Evaluation of Feeding Barley and Flaxseed Germinated with Beetroot powder on Heart Disorders in Rats

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RECAP

This new research wanted to determine the effect of barley and flax seeds germinated with beetroot powder in equal amounts added to the standard diet on cardiovascular disease risk factors. Thirty-six adult male Sprague-Dawley rats were separated into two main groups; the first group, consisting of 6 rats, served as the negative dominance set and was fed a standard diet. To induce cardiovascular disease (CVD) second group of 30 rats received a diet and were injected with Adriamycin (ADM) twice weekly via intraperitoneal (IP) injection. The second group was divided into five groups as a following: 6 rats in the first grouping followed a diet as a representative of a positive control group. Flaxseed and barley germinate individually mixed with beetroot (in similar amounts) were added to the diet at different levels (5 and 10%), respectively, for 28 days in the 2ed, 3ed 4th, and 5th groups, respectively. The findings showed that previously fortified diet feeding of rats improved sera antioxidants and liver enzymes, and lowered lipid profile levels by a significant (P <0.05) degree. In conclusion, the present study indicated that barley and flaxseed germinated with beetroot ameliorate blood vessel health and lower the risk of CVD. The histological examination supported the improved impact of this mixture on both liver and cardiac status.

Keywords*:* *Germinated barley, Germinated flaxseed, Beetroot, cardiovascular disease.*

INTRODUCTION

Globally, CVD accounts for 30% of deaths. More than three percent of these fatalities are attributable to coronary heart disease and stroke **(WHO, 2014)**. According to estimates from 2018, Trans fats contributed to more than 500,000 annual deaths **(WHO, 2018).** The removal of trans-fat from diets has been widely promoted at the same time. **Hooper et al., (2020)** discovered that high trans-fat intake has negative impacts on blood lipids and circulating inflammatory markers. According to estimates, dietary risk factors account for 53% of deaths from CVD **(Petersen and Kris-Etherton, 2021)**. This may be brought on by, among other things, high blood pressure, smoking, diabetes, obesity, high blood cholesterol, poor diet, excessive alcohol use, and lack of sleep **(Jackson et al., 2015)**. The critical to prevent heart failure is to lower risk factors. Making healthy lifestyle changes and a healthy diet can minimize or even completely get rid of numerous risk factors for heart disease. One of the best methods to prevent heart disease, heart attacks, and strokes is to have a healthy lifestyle **(Uthman et al., 2015).**

Tesby et al., (2021) illustrated that germination of barley (BG) enhanced the amount of folic acid, vitamin E, and vitamin B complex. **Nelson et al., (2013)** concluded that the use of germination in grains helps to increase the nutritional and health value of whole grains, as well as its widespread use may affect reducing many diseases.

Many studies indicated the healthy benefits of barley and flaxseeds germinated (FX G). As they may reduce the risk of CVD, may improve liver and kidney function, act as antioxidants and improve blood lipid profiles **(Parikh et al., 2018; Islam et al., 2021; Kim et al., 2021)**.

Georgiev et al., (2010); Agency et al., (2012); Wruss et al., (2015), reported that beetroot (BR), a rich dietary supply, is thought to possess health-promoting qualities, anti-oxidant and anti-inflammatory effects, anti-carcinogenic and anti-diabetic activities, hepato-protective, hypotensive, and wound healing properties. According to **Lundberg et al. (2011),** beetroot juice lowers blood pressure in hypertensive animals, which may have an impact on the mechanisms underlying cardiovascular disease **(Hobbs et al., 2012; Siervo et al., 2013).**

This study uses experimental rats as a module to assess the effects of consuming flax seed and barley germinated mixed with beetroot on cardiac problems.

MATERIALS & METHODS

Materials:

A standard diet was prepared according to (**Reeves et al., 1993).** Beetroot, flax seed, and barley were purchased from the local market of Matrouh governorate, Egypt.

Adriamycin injectable solution (25 mg/ml) was purchased from Mina pharm Co., Cairo Egypt. All other chemicals, kits, and reagents were obtained from El-Gomhoreya Company, Cairo, Egypt. Thirty-six (36) male albino rats, Sprague Dawley strain, weighing 150 ±10g, were obtained from Serum and Vaccine Center-Cairo.

Experimental design:

For at least seven days before the studies, 36 Sprague-Dawley male albino rats were placed in group cages and provided a standard diet for adaption. The six groups, each with six rats, were divided as follows: Group 1 was fed only a standard diet (negative control group, -ve). According to **Hong et al., (2002),** thirty (30) rats (Heart problem groups) were

administered Adriamycin (ADM) two days per week for two weeks by intraperitoneal injection (5 mg/kg BW). The rats were then separated into five groups. Group 2 was a positive control group (+ve); they were only given a diet after injection. Group 3 received a mixture of beetroot powder and 5% barley germinated in equal amounts was added to the standard diet. Group (4) intake a diet in addition to 10% barley germinated and beet powder in the same amount. Group (5) consumed a meal plus 5% of blend flaxseed germinated and beetroot powder in equivalent amounts. Group (6): fed a diet along with 10% flaxseed germinated with beetroot powder at an equal rate. Rats were slaughtered, and blood samples were collected for biochemical examination after the study period (4 weeks).

Biochemical analysis:

Blood samples were used for the determination of the following parameters by commercially available (Bio Merieux) kits: Determination Alanine aminotransferase (ALT), Aspartate amino-transferase (AST) were determined according to the method described by **Huang et al., (2006),** Malon-dialdehyde (MDA) as stated by **Ohkawa et al., (1979),** Glutathione peroxidase (G Px) by **Flohe and Gunzler, (1984),** Total cholesterol (TC) by **Richmond, (1973),** Triglyceride (TG) estimated as claimed by **Wahlefeld, (1974),** High-density lipo-protein cholesterol (HDL– c) by **Albers et al., (1983)** and Low-density lipoprotein cholesterol (LDL–c) calcu-lated by **Friedewald et al., (1972)** and very low-density lipoprotein cholesterol (VLDL-c) were carried out according to the methods of **Lee and Nieman, (1996).** Atherogenic coefficients (AC), cardiac risk ratio (CRR), and atherogenic

index of plasma (AI) were calculated as follows the equation: AC= TC-HDL-c/HDL-c, CRR= TC/HDL-c, and AI= log of TG/HDL-c.

Chemical analysis:

Total Phenolic Estimation:

The qualitative and quantitative determinations of phenolic compounds were carried out following a modified procedure by **Croci et al., (2009).**

Histopathology examinations:

From each experimental animal, small samples of the liver and heart were removed, fixed in buffered formalin (10%), dehydrated in increasing concentrations of ethanol (up to 70%), cleaned in azylene, and then embedded in paraffin. According to **Bancroft et al., (1996)** sections of 4- 6 m thickness were produced and stained with hematoxylin and eosin.

Statistical study:

Utilizing the software statistical package for social science (SPSS) Ver. 10, statistical analysis was carried out, and results were compared using the appropriate tests. The mean and SD were shown. To assess differences between the rat groups and their respective controls, a paired T. test was performed. Time course experiments were statistically analyzed using multiple measures ANOVA **(Snedecor and Cochran, 1989).**

RESULTS & DISCUSSION

Table 1 displays the bioactive compound composition of a blend of barley (BG), flaxseed (FX G), and beetroot powder (BR). The information provided values for the amounts of T. phenols (607.0, 621.0mg/100g) and T. flavonoids (120.0, 129.0mg/100g), respectively. According to data comparable to those from **Wang et al. (2015),** the germination of flax seeds for 8 days results in a considerable increase in the phenolic components and

overall antioxidant activity. Furthermore, **Wang et al., (2016)** demonstrated that 10-day flaxseed geminated contained the highest levels of total phenolics and total flavonoids, which are what give flaxseed its antioxidant properties. **Tesby and others (2021)** observed an increase in barley germinated content of flavonoids and phenolic contents along with the antioxidant activity. Also, during germination the content of phenolic acid differed greatly, and also the germinated barley showed great activity in removing free radicals. **Donkor et al., (2012)** stated that germinated barley contained substantial amounts of total phenolics.

Results summarized in Table 2 demonstrated that Adriamycin administration (positive control group) significantly (P 0.05) increased the mean value SE of serum concentrations of AST and ALT when compared to the negative control group. For all groups fed on a diet substituted with BR & BG and BR & FX G mixture at various doses, a significant (P 0.05) reduction in serum AST and ALT levels was seen. These results concur with those of **Quan et al. (2018),** who demonstrated the effective-ness of barley phenolic extract as a liver injury-preventative measure. Additionally, Nida et al. (2019) discovered that adding flaxseed or its protein to meal formulations can reduce hepatotoxicity. **Albasher et al., (2019)** illustrated that red beetroot (RBR) prevents liver injury by attenuating oxidative stress, and inflammation and reducing hepatotoxicity. The highest improvement of ALT serum was observed in rat feeding diet replaced with BR& FX G (10%).

As shown in Table 3, the serum malondialdehyde (MDA) level was significantly higher (P 0.05) in the positive control group compared to the negative

control group, whereas it was significantly lower (P< 0.05) in all treated groups when barley and flaxseed were mixed with beetroot at various amounts. On the other hand, it was evident that all treatment groups with the barley and flaxseed mixture germinated with beetroot at varied levels showed a substantial (P 0.05) increase in blood GPX activity in comparison to the positive control group. The rats whose diets were changed with BR & B G showed the greatest improvement in MDA and GPx levels (5%). According to **Dos Santos et al., (2018),** ADM raises the levels of MDA and lowers antioxidant enzymes in the blood. While MDA levels induced by ADM activity in red blood cells are decreased by antioxidants like flavonoids and phenols. Flaxseed that has been germinated exhibits significant free radical scavenging action **(Herchi**a, **et al., 2015).** According to **Ahmad, et al., (2016),** the germination of barley can be a useful strategy for boosting the antioxidant capacity of -d-glucan. Also, **Islam et al., (2021)** found that the antioxidant properties mainly increased during the germination period of barley.

Data in Table (4) showed the effect of germinated barley and germinated flaxseed with beetroot mixture powder on the lipids profile of negative control and Adriamycin groups. Exposure of rats to ADM led to significantly higher values of TC, TG, LDL-c, and VLDL-c in the blood sera, in contrast to HDL which showed a significantly lower matching with the normal control (P≤ 0.05). The same results were obtained by **Hong et al., (2002)** who said that ADM caused an increase in serum total cholesterol, triglyce-rides, and LDL cholesterol. Also, **Sunanda and Anand, (2009)** found that ADM increases levels of lipids in the blood, but reduces the amount of HDL-c. Feeding

rats on diet replaced with BR & B G, and BR & FX G at different concentrations led to a significant down the level of TC, TG, and VLDL-c blood. This may be due to containing B G, FX G, and BR on fiber, total phenols, and flavonoids. These findings are similar to **McRae, (2017)** who found the dietary fibers' actions in reducing total cholesterol and LDL-c concentrations. Furthermore, **Anna et al., (2017)**'s research showed that higher dietary polyphenol intake was negatively related to cardio-vascular disease in post-menopausal women, poin-ting to the advantages of consuming more poly-phenols in food.

The effect of barley and flaxseed germinated mixed with beetroot powder on atherogenic factors of negative control and Adriamycin groups are shown in **table (5).** Rats' injection with ADM led to a significant raise in the values of AC, CRR, and AI versus the negative control (P≤ 0.05). According to **Niroumand et al., (2015),** AI can be utilized in routine practice as a compliance monitoring index of CVD. Additionally, **Jing et al. (2021)** hypothesized that in the adult population, a higher AI score would be causally related to coronary artery disease. Rats were fed diets that were substituted with BR & G B and BR & G FX at various conce-ntrations, and this resulted in a significant reduction (p 0.05) in the levels of AC, CRR, and AI. These results support the hypothesis made by **Da Silva et al., in 2022** that the betanin content of beetroot, which is known for its capacity to scavenge free radicals, is responsible for the adjuvant effect of beetroot in lowering cardiovascular diseases. The lowest reduction of AI level was found in rats' intake of a diet supplemented with BR& B G (10%).

Figure (1) showed the effect of barley and

germinated flaxseed with beetroot powder on heart histopathology of negative control and Adriamycin groups. Microscopical examination of heart sections of negative control rats revealed normal histological structure while, positive control showed 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 the blood vessel. it was observed that rats treated with BR& G B (5%), BR& G B (10%), BR& G FX (5%), and BR& GFX (10%) resulted in pronounced protection against ADM-induced alterations and the heart tissue appeared nearly within a normal pattern. This data is similar to **Abulnaja and El Rabey (2015)** who illustrated that administration of barley bran to hypercholesterolemic rats improved the tissues of the heart, liver, and kidneys until they returned to almost normal.

Figure (2) showed the Effect of barley and flaxseed germinated with beetroot powder on liver tissues of negative control and Adriamycin groups. Microscopical examination of liver sections of negative control rats revealed normal histological structure while, positive control showed vacuolar degeneration of hepatocytes, fibroplasia in the portal triad, and portal infiltration with inflammatory cells. Also, the hepatocytes showed variable degrees of hydropic degeneration of hepatocytes and focal hepatic necrosis associated with inflammatory cell infiltration. However, examined sections that replaced diet with BR& G B (5%) and BR& G FX (10%) showed no changes except vacuolar degeneration of some hepatocytes. According to **Al-Shali and Ramadan (2020**), adding germinated barley (GB) to a

high-fat diet prevented liver damage, altered lipid profiles, and modified liver structure. It also reduced related hepatic inflammation and downregulated SDC1 in liver tissue.

CONCLUSION

In conclusion, consuming diets rich in germinated barley, flaxseed, and beetroot can enhance blood vessel health and decrease the risk of CVD by lowering the bad cholesterol profile and raising blood antioxidant levels. The improvement of both liver and heart health that sprouted barley, flaxseed, and beetroot caused was validated by histopathological evaluation.

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Table (1): Active phenolic compounds content of germinated barley, germinated flaxseed, and beetroot mixture powder (mg / 100gm).

Phenolic compound	BR & G B (mg / 100gm)	BR & GFX. (mg / 100gm)
Total Phenols	**607.0**	**621.0**
Total Flavonoids	**120.0**	**129. 0**

GB: germinated barley, G FX: germinated flaxseed, BR: beetroot.

Table (2): Means ± SD values of liver functions in rats fed on basal diet and diet supplemented by germinated barley, germinated flaxseed, and beetroot mixture powder.

Groups	Liver Enzymes	
	ALT (U /L)	AST (U /L)
G1: (-) Ve	35.00^{e} ±2.16	30.00^{e}±4.00
G2: (+) Ve	60.25^{a}±6. 85	56.75^{ab}±5.68
G3: BR& G B (5%)	43.50^{cd}±7.59	51.00^{bcd}±4.55
G4: BR& G B (10%)	41.50^{cde}±6.03	54.00^{bc}±3.37
G5: BR& G FX (5%)	54.00^{ab}±7.35	49.50^{cd}±1.25
G6: BR& G FX (10%)	39.00^{de}±7.12	51.00^{bcd}±4.08

Mean values are expressed as means ± SD.

Means in the column that have a unique superscript letter are significantly different at P 0.05.

Table (3): Means ± SD values of GPX and MDA in rats fed on basal diet and diet supplemented by germinated barley, germinated flaxseed, and beetroot mixture powder.

Groups	**GPX (mu/ml)**	**MDA (nmol/mL)**
G1: (-) Ve	15.94^{de}±1.28	**15.62^{d}±1.55**
G2:(+) Ve	5.22^{f}±1.55	**38.90^{a}±4.23**
G3: BR& B G (5%)	20.28^{b}±1.89	**30.89^{c}±0.47**
G4: BR& B G (10%)	18.68^{bcd}±2.50	**35.15^{abc}±3.23**
G5: BR& FX G (5%)	19.29^{bc}±0.57	**35.28^{ab}±3.13**
G6: BR& FX G (10%)	**19.36^{bc}±1.72**	**37.20^{a}±0.95**

Mean values are expressed as means ± SD.

Means in the column that have a unique superscript letter are significantly different at P <0.05.

Table (4): Means ± SD values of lipids profile in rats fed on basal diet and diet supplemented by germinated barley, germinated flaxseed, and beetroot mixture powder.

Groups	Lipid profile				
	T.C (mg/dl)	T.G (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c
G1: (-) Ve	57.50^{c} ±5.69	67.94^{c} ±7.19	27.00^{a} ±0.82	16.91^{e} ±4.08	13.59^{c} ±1.4
G2:(+) Ve	239.00^{a}±6.5	187.50^{a} ±11.9	23.75^{bcd} ±1.7	177.8^{a} ±8.54	37.50^{a} ±2.8
G3: BR& G B (5%)	104.50^{b} ±4.2	111.75^{b} ±11.4	25.68^{abc} ±2.26	56.48^{d} ±6.06	22.35^{b} ±2.3
G4: BR&G B (10%)	106.75^{b} ±3.1	60.25^{c} ±4.99	26.25^{ab} ±1.26	68.45^{bc} ±2.8	12.05^{c} ±0.9
G5: BR&G FX (5%)	110.00^{b} ±8.0	65.00^{c} ±1.41	24.50^{abcd} ±1.3	72.50^{b} ±8.1	13.00^{c} ±0.3
G6:BR&GFX (10%)	110.50^{b}±8.4	69.25^{c} ±4.50	23.75^{bcd} ±1.7	74.40^{b} ±9.5	13.85^{c} ±0.9

Mean values are expressed as means ± SD.

Means in the column that have a unique superscript letter are significantly different at P 0.05.

Table (5): Means ± SD values of atherogenic in rats fed on a basal diet and diet supplemented by germinated barley, germinated flaxseed, and beetroot mixture powder

Groups	AC (mg/dl)	CRR (mg/dl)	AI (mg/dl)
G1: (-) Ve	1.29^{d}±0.18	2.13^{d} ±0.18	**0.40^{ef}±0.05**
G2:(+) Ve	9.10^{a}±0.75	10.10^{a}±0.75	**0.90^{a}±0.05**
G3: BR& G B (5%)	3.10^{c}±0.46	4.10^{c}±0.46	**0.64^{c}±0.06**
G4: BR& G B (10%)	3.07^{c}±0.19	4.07^{c}±0.19	**0.36^{f}±0.02**
G5: BR& G FX (5%)	3.50^{bc}±0.37	4.50^{bc}±0.37	**0.42^{e}±0.03**
G6: BR& G FX (10%)	**4.01^{b}±0.74**	**5.01^{b}±0.74**	**0.49^{d}±0.05**

Values are expressed as means ± SD; means in the same columns with the different letters are significant ($p < 0.05$).

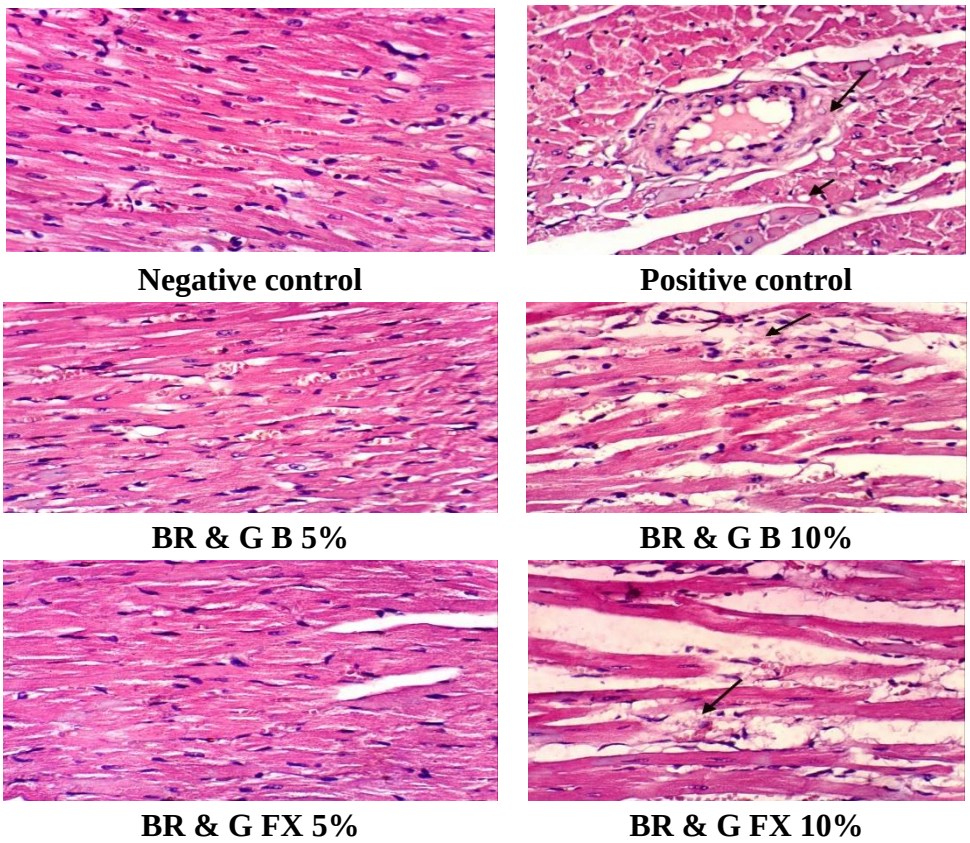

Figure (1): Effect of germinated barley, germinated flaxseed, and beetroot mixture powder on heart histopathological of negative control and Adriamycin groups.

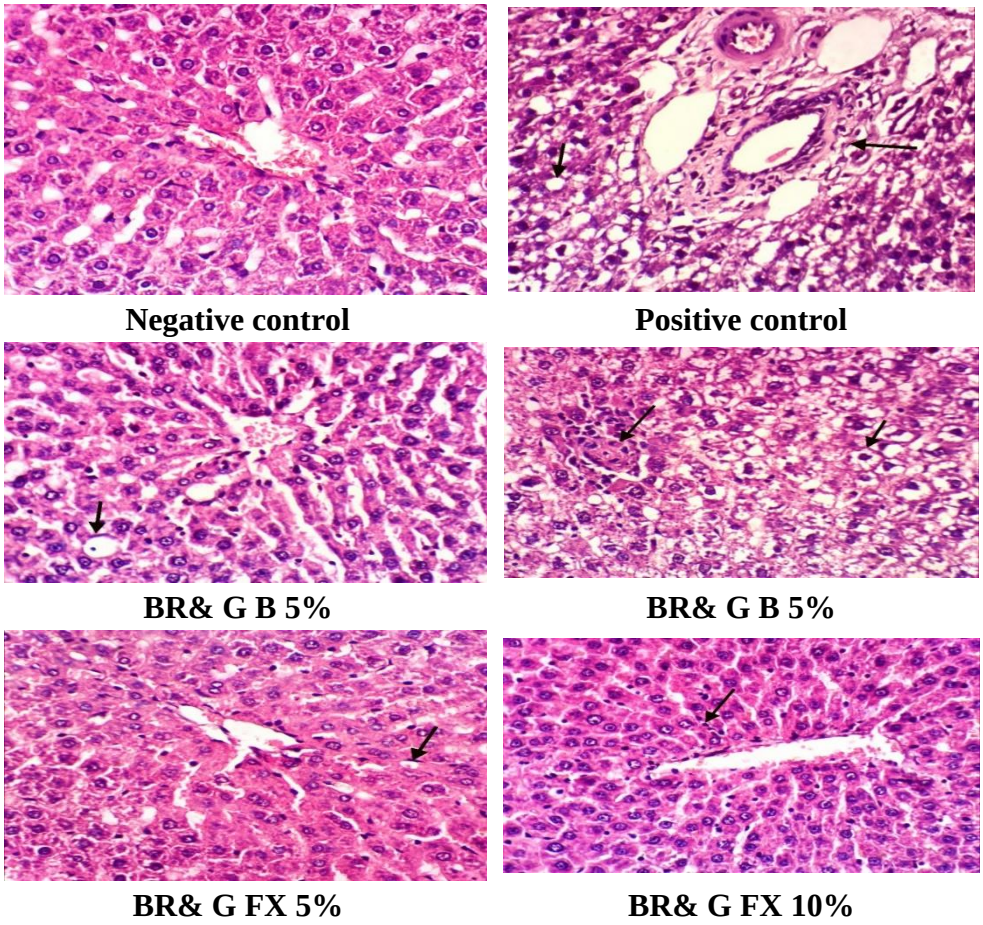

Figure (2): Effect of germinated barley, germinated flaxseed, and beetroot powder on liver histopathological of negative control and Adriamycin groups

تقييم التغذية بالشعير وبذور الكتان المنبتة مع مسحوق البنجرعلى اضطرابات القلب في الجرذان

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الملخص العربى

هدف هذا البحث تحديد تأثير الشعير وبذور الكتان المنبته مع مسحوق البنجر المستخدمه فى الغذاء على العوامل المسببة لامراض القلب الوعائيه فى الجرذان. تم تقسيم ستة وثلاثون جرذاً سلالة سبراغ داولي إلى مجموعتين رئيسيتين. تم تغذية المجموعة الرئيسية الأولى (6 فئران) على الغذاء الأساسى كمجموعة ضابطة سالبه. للحث على أمراض القلب والأوعية الدموية (CVD) ، تلقت المجموعة الثانية المكونة من 30 جرذًا نظامًا غذائيًا قياسي وتم حقنها بـ Adriamycin (ADM) مرتين أسبوعياً عن طريق الحقن داخل الصفاق (IP)، وأضيف مخلوط من بذور الكتان والشعير المنبته مع مسحوق البنجر (مخلوطه بكميات متساويه لكلا منها) إلى النظام الغذائي الأساسى. بمستويات مختلفة (5 و 10٪) على التوالي و لمدة 28 يوم في المجموعة الثانية والثالثة والرابعة والخامسة على التوالي. أشارت النتائج إلى أن تغذية الجرذان على النظام الغذائى المدعم ، أدت إلى تحسن معنوي في مضادات الأكسدة في مصل الدم ، وظائف الكبد ، خفض مستويات الدهون. خلصت الدراسة إلى أن الشعير وبذور الكتان المنبته مع مسحوق البنجر لهما القدرة على تحسين حالة الأوعية الدموية وتقليل مخاطر الإصابة بأمراض القلب من خلال تقليل الدهون الضارة وزيادة مستويات مضادات الأكسدة في الدم. كما أكدت دراسة التغيرات الهستوباثولوجيه التأثير المعزز للشعير وبذور الكتان المنبته مع مسحوق البنجر على حالة الكبد والقلب .

الكلمات المفتاحيه : الشعير المنبت – بذورالكتان المنبت – البنجر – امراض القلب الوعائية