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Effect of germinated flaxseed, barley and beetroot on rats inducing cardiovascular failure disease.

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Abstract:

The present study aimed to investigate the effect of some phytochemicals in different plants, such as germinated barley (*Hordeum vulgare*) and germinated flaxseed (*Linum usitatissimum*), and beetroot (*Beta vulgaris*), on rats inducing cardiovascular failure disease. Sixty adult male albino rats weighing 150 ± 10 g were divided into ten groups, six rats for each. One was kept as a (-ve) control group, while the other nine groups were treated with adriamycin (ADM) for two weeks via injection. Germinated seeds mixed with beetroot were added to eight groups in different quantities (5 and 10%). The treatment lasted for 28 days. From the obtained results, it could be shown that treatment with different plants in different quantities (5% and 10%), respectively, for 28 days caused a significant ($P \leq 0.05$) increase in weight gain%, relative organs weights, liver functions, kidney functions, serum antioxidants, TC, TG, LDL, and VLDL. The best treatment was for germinated barley at 5%, germinated barley and beetroot at 5%, and germinated flaxseed and beetroot at 10%. Therefore, it can be concluded that germinated seeds have the ability to improve the condition of blood vessels and reduce the development of heart diseases. The histological investigation confirmed the enhancement of both of liver and kidney functions as well as heart status

Key words: *Germinated barley, Germinated flaxseed, Beetroot, Lipoproteins, Cholesterol*

Introduction:

Cardiovascular disease are the most common cause of death globally as of 2008, accounting for 30% of deaths in the united states. ⁽¹⁾ The heart function as a pump in the

circulatory system to provide a continuous flow of blood throughout the body. The systemic circulation then transports oxygen to the body and returns carbon dioxide and relatively deoxygenated blood to the heart then transfer to the lungs. ⁽²⁾ More than three quarters are a result of coronary artery disease and stroke. ⁽³⁾ Cardiovascular diseases (CVD), are the leading cause of death worldwide. The majority of cardiovascular disease is noncommunicable and related to lifestyle and other factors, becoming more prevalent with ageing. ⁽⁴⁾

According to Lundberg *et al.*, ⁽⁵⁾ beetroot juice reduced blood pressure in hypertensive animals and so may have an effect on mechanisms of cardiovascular disease. ^(6,7) On the other hand germination an effective natural technique for increasing the health benefits and nutritional value of grains, with potentially widespread effects across populations in attenuating adverse lifestyle disease outcomes. ⁽⁸⁾ Tentative evidence has found that dietary nitrate supplementation such as from beets and other vegetables results in a small to moderate improvement in endurance exercise performance. ⁽⁹⁾ According (FAD), consuming at least 3 grams per day of barley beta-glucan or 0.75 grams per serving of soluble fiber can lower the levels of blood cholesterol which is a risk factor of cardiovascular diseases. ⁽¹⁰⁾ Also that phytochemical constituents isolated from *portulaca oleracea* including steroids, vitamins, minerals, fatty acids, alkaloids and saponins played an important role in its antioxidant activity and hepatoprotective effects. ⁽¹¹⁾ Flax contains hundreds of times more lignans than other plant foods. ⁽¹²⁾ On the same time and whole grain barley also contains phytochemicals including phenolic acids, flavonoids, lignans, tocols, phytosterols, and folate. These phytochemicals exhibit strong antioxidant, antiproliferative, and cholesterol lowering abilities, which are potentially useful in lowering the risk of certain diseases. Therefore, the high concentration of phytochemicals in barley may be largely responsible for its health benefits. ⁽¹³⁾

According to Goyal *et al.*, (2014) ⁽¹²⁾ flaxseeds are especially rich in thiamine, magnesium, potassium, and phosphorus (DVs above 90%). Also it was found that a germination process of one day at 20C° led to an improvement in the nutrient composition while fatty acid profiles of whole flaxseed remained unchanged. Antioxidant capacity increased from 210 to 442 µmol Trolox equivalent/g dry matter, lignans also was increased from 12.4 to 13.7 mg/g dry matter and free essential amino acids from 115 to 331 µg/100 g dry matter. ⁽¹⁴⁾ Ten grams of flaxseed contains one gram of water-soluble fiber (which lowers blood cholesterol) and three grams of insoluble fiber (which helps prevent constipation). ⁽¹⁵⁾ At the same time, it was found that germination process could reduce the total content of cyanogenic glucosides, linustatin, neolinustatin and lotaustralin which decreased significantly, level had increased. ⁽¹⁶⁾

Materials and methods:

Materials:

Flaxseed, barley and beetroot were obtained from Ministry of Agriculture. It were germinated, and dried at 40°C in a vacuum oven, then milled. The powders stored in dark glass jars and kept at less than 30°C till use.

Adriamycin injectable solution (25 mg/ml) was purchased from Mina pharm Co., Cairo Egypt. Formalin, and all other chemicals were obtained from El-Gomhoryia Company for Trading Drugs and Medical Instruments.

Experimental design:

This study was carried out in the animal house of the faculty of Home Economics, Menoufia University using sixty male albino wistar rats weighting 150 ± 10 g. All rats were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. Rats were housed in clean wire mech cages under standard conditions of humidity ($50 \pm 5\%$), temperature (25 ± 3 C°), light (12h light / 12 h dark cycle) and accumulated for one week before starting the experiment using free access standard basal diet and water and labium.

Group 1: Rats were fed the basal diet for further 21 days and served as control negative.

Group 2: Rats were fed the basal diet for further 21 days and served as control positive and injected with Adriamycin.

Group 3: Rats were fed the basal diet containing 5% of germinated barley powder.

Group 4: Rats were fed the basal diet containing 10% of germinated barley powder.

Group 5: Rats were fed the basal diet containing 5% germinated flaxseed powder.

Group 6: Rats were fed the basal diet containing 10% germinated flaxseed powder.

Group 7: Rats were fed the basal diet containing 5% of germinated barley and beetroot powder.

Group 8: Rats were fed the basal diet containing 10% of germinated barley and beetroot powder..

Group 9: Rats were fed the basal diet containing 5% germinated flaxseed and beetroot powder.

Group 10: Rats were fed the basal diet containing 10% germinated flaxseed and beetroot powder.

Basal diet composition of rats:

Basal diets were prepared according to ⁽¹⁷⁾. The experiment was carried – out in accordance with the national regulations on animal welfare and Institutional Animal Ethical committee (1309/2013), Menoufia, University, Egypt.

Rats were divided into 10 groups each with 6 rats. The first group (1) was fed on the basal diet and served as negative control (-ve). All other groups were injected for two consequence weeks each with 1mg/kg of ADM injectable solution. The second group (2)

was also left the positive control group, while eight groups were fed the basal diet, in addition to the germinated seeds and beetroot under study, as explained previously for a period 28 days. Then dissected the rats and serum samples were taken for analysis by both the collect of the blood as well as liver and kidney enzymes and antioxidants MAD and GPX, and level of lipoproteins in blood as well as keeping organs in formalin 8% until examined.

Statistical analysis:

All values were expressed as \pm SD. The statistical significance of differences between groups was assessed using one-way analysis of variance (ANOVA). The Mann-Whitney U test was used to compare the difference between two groups. A value of $p < 0.05$ was considered significant. Statistical analysis was performed using SpSS 17 for windows software (SPSS Inc, Chicago, USA).⁽¹⁸⁾

Results And Discussion:

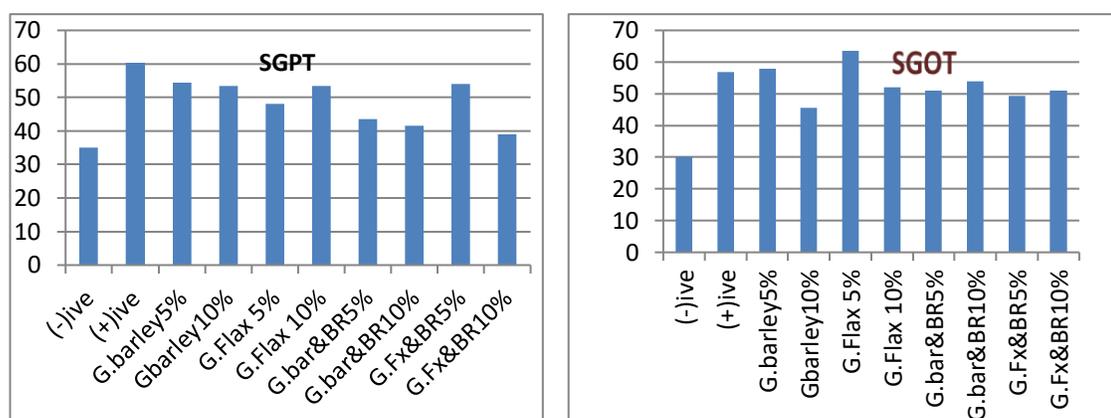


Fig (1):Effect of ADM and Germinated seeds and beetroot on serum GPT and GOT.

Fig. (1) illustrated the activities of liver enzymes (GPT) of all experimental groups. As shown in this figures the best treatment for GPT enzyme was observed for group 10 (10% G.FX & BR) with a significant difference with (-ve) control group, the better treatment for GPT enzyme was noticed for G6 & G5 with non-significant difference between them, and the best treatment of serum GPT enzyme recorded to group G5, G7, G8 & G10, in comparison with (+ve) control group.

These data were in agreement with results obtained by Gadah *et al.*, (2019)⁽¹⁸⁾ who found the red beetroot (RBR) prevented chlorpyrifos (CPF)-induced liver injury via attenuation of oxidative stress, inflammation and apoptosis. RBR enhanced antioxidant defenses,

suggesting that it could be used as a potential therapeutic intervention to minimize CPF hepatotoxicity. At the same time found that incorporation of flaxseed or its protein in food formulations can prevent hepatotoxicity. ⁽¹⁹⁾

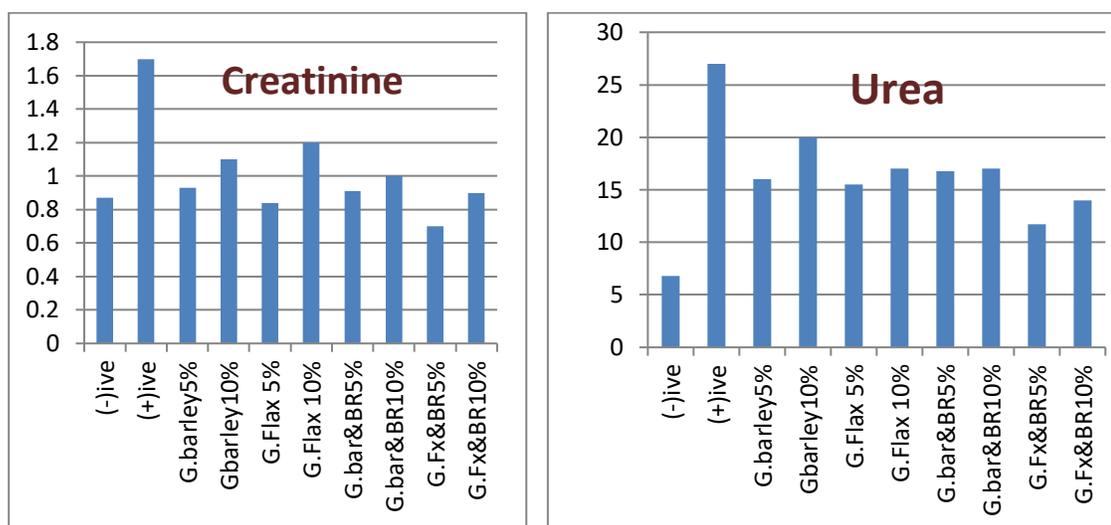


Fig.(2): Effect of ADM and Germinated seeds and beetroot on serum Creatinine and Urea.

Fig. (2) Illustrated the activities of renal serum (Creatinine and Urea) of all experimental groups. As shown in this figures the best treatment for serum Creatinine was observed for group 9 (5% G.FX &BR) with a significant difference with (-ve) control group, G3, G7 & G10, the better treatment for serum urea was noticed for G9 & G10 with non-significant difference between them, in comparison with (+ve) control group.

According to Kaoru *et al.*, (2010) ⁽²⁰⁾ adriamycin-associated nephropathy (AAN) remains poorly understood. Also flavonoids have shown renal protective effects against many nephrotoxic agents that frequently cause acute kidney injury (AKI) or chronic kidney disease (CKD). Flavonoids also improve cisplatin- or methotrexate-induced renal damage, demonstrating important actions in chemotherapy, anticancer and nonprotective effects. ⁽²¹⁾

Fig. (3) Illustrated the activities of fat serum (cholesterol, triglyceride, LDL and VLDL) of all experimental groups. As shown in this figures the best treatment for serum Cho was observed for group 7 (10%germinated barley and beetroot) with a-significant difference with (-ve) control group, G9, the better treatment for serum Trig was noticed for (G3 & G8) with non-significant difference ($P > 0.5$) between them, and the best treatment of

serum LDL recorded to group 3 (5% germinated barley). On the other hand, the best treatment of serum HDL recorded to group 10 (10% germinated flaxseed and beetroot), the best treatment of serum VLDL recorded to group 8 (10% germinated barley and beetroot) in comparison with (+ve) control group.

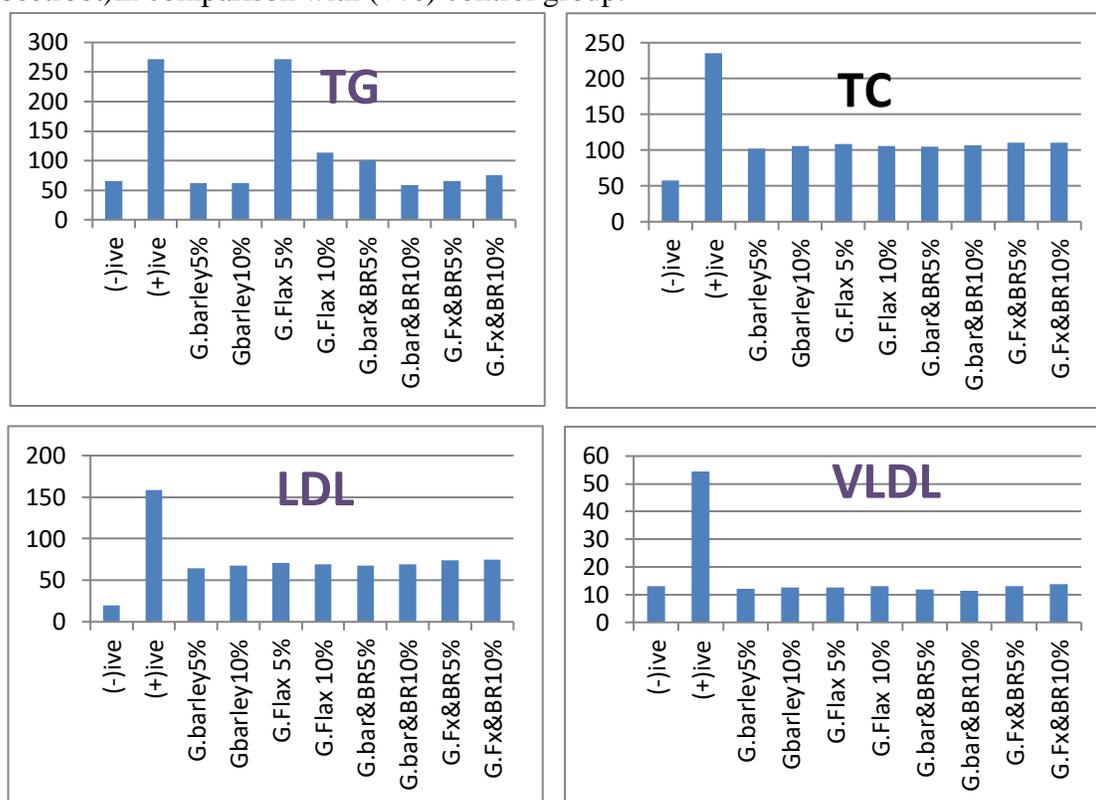


Fig.(3): Effect of ADM and Germinated seeds and beetroot on serum cholesterol, triglyceride, LDL and VLD

In similar study found the supplementation with omega-3 polyunsaturated fatty acids (PUFA) has been shown to improve vascular function. ⁽²²⁾ Also the flavonoids show the potential to improve HDL function through their well-documented effects on cellular antioxidant status and inflammation. Also many flavonoids (e.g., anthocyanidins, flavanols, and flavone subclasses) influence reverse cholesterol transport (RCT) and HDL function beyond simple HDL cholesterol concentration by regulating cellular cholesterol efflux from macrophages and hepatic paraoxonase expression and activity. ⁽²³⁾ According to Anna *et al.*, (2017) ⁽²⁴⁾ higher dietary polyphenol intake, was inversely

associated with CVD in postmenopausal women, which points to the health benefits of increased polyphenol intake from food sources for these women. Also the increasing long-chain omega-3 (LCn3) reduces serum triglycerides (evidence mainly from supplement trials). Increasing ALA slightly reduces risk of cardiovascular events and arrhythmia.⁽²⁵⁾

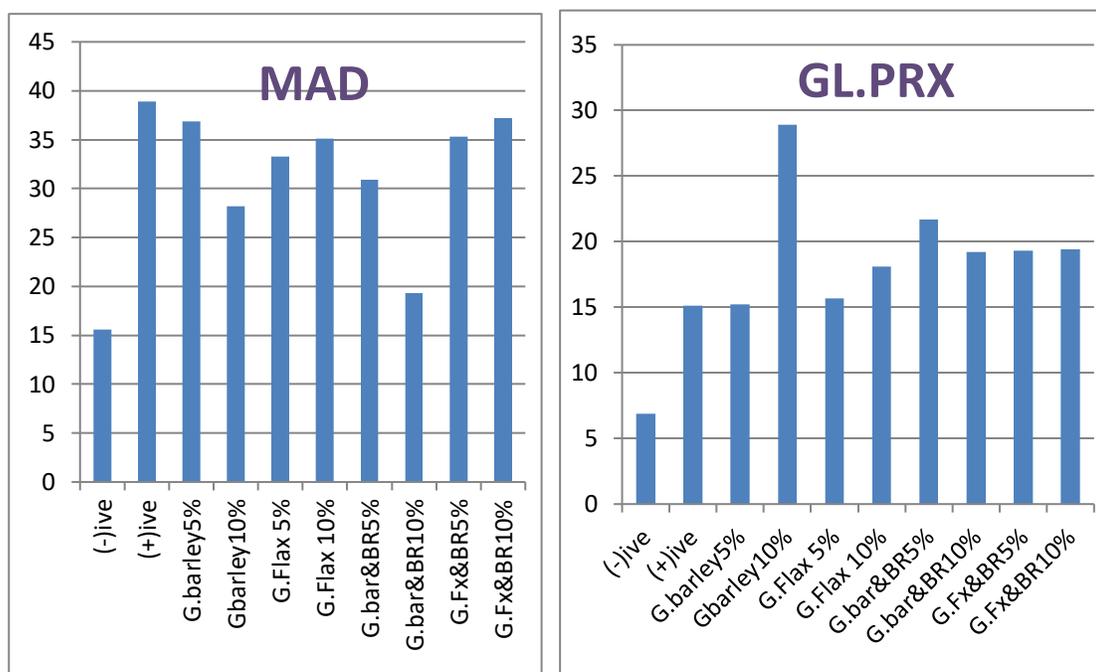


Fig.(4): Effect of ADM and Germinated seeds and beetroot on antioxidant enzyme (MAD & G.PRX).

Fig (4) illustrated the significant increases in antioxidant enzyme (MAD&G.PRX) (-ve) for (+ve) control group as compared with (-ve) control group. As shown in this figures the best treatment for serum MAD was observed for group 4&8 with nonsignificant difference between them. The best treatment for serum G.PRX was observed for group 3 with a significant difference with (-ve) control group.

That the ADR produce large amounts of reactive oxygen species (ROS) in vivo, that ADR administration causes a peripheral increase in tumor necrosis factor α (TNF- α), which migrates across the blood brain barrier (BBB) and leads to inflammation and oxidative stress in brain, most likely contributing to the observed decline in cognition.⁽²⁶⁾ On the other hand found that was positive correlation was observed between antioxidant activity (AOA) and total phenolic content (TPC) where with increasing the duration of

germination to 24 hours.⁽²⁷⁾ Also that germination of barley can be a good approach for enhancing the antioxidant potential of β -d-glucan.⁽²⁸⁾ On the same time and according to Guine *et al.*, (2018)⁽²⁹⁾ Beetroot is an important source of polyphenols as well as betalains, which are compounds that possess high antioxidant effect and radical scavenging capacity. Also germination process also reduced the total content of cyanogenic glucosides, the content of linustatin, neolinustatin and lotaustralin decreased significantly, but caused an increase in the content of linamarin. Therefore, germination can be utilized as an effective method to improve the nutritional value of flaxseed.⁽¹⁶⁾

Histopathological Results:

Liver:

The histopathological effect of ADM on liver tissue figs 5 to 8 where there were significant changes on the liver of control negative was compared hydroptic degeneration of hepatocytes and focal hepatic necrosis associated with inflammatory cells infiltration. The treated sections were also examined, and it was found that the best results obtained were for sections treated with germinated flaxseed 5% and a mixture of germinated flaxseeds and beetroot 10%.

Heart:

The histopathological effect of ADM on heart tissue figs 9 to 12 showed normal histological structure on control negative, also there were significant changes on the heart of control positive, compared massive hemorrhage in between the cardiac myocytes, focal necrosis of cardiac myocytes associated with inflammatory cells infiltration, vacuolation of the sarcoplasm of cardiac myocytes and hyalinosis in the wall of blood vessel. On the other hand, heart tissue that treated with both germinated barley 10% and germinated flaxseed 10% significantly improved the condition of heart tissue

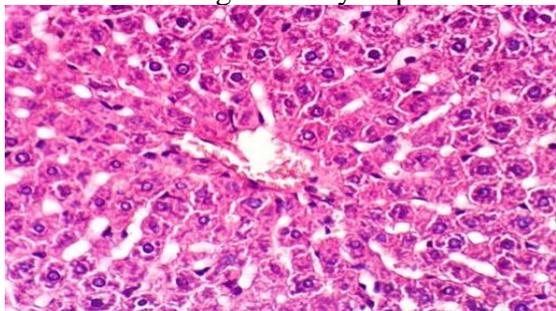


Fig. (5): Liver of rat from group 1: showing the normal histological structure of hepatic lobule.

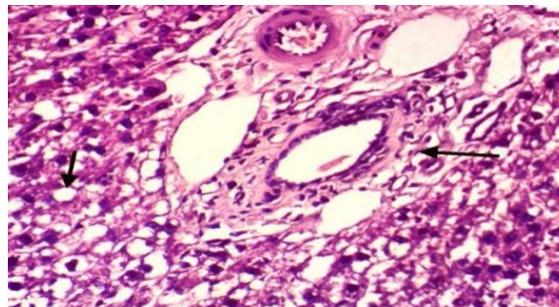


Fig. (6): Liver of rat from group 2: showing vacuolar degeneration of hepatocytes and fibroplasia in the portal triad.

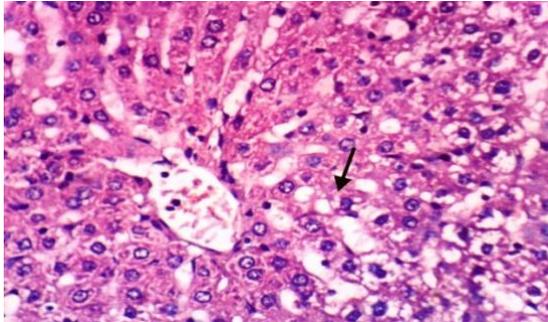


Fig. (7): Liver of rat from group 5 (G. FX 5%) showing vacuolar degeneration of some hepatocytes.

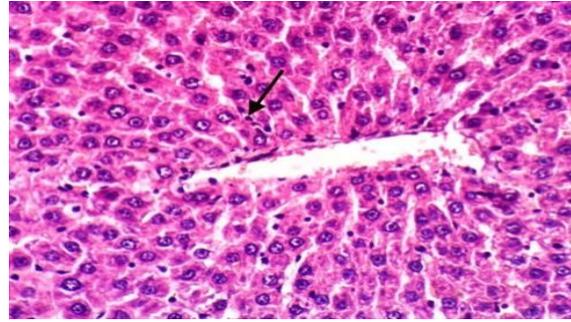


Fig. (8): Liver of rat from group 10(BR& G.FX10%) showing slight activation of Kupffer cells.

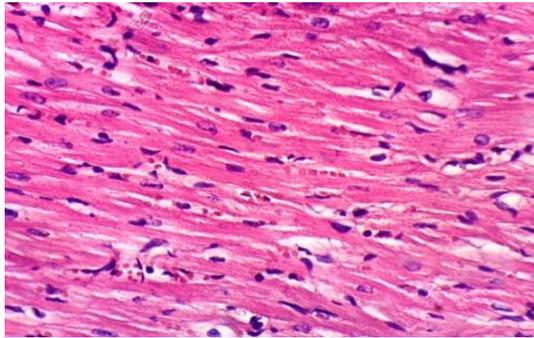


Fig. (9): Heart of rat from group 1 showing the normal histological structure of cardiac myocytes.

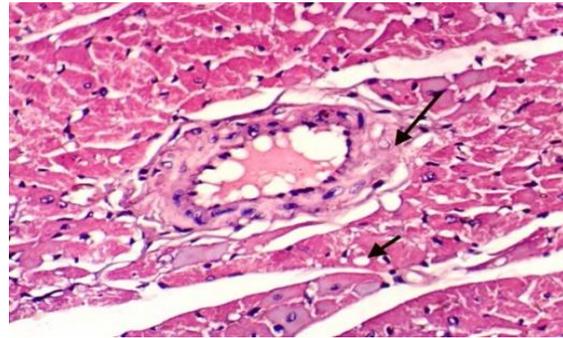


Fig. (10): Heart of rat from group 2 showing vacuolation of the sarcoplasm of cardiac myocytes and hyalinosis in the wall of blood vessel.

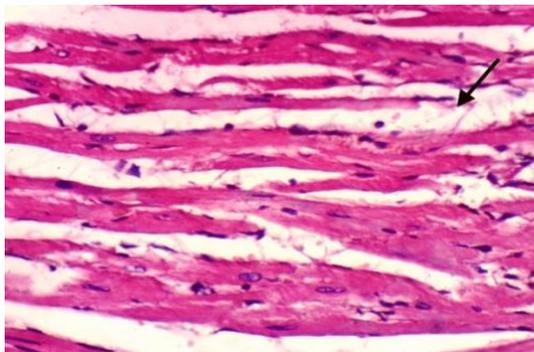


Fig. (11): Heart of rat from group 4 (G.B 10%) showing slight intramyocardial oedema.

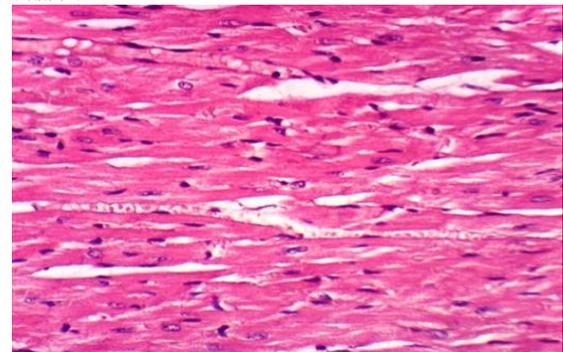


Fig. (12): Heart of rat from group 6 (G. FX 10%) showing no histopathological alterations.

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تأثير كلاً من الشعير والكتان المنبت، والبنجر على فئران التجارب المحدث لها الفشل في أمراض القلب

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الملخص العربي:

تهدف هذه الدراسة إلى معرفة تأثير المواد الكيميائية النباتية في نباتات مختلفة مثل الشعير المنبت (Hordeum vulgare)، بذور الكتان المنبت (Linum usitatissimum)، والبنجر (Beta vulgaris) على أمراض القلب والأوعية الدموية. تم تقسيم ستون من ذكور فئران الألبينو وزنها 10 ± 150 جم إلى عشر مجموعات كل منها ستة فئران. تم الاحتفاظ بإحدى المجموعات كمجموعة ضابطة (-)، بينما تم حقن المجموعات التسع الأخرى بواسطة الأدياميسين للإصابة بخلل في وظائف القلب لمدة أسبوعين متتاليين. أضيفت البذور النابتة المختلفة بنسبة 5% و 10% من النظام الغذائي الأساسي وتم إعطاؤها كمجموعة مفردة إلى 4 مجموعات على التوالي بينما أخذت المجموعات الأربع الأخرى البذور النابتة مخلوطة بالبنجر بنفس النسب السابقة. بعد انتهاء فترة التجربة تم التشريح وإجراء التحاليل المعملية التالية: ناقلات أمين الجلوتاميك أو كسالوأسيتيك، ناقلات أمين الجلوتاميك بيروفيك، الكرياتينين، واليوريا، وحالة مضادات الأكسدة (المالونالدهيد و الجلوتاثيون بيروكسيداز)، وصورة الدم الكاملة (CBC) والتغيرات النسيجية. أظهرت النتائج أن كلاً من المجموعتين المعالجتين بالشعير النابت بنسبة 5% وبذور الشعير النابتة المخلوطة بالبنجر بنسبة 5% وبذور الكتان النابتة المخلوطة بالبنجر بنسبة 10% مهمان في حالة مضادات الأكسدة والبروتينات الدهنية والكوليسترول في الدم. كما أكدت دراسة الأنسجة المرضية التغيرات البيوكيميائية في وظائف الكبد والقلب.

الكلمات الكاشفة: الشعير المنبت، بذور الكتان، الشمندر، البروتينات الدهنية، الكوليسترول..