralizing superoxide radicals. Doxor therapy led to a rise in the levels of MDA, which is a byproduct of lipid peroxidation. The rise in lipid hydroperoxides signifies damage to the heart, and lipid peroxidation is an important pathological occurrence in myocardial necrosis.[23]. MDA levels decreased as a result of elevated lipid peroxides during Doxor-induced *A. porrum* treatment. Scavenging or neutralizing free radicals, inhibiting hydrogen peroxide and tumor necrosis factor-alpha, inhibiting xanthine oxide, interacting with the oxidative cascade and preventing its outcome, quenching oxygen and reducing its availability for oxidative reactions, and inhibiting cytochrome P450 are the mechanisms that account for A. porrum's antioxidant action[24].

A cumulative dose of Doxor has been linked to acute cardiotoxicity in humans. The accumulation of ROS has been shown to cause cellular damage in the absence of scavenger activity; free radicals implicated in the pathogenesis include superoxide, hydroxyl radicals, and nitric oxide[25]. Doxor not only rises the production of ROS nevertheless besides lowers levels of antioxidant enzymes like GSH and TAC, which results in toxicity. One of these enzymes' main functions is to protect membrane lipids against peroxidation by inhibiting peroxidation chain reactions and scavenging ROS[26].

Although a large body of evidence suggests that Doxor administration is associated with a decrease in endogenous antioxidants and an increase in oxygen-free radicals, leading to increased oxidative stress, which is followed by the development of various sub-cellular changes in the myocardium, typical of doxorubicin-induced cardiac injury, the exact mechanism of cardiotoxicity induced by a Doxor is unclear from the current study[27]. We observed a significant rise in MDA levels in the heart tissue of Doxor-treated rats, indicating enhanced lipid peroxidation, and a reduction in GSH, SOD, and CAT concentrations[28]. *A. porrum* pretreatment effectively prevented the cardiac tissue damage caused by doxorubicin by causing a considerable drop in MDA and an increase in GSH, SOD, and CAT levels[29]. As previously established, depletion of antioxidants and increasing oxidative stress may be the cause of cardiac tissue damage[30].

Allium porrum is a medicinal plant with high levels of carotenoids, flavonoids, and polyphenols. Its anti-inflammatory and antioxidant properties have been researched. Lipid peroxidation was reduced and the antioxidant state of the cells was significantly increased

by the co-administration of the leek extract[31]. Because leek extract has a high level of polyphenols and flavonoids, it appears to have strong antioxidant activity, which contributes to its cardioprotective effects. The polyphenols have a significant ability to scavenge free radicals and are powerful reducing agents. This likely explains why leek has so strong anti-Doxor cardiotoxic properties[32].

ROS have been linked to the etiology of numerous different illnesses, including Doxor-induced cardiotoxicity. Under physiological conditions, the effect of antioxidant defense mechanisms counterbalances the effect of ROS generated by various processes. When this equilibrium shifts in favor of ROS, lipid radicals form in the cellular membrane, which is followed by the formation of lipid hydroperoxides, which is then followed by the formation of toxic products like aldehyde, alkenes, and MDA[33]. This leads to oxidative stress and tissue damage. Stated differently, MDA is a byproduct of tissue damage[34]. The pathogenesis of Doxor-associated cardiotoxicity has been linked to higher cardiac tissue MDA levels in numerous prior studies. Furthermore, it has been shown that various antioxidant compounds can diminish Doxor's cardiotoxic effects by decreasing tissue MDA levels. [28]. In our research, we found that elevated MDA levels occurred after Doxor. Therapy diminished following leek treatment. Based on these results, Doxor induces tissue harm by elevating lipid peroxidation in tissues, while Leek offers biochemical protective advantages.

According to a histopathological report, the group that had leek pre-treatment was able to reduce the amount of myofibrils lost, cytoplasmic vacuolization, and mitotic swelling caused by doxorubicin. The rats treated with doxorubicin showed comparable histological alterations to those previously documented[35]. When taken as a whole, these histological and biochemical, data suggest that there may be a potential cardioprotection against Doxor toxicity. Thus, sulfur-free polyphenolic substances such anthocyanins, flavonols, tannins, flavonoids, phenolic acids, phytosterols, carotenoids, and saponins may be a part of the leek's antioxidant mechanism[36]. Antioxidants called polyphenols are crucial in preventing oxidative damage to biological components, which may help lower the chance of developing chronic illnesses[37]. Leek therefore successfully avoided tissue damage in our study by lowering oxidative stress and regaining antioxidant state.

5. Conclusion

Leek extract prevents cardiac damage by reducing Doxorubicin-induced cardiotoxicity, making it a promising agent for cardioprotection. While a positive impact on cardiotoxicity is noted, the antioxidant characteristics of the extract must be considered, and lower concentrations should be evaluated.

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