Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

EFFECT OF FLAXSEEDS (LINUM USITATISSIMUM L.) EXTRACT IN MALE REPRODUCTIVE SYSTEM OF ALBINO RAT TREATED WITH SODIUM NITRATE

Rawaa Hamid Abdulshahed M.Sc[1,2],
Sarah Saad Abdul Ameer M.Sc[1,2]
[1]Assistant teach. Directorate of education of kerbala, Ministry of Education,Iraq.
[2]Department of Biology, College of Education for pure Sciences, Univ. of Kerbala Kerbala, Iraq.
rawaa_hameed@karbala.edu.iq.
aliroza353@gmail.com.

Abstract

The objective of the present study is assessing the potential protective role of the aqueous extract of the flax seeds on the Sodium Nitrate mediated reproductive dysfunction of the adult albino rats. Albino rats (36) have been placed randomly to 6 groups 6 rats in each. Normal control rat has been the first group that was given distil water (D.W.), while the second group was given Sodium Nitrate 150 200mg⁄kg. Third group (FSE 200) received200mg⁄kg. Fourth group (FSE 300) received (300 mg/kg) of aqueous extract of flax seeds, the other protective groups fifth and six group have been treated with 200&300mg/kg of the flaxseeds extract respectively with Sodium Nitrate 150mg ⁄kg. The. All of the groups have been treated orally for a period of 30 days by the use of the gavages tube. In Sodium Nitrate group, the result had shown significant decrease (P≤0.050) in LH, FSH, testosterone, HDL level, significant increase (P≤0.05) in triglycerides TG, LDL and cholesterol level in comparison with the control group. Treatment by the flaxseeds 200&300mg/kg had resulted in a significant raise in LH, FSH (P≤0.05)in comparison to the control group, but testosterone level in (FSE 200) record non-significant (P>0.05) in comparison to the D.W, while HDL and TG record non – significant changes as compare with control group while LDL & cholesterol level record significant reduction (P>0.05) as compared to D.W. The protective groups treated with flaxseeds extract 200 & 300mg/kg with Sodium Nitrate record significant raise in LH, FSH and testosterone (P≤0.05) compared to Sodium nitrate group, whereas the cholesterol make non-significant changes (P>0.05) in cholesterol level as compared with Sodium nitrate groupmate & LDL displayed significant reduction (P≤0.05) compared to the Sodium nitrate group and significant raise in HDL level (P≤0.05) in these groups. In addition to that, the oral administration of flaxseeds extracts with the Sodium Nitrate enhanced most histological changes of testes caused by Sodium Nitrate in FSE (200&300) GROUPS WHILE, oral administration of the Sodium nitrate cause degeneration of the constituent layers of sperm cells and congestion in the interstitial tissue. In conclusion, Sodium Nitrate (150mg/kg) induce degenerative changes in reproductive organs of the male rats and aqueous extract of the flaxseeds enhance the adverse effects of the Sodium Nitrate on male reproductive system.

Introduction

A medicinal plant that has natural properties and is one of the spring crops known as flax seed or line seeds. Flaxseed has a medicinal effect. It is used to protect the liver [1], and it decreases risks of CVD, like the coronary heart disease and strokes [2]. It reduces blood pressure and increases anti-

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

inflammatory activity. It reduces cases of chronic inflammation [3] and has a protective effect on the testicles, [4] being an extract that can be used as a natural antioxidant, [5] by affecting the liver by inhibiting oxidative stress, and this in turn leads to protection against Chronic diseases such as renal and hepatic failure, hyperlipidemia and diabetes that flax extract has a high efficacy in the formation of steroids and very effectively [6].

Eating behaviors play an essential role in the metabolic and diet-related disorders, as the consumption of appetizing substances in mammals protects them from essential nutrient deficiencies [6]. On the other hand, excessive consumption of appetizers or palatable or appetizing food containing food additives leads to unbalanced amounts of nutrients, as food additives are commonly utilized for a variety of the purposes, which include preservation, sweetening and coloring [7]. Sodium nitrate is used as a preservative for processed cheese and meat Sodium nitrate is added to canned and processed meat such as sausages, pepperoni, mortadella, and bacon. It is also sometimes added to fresh, chilled meat, to preserve it and limit the growth of bacteria on it. It is also added to meat to keep the red or pink color, as the European Union granted sodium nitrate No. (E251)[8,9] .

Animal samples

The present study has been conducted in the animal facility of College of Education of pure sciences\ University of Karbala, Ethical permission was granted by the research and experimental group College of Education Ethics Committee for Pure Sciences – Dept. of Life Sciences under Ethical Approval No.654 in December 2022 where 36 male rats ranging from 200 to 225 g and ages of approximately (10 to 12) weeks were utilized. The sample the animals received a month's adaptation to the place and nature of food before the experience. The animals were kept in plastic cages covered with metal (measure 40cm × 20cm × 20cm) with floors covered by sawdust, rats have been subjected to appropriate lab conditions, like temperature ($22^{o} \pm 5^{o}$) and light exposure time and darkness (10hrs-14hrs). In addition, animals have been provided with freely ad libitum water and a food supply with standard pellet diet.

Collection and extraction of plants

Flaxseeds were obtained from the local markets in Karbala Iraq, medically diagnosed at the University of Karbala, Iraq, then cleaned, by removing foreign matter. Then it was dried in the shade with a constant stirring. After crushing inside the blender, a fine powder is made, (20g) of which are mixed with (400ml) of the distilled water and left for a period of (24hrs) at the room temperature. Subsequently, this mix has been filtered through a multi-layered medical gauze for the purpose of remove impurities and spun rapidly at 3000 revolutions/min for ten minutes, cleaned with filter paper, oven-dried at 40 °C, and finally refrigerated for use [10].

Experimental design

The rats have been divided randomly to 6 experimental groups (n=6), which are: control, Sodium Nitrate (150mg/kg), FSE (200 mg/kg), FSE (300 mg/kg), FSE (200 mg/kg)+ Sodium Nitrate and, FSE

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

(300 mg/kg)+Sodium Nitrate. FSE has been administered by oral gavage daily for a period of 30 days. The Nitrate has been administered by an oral dosage for a period of 30 days. FSE (200 mg/kg)+ Sodium Nitrate rats were administered orally with FSE (200 mg/kg)before administrated with Sodium Nitrate (150 mg\kg)for 30 days and FSE (300 mg/kg)+ Sodium Nitrate rats were administered orally with FSE (300 mg/kg)before administrated with Sodium Nitrate (150mg\kg) for a period of 30 days, 30 days later, the animals have been sacrificed, dismembered, with blood directly taken from the heart through the centrifugation at 3,000 cycles/min for 15min. The sera have been placed in tubes then refrigerated at a temperature of (-20^{o}C). The testis was removed and fixed in 10% formalin for a period of 48hrs for the histological examinations.

Measurement of serum LH, FSH, Testosterone, TG, LDL, HDL, and Cholesterol levels

Testosterone

Hormone concentration levels have been measured based on [11].

luteinizing hormone

levels of hormone concentration have been measured based on [12].

Follicle- stimulating hormone

Hormone concentration levels have been measured based on [13].

Cholesterol

THE cholesterol concentration level was measured according to[14].

Triglycerides (TG)

THE Triglycerides concentration level was measured according to [15].

High density lipoprotein (HDL)

The HDL concentration level was measured according to **Burstein,** [16].

Low density lipoprotein (LDL)

The LDL concentration level was measured according to [17].

Histopathological Characteristics

After sacrificing the animals, at the end of the experimentation, some organs like the testes have been collected for the histopathological examinations. Small biopsies from every animal's organs have been fixed in 10% formalin and histopathological processing had been carried out with the use of the hematoxylin and eosin staining. Sectioned tissues have thus been examined with the use of 10X light microscopy objectives. [18].

Statistical analyses

Data have been analyzed with the use of the SAS software and the possible outcomes have been compared by using the Least Significant Difference (LSD) or Duncan test at a probability level of 0.05 [19].

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

Results

Effects of FSE and sodium nitrate on the sex hormones

Table (1) had shown a significant decrease in levels of LH, FSH & Testosterone in sodium nitrate group, when compared with the control group, FSE group (200, 300 mg/kg) had resulted in significant increase in FSH and LH levels, while testosterone concentrations were recorded Statistically significant differences only in the FSE 300 mg/kg group, while (FSE 200 & 300 mg/kg +Sodium Nitrate) had shown a significant increase in T, FSH, and LH, in comparison with the FSE and sodium nitrate group

Treatments	Means± S.E		
	LH mIU / ml	FSH mIU / ml	Testosterone ng / ml
Control D.W	24.39± 0.38 C	21.94± 0.30 C	4.16± 0.03 B
1 Sodium Nitrate 150 mg/kg	13.45± 0.30 F	11.88± 0.18 F	0.50± 0.007 E
2 FSE 200 mg/kg group	27.70± 0.36 B	32.39± 0.60 B	4.33± 0.02 B
3 FSE 300 mg/kg group	29.37± 0.20 A	36.01± 0.51 A	4.80± 0.03 A
4 FSE 200 mg/kg + Sodium Nitrate	16.92± 0.22 E	13.74± 0.56 E	1.92± 0.09 D
5 FSE 300 mg/kg + Sodium Nitrate)	20.94± 0.28 D	17.26± 0.70 D	3.37± 0.10 C
LSD	0.8659	1.4774	0.1753
P (VALUE)	0.050	0.050	0.050

Table1. statistical analyses. The data have been analyzed with the use of SAS software and the results were compared using the Least Significant Difference (LSD) or Duncan's test at a probability level of 0.05. • This note is written at the bottom of each table when choosing the Dunkin test Averages bearing common or similar letters did not differ significantly

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

Treatments	Means± S.E			
	Tg (mg/dl)	LDL (mg/dl)	HDL(mg/dl)	Cholesterol(mg /dl)
Control D.W	90.83± 0.30 D	12.40± 0.09 C	40.66± 0.61 A	71.50± 0.56 C
1 Sodium Nitrate 150 mg/kg	211.66± 4.66 A	17.70± 0.25 A	23.16± 0.30 D	77.16± 0.47 A
2 FSE 200 mg/kg group	91.83± 0.70 D	10.10± 0.10 D	40.83± 0.47 A	68.83± 0.30 D
3 FSE 300 mg/kg group	90.83± 0.16 D	7.21± 0.06 E	41.33± 0.49 A	65.33± 0.33 E
4 FSE 200 mg/kg + Sodium Nitrate	135.00± 1.36 B	15.36± 0.29 B	27.66± 0.42 C	76.50± 0.61 A
5 FSE 300 mg/kg + Sodium Nitrate	122.50± 1.20 C	15.01± 0.20 B	33.33± 0.42 B	73.50± 0.34 B
LSD	5.9788	0.5452	1.3444	1.3183
P (VALUE)	0.050	0.050	0.050	0.050

Table 2 statistical analysis the data were analyzed using SAS software and results have been compared by using LSD or Duncan's test at a probability level of 0.05. • This note is written at the bottom of each table when choosing the Dunkin test Averages bearing common or similar letters did not differ significantly.

Effect of FSE and sodium nitrate on lipid profile

Table No. (2) had shown a significant increase in the level of the HDL and in the FSE 200 & 300 mg/kg group, but no significant difference was recorded between their concentrations in the FSE 200 and FSE 300 groups, but significant differences were recorded in FSE 200 & 300 mg/kg + Sodium Nitrate compared with the nitrate group Table (2) also showed a significant decrease in LDL level in FSE 200 & 300mg/kg group in comparison to the control group, and a significant decrease in (FSE 200 & 300 mg/kg + Sodium Nitrate) groups when compared with the nitrate group

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

Table No. (2) recorded a significant decrease in the TG group in the (FSE 200 & 300 mg/kg) group, and a significant decrease in the two groups (FSE 200 & 300 mg/kg + Sodium Nitrate) compared to the nitrate group. The results are also recorded in Table No.(2) A significant decrease in the concentration of HDL and a significant increase in the concentration of LDL and TG compared to the control group, and a significant increase in the level of cholesterol in the nitrate group and a significant decrease in the FSE 200 & 300 mg/kg group) and a significant increase in the FSE 200 & 300 mg group \kg +Sodium Nitrate) in comparison with the nitrate group

The effect of FSE and sodium nitrate on testicular tissue

. Image 2 shows a cross-section of testicular tissue for a group treated with sodium nitrate, in which it is noted that there are interfacial spaces between the seminiferous tubules, lack of Leydig cells, lack of sperm, an increase in the diameter of the medial lumen, necrosis of the cells lining the tubules, a decrease in the size of the germ cell layer, and destruction tubules;

As for the cross-section in image(3) &(4) of the testis tissue in the group treated with aqueous extract of flaxseeds with a concentration of FSE 200 & 300mg/kg) of body weight, the normal tissue of the testis and seminiferous tubules are noted and filled with sperm, with the presence of Leydig cells in the interstitial tissue and a lack of diameter tubule cavity While image No. (5) showed a cross-section of the testicular tissue in the group treated with aqueous extract of flaxseeds at a concentration of (FSE 200 mg/kg + Sodium Nitrate) a concentration of body weight, in which it is noted that there are few interstitial spaces between the seminiferous tubules, the irregularity of some tubules, and the low number of sperms In it with the presence of fibrous cells in the interstitial tissue and an increase in the diameter of the middle cavity, while Image (6) showed a cross-section of testicular tissue in the FSE 300 mg/kg + Sodium Nitrate (150 mg / kg) group, showing a clear improvement in the seminiferous tubules, an increase in the number of sperms(black star), with the presence of Leydig cells in the interstitial tissue and a decrease in the diameter of the medial lumen.

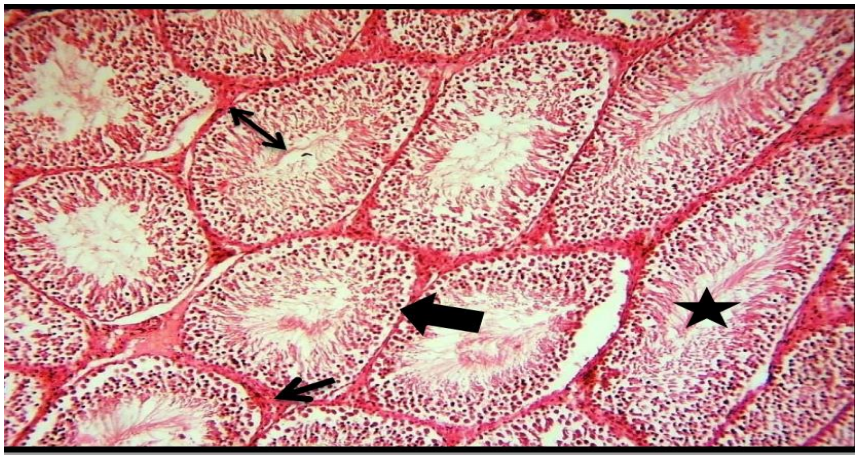

Image (1) of a transverse section of the testicular tissue of the control group, in which the normal tissue of the testis with the seminiferous tubules (thick black arrow) with lydig cells (thin black arrow) and

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

the presence of the middle cavity with sperm (black star) and the layer of epithelial germ cells(double side arrow) (100X) H & E

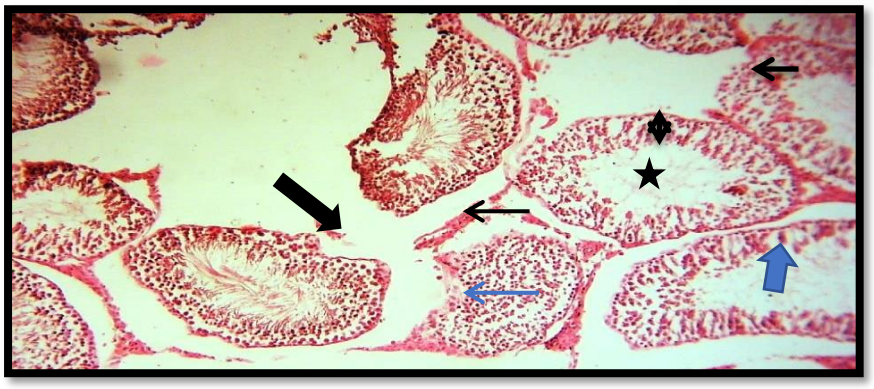

Image (2) of a transverse section of the testicular tissue of the group treated with sodium nitrate, in which it is noted that there are interfacial spaces between the seminiferous tubules (thick black arrow), decreased in lydig cells(thin black arrow), increased medial cavity diameter (black star), and necrosis of the lining cells tubules (thick blue arrow) and a decrease in the size of the germ cell layer (double side arrow) and the breakdown of the tubules (thin blue arrow) 100X E & H

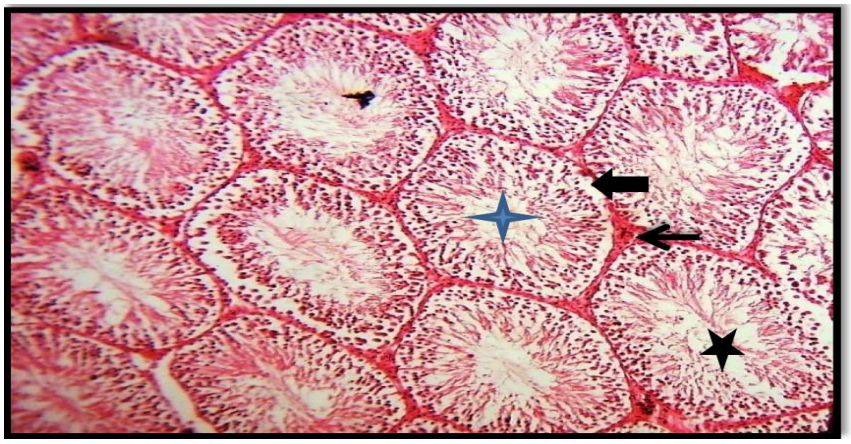

image (3) cross section of testicular tissue in the group treated with aqueous extract of flaxseeds at a concentration of (200 mg / kg (of body weight) in which the normal tissue of the testis and seminiferous tubules (thick black arrow) is observed and filled with sperm (black stars) with the presence of Leydig cells in the interstitial tissue (thin black arrow) and the decrease in the diameter of the tubule lumen (blue star) (100X E & H).

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

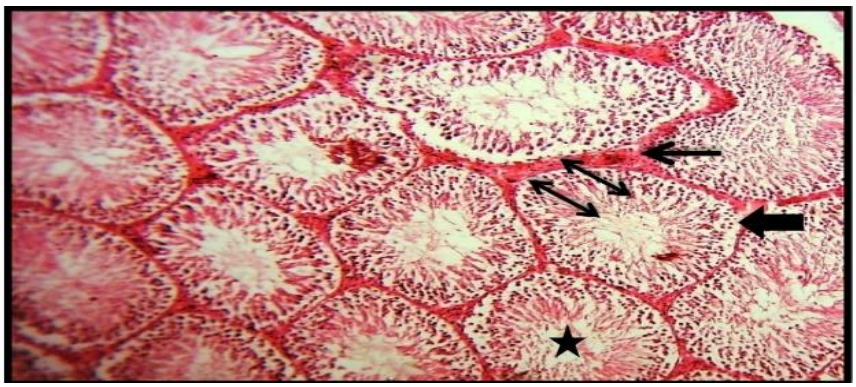

Image (4) of atransverse section of the testis tissue in the group treated with aqueous extract of flax seeds at a concentration of (300 mg / kg) of body weight, in which the normal structure of the testis and seminiferous tubules (thick black arrow) and the increase in the number of sperms (black star) with the presence of Lydig cells in the interstitial tissue (thin black arrow) And an increase in the number of layers of germ cells forming sperm (double side arrow) (E & H) 100

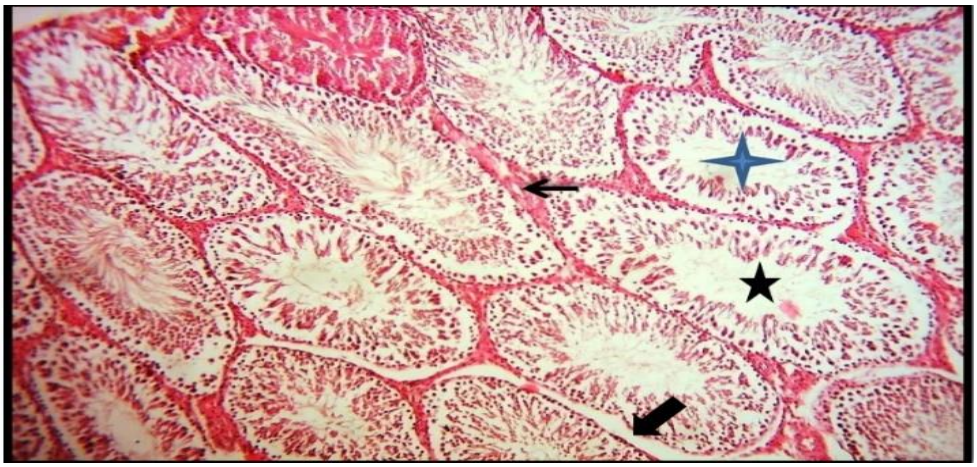

image (5) cross section of testicular tissue in the group treated with aqueous extract of flaxseeds at a concentration of (200 mg / kg) with sodium nitrate at a concentration of (150 mg / kg) of body weight, it is noted that there are few interstitial spaces between the seminiferous tubules (thick black arrow) Irregularity of some tubules and a decrease in the number of sperms in them (black star) with the presence of Lydig cells in the interstitial tissue (thin black arrow) and an increase in the diameter of The middle lumen (blue star)(H & E 100X)

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

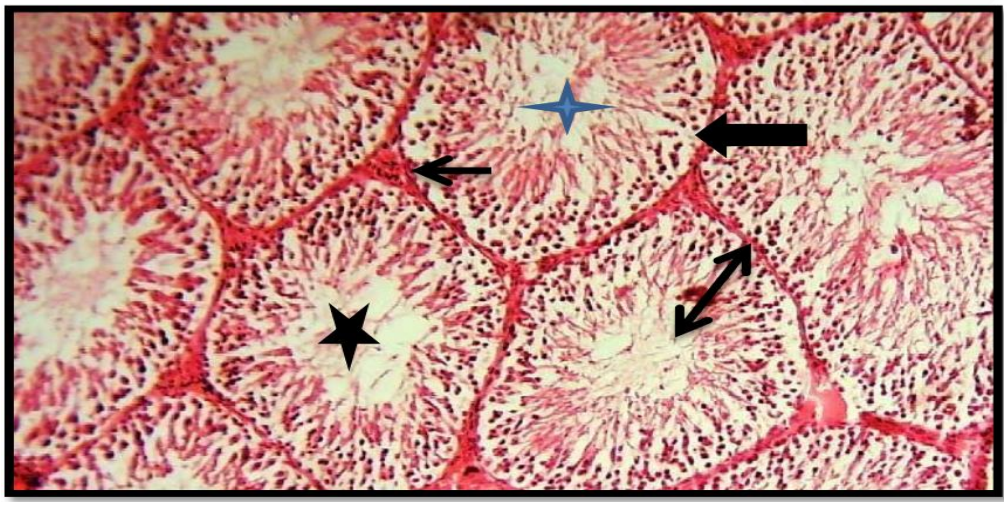

Image (6) showed a cross-section of testicular tissue in the FSE 300 mg/kg + Sodium Nitrate (150 mg / kg) group, showing a clear improvement in the seminiferous tubules(thick black arrow), an increase in the number of sperms(black star), with the presence of Leydig cells in the interstitial tissue((thin black arrow), a decrease in the diameter of the medial lumen(blue star), and an increase in the number of cell layers. germ-forming (double side arrow))(H & E 100X)

Discussion

The decrease in the levels of reproductive hormones in the serum can most likely be explained by the oxidative stress caused by exposure to sodium nitrate, which causes damage to the testicular tissue with degeneration of the germ cells, in addition to the decrease in the level of FSH and LH, due to lipid peroxidation, which leads to a decrease in the production and secretion of GnRH from under the skin. The hypothalamus leads to the failure of the pituitary gland to produce and secrete both (FSH) and (LH), and thus Leydig cells fail to manufacture testosterone, and Sertoli cells are also unable to function normally [20,21].

Or it may be due to atrophy of Leydig cells due to oxidative stress and increased free radicals in the testicle. [22], which leads to testicular dysfunction. The reason for the decrease in these hormones may be due to the increase in the active oxygen species (ROS), which coincides with a decrease in antioxidants and thus leads to oxidation of proteins and fats in cell membranes. The body, including the pituitary gland [23,24]. Plant effect The current results indicated that natural products that contain antioxidants have the ability to protect testes from oxidative damage and lipid peroxidation caused by exposure to sodium nitrate. Testosterone hormone plays an important role in the formation of sperm and therefore can be used as herbs that enhance sexual activity, fertility, vitality, movement and number of sperm. [25,26,27].

The high levels of FSH, LH, and testosterone in the groups that were dosed only with seeds is attributed to the presence of cytokines and steroids present in flax that protect all tissues from damage and enable them to perform their functions normally and thus secrete hormones that enhance fertility and give as

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

a treatment for impotence. Testosterone is one of the most important synthetic androgens [25]. And secreted from interstitial cells, also known as Leydig cells, and these cells are located under the influence and control of the hormone testosterone in showing secondary sexual characteristics, as well as in the activity and development of the reproductive system, which has a crucial role in stimulating and regulating the process of spermatogenesis and sperm metaplasia (spermiogenesis). [13,26].

As for the decrease in the concentration of HDL and the increase in the level of LDL, cholesterol, and triglycerides in the sodium nitrate group, it was in agreement with [28]. The reason for these significant differences may be due directly or indirectly to the effect of nitrates on lipid metabolism or lipid peroxidation, and the reason for the increase in HDL and the decrease in the level of LDL, cholesterol, and triglycerides in the flaxseed groups agrees with [29]. The reason for the improvement of lipids in flaxseeds was attributed to the presence of high levels of Alpha lipoic acid (ALA), lignans, polyunsaturated fatty acids and fiber in flaxseeds.Lipid levels were improved by increasing the dose of flaxseed in the diet [30,31,32].

Our results agree with [33] and his group (2010), who observed an improvement in the level of lipids when they were fed a diet with a concentration of 25% of dry flaxseeds for a period of 21 days. It also agrees with what was indicated by the study of [34] in reducing total cholesterol and triglycerides, by reducing lipid peroxidation and producing antioxidant activity [35].

As for the cause of shedding of the layers of cells that make up the sperm cells and congestion in the interstitial tissue, it may be attributed to the increase in the level of lipid peroxidation and the decrease in the activity of antioxidants after the administration of sodium nitrate, which leads to damage to the testicular tissue The testes shown in Picture No. (7-7) of the group dosed with sodium nitrate, and this is consistent with x and cause a change in sperm production and maturation [36,37].

The results of the histological examination of the two groups of rats that were given aqueous flaxseed extract at a dose of 200 and 300 mg/cvm for 30 days showed normal testicular tissue with high sperm density in the seminiferous tubules and an abundance of Leydig cells in the interstitial tissue, as well as an increase in the number of constituent germ cell layers. For sperm, as shown by the current results of the histological examination of the protective groups that were dosed with flaxseed extract at a concentration of 200 mg / kg with sodium nitrate at a concentration of 150 mg / kg. Picture (5) Positive effects of the extract against the toxicity of the preservative preservative, where an improvement in the degenarative layer of cells that make up the sperm is noted with no irregularity in some tubules and an improvement in the number of sperm and Leydig cells appear in numbers less than normal, and we show the picture (6) the preventive dose that was dosed with flaxseed extract at a concentration of 300 mg / kg with sodium nitrate at a concentration of 150 mg / kg, the return of the tissue to its almost normal shape, with filling of the cavities with appendages, a clear improvement of the twisted tubules, an increase in the number of germ cell layers, and an increase in their effectiveness, this is a result of its active photochemical compounds that are present in the extract as phytoestrogen. and the results of our current study are consistent[23,38].

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

Conclusion

This study showed that the aqueous extract of Linium usitatissimum (flaxseed) has a protective effect against Sodium Nitrate-induced liver damage The hepatoprotective effect of the extract may be By increasing antioxidant defense mechanisms.

References

1. Shakir, K. A. F. and Madhusudhan, B. (2007). Hypocholesterolemic and hepatoprotective effects of flaxseed chutney: Evidence from animal studies. Indian Journal of Clinical Biochemistry, 22(1): 117.
2. Hall, Louise & Johnson, Judith & Watt, Ian & Tsipa, Anastasia & O'Connor, Daryl. (2016). Healthcare Staff Wellbeing, Burnout, and Patient Safety: A Systematic Review. PloS one. 11. e0159015. 10.1371/journal.pone.0159015.
3. Nguyen, J. Y.; Rock, C.; Gray, V.; Claver, M. and Costa, C. (2018). Product Development Considerations of Flaxseed (Linum Usitatissimum) Supplementation for the Aging Population: A Pilot Study. California State University, Long Beach.
4. , Abdel-Moneim, A. E., Dkhil, M. A., & Al-Quraishy, S. (2010). Protective role of flaxseed oil against lead acetate induced oxidative stress in testes of adult rats. African Journal of Biotechnology , 9(42), 7216-7223.
5. , Saleem, M. H., Kamran, M., Zhou, Y., Parveen, A., Rehman, M., Ahmar, S., ... & Liu, L. (2020). Appraising growth, oxidative stress and copper phytoextraction potential of flax (Linum usitatissimum L.) grown in soil differentially spiked with copper. Journal of environmental management, 257, 109994.
6. El-Sayed, H. H.; Darwish, A. H.; Ysein, E. M. and Zehairy, G. D. (2014). Biochemical and biological study on the effect of flaxseed on rats Suffer from nephropathy. Journal of Environmental Science, Toxicology and Food Technology, 8,: 59–66.
7. Anand, S. P., & Sati, N. (2013). Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. International Journal of Pharmaceutical Sciences and Research, 4(7), 2496.
8. Sun, B., & Wang, J. (2017). Food additives. Food Safety in China: Science, Technology, Management and Regulation, 186-200.
9. Nujić*,M; Habuda-Stanić ,M.(2017) .Nitrates And Nitrites, Metabolism And Toxicity. Food in Health and Disease, scientific-professional journal of nutrition and dietetics (2017) 6 (2) 48-89 .
10. Hernández-Salazar, M.; Guevara-González, R. G.; Cruz-Hernández, A.; Guevara-Olvera, L.; Bello-Pérez, L. A.; Castaño-Tostado, E. and Loarca-Piña, G. (2013). Flaxseed (Linum usitatissimum L.) and its total non-digestible fraction influence the expression of genes involved in azoxymethane-induced colon cancer in rats. Plant Foods for Human Nutrition, 68(3): 259–267
11. Tietz, N. W. (1995). Clinical guide to laboratory tests. 3rd ed. Philadelphia, WB Saunders Co.
12. Kosasa, T. S. 1981. "Measurement of Human Luteinizing Hormone." Journal of Reproductive Medicine 26:201–6

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

13. Simoni, Manuela, Jörg Gromoll, and Eberhard Nieschlag. 1997. "The FollicleStimulating Hormone Receptor: Biochemistry, Molecular Biology, Physiology, and Pathophysiology." Endocrine Reviews 18(6):739–73.
14. Allain.(1974).Measurement of cholesterol.Clin.Chem. 20: 470-475
15. Fassati, P. and Principe ,L. (1982). Measurement of Triglyceride.Clin. Chem. 28(20):77-80
16. Burstein, M. J. (1970). Measurement of HDL. Lipid Res. , 11:583
17. Friedewald Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry, 18(6), 499-502
18. Suvarna, K. S., Layton, C., & Bancroft, J. D. (2013). Bancroft's Theory and Practice of Histological Techniques .7th ed. Elsevier Health Sciences.
19. SAS 2012. Statistical Analysis System, User,s Guide. Statistical. Version 9.1th ed. SAS. Institute Incorporated Cary. N.C. USA
20. Alyoussef, A., & Al-Gayyar, M. M. H. (2016). Thymoquinone ameliorates testicular tissue inflammation induced by chronic administration of oral sodium nitrite. Andrologia, 48(5), 501-508.
21. Noureen F., Khan M.R., Shah N.A., Khan R.A., Naz K. and Sattar S. (2017) . Pistacia chinensis: Strong antioxidant and potent testicular toxicity amelioration agent. Asian Pacific J. Trop Med., 10 (4): 380–89.
22. Momeni HR, Oryan S, Eskandari N (2012). Effect of vitamin E on sperm number and testis histopathology of sodium arsenite-treated rats. Reprod. Biol. 12(121): 171–181
23. Al-Harbi, N. Y., Hadi, M. A., & Abbas, F. A. (2017). Effect of Flaxseeds (Linum usitatissimum L.) Extract in Male Reproductive System of Albino Rat Treated with Cyproterone Acetate. Journal of Global Pharma Technology, 9(8-9), 2017.
24. Hoesla C. E., Saadb F., Po¨ppela M., Altweina J. E. (2005). Reversible, NonBarrier Male Contraception: Status and Prospects. European Urology 48: 712–723
25. Abbaspour, B., Sharifi, S. D., Ghazanfari, S., Honarbakhsh, S., & Mohammadi-Sangcheshmeh, A. (2019). The effect of l-arginine and flaxseed on plasma testosterone concentration, semen quality and some testicular histology parameters in old broiler breeder roosters. Theriogenology, 128, 101-109.
26. Romero, D. C. M., Barbosa, L. P., Machado, W. M., da França, C. S., Vieira, R. L. A., Mendes, C. S., ... & Souza, R. S. (2020). Evaluation of the seminal quality of rabbits fed diets containing different inclusion levels of flaxseed (Linum usitatissimum). Ciencia y Tecnología Agropecuaria.
27. Simoni, Manuela, Jörg Gromoll, and Eberhard Nieschlag. 1997. "The FollicleStimulating Hormone Receptor: Biochemistry, Molecular Biology, Physiology, and Pathophysiology." Endocrine Reviews 18(6):739–73.
28. Helal, E. G., Almutairi, A., Abdelaziz, M. A., & Mohamed, A. A. (2020). Adverse Effects of Fast Green, Sodium Nitrate and Glycine on Some Physiological Parameters. The Egyptian Journal of Hospital Medicine, 80(3), 964-970.

ISSN: 2776-1010 Volume 4, Issue 8, August 2023

29. Thakur, G.; Mitra, A.; Pal, K. and Rousseau, D. (2009). Effect of flaxseed gum on reduction of blood glucose and cholesterol in type 2 diabetic patients. International Journal of Food Sciences and Nutrition, 60(sup6): 126–136.
30. Riediger, N. D.; Othman, R.; Fitz, E.; Pierce, G. N.; Suh, M. and Moghadasian, M. H. (2008). Low n-6: n-3 fatty acid ratio, with fishor flaxseed oil, in a high fat diet improves plasma lipids and beneficially alters tissue fatty acid composition in mice. European Journal of Nutrition, 47(3): 153–160.
31. Cho, Y.-Y.; Kwon, E.-Y., Kim, H.-J.; Park, Y.-B.; Lee, K.-T.; Park, T. and Choi, M.-S. (2009). Low trans structured fat from flaxseed oil improves plasma and hepatic lipid metabolism in apo E−/− mice. Food and Chemical Toxicology, 47(7): 1550–1555.
32. Johnsson, P., Peerlkamp, N., Kamal-Eldin, A., Andersson, R. E., Andersson, R., Lundgren, L. N., & Åman, P. (2002). Polymeric fractions containing phenol glucosides in flaxseed. Food Chemistry, 76(2), 207-212.).
33. Daleprane, J. B.; Batista, A.; Pacheco, J. T.; Da Silva, A. F. E.; Costa, C. A.; de Castro Resende, Â. and Boaventura, G. T. (2010). Dietary flaxseed supplementation improves endothelial function in المصادرReference 84 the mesenteric arterial bed. Food Research International, 43(8): 2052–2056.
34. Khalesi, S.; Jamaluddin, R. and Ismail, A. (2011). Effect of Raw and Heated Flaxseed (Linum Usitatissimum L.) on Blood Lipid Profiles in Rats. International Journal of Applied Science and Technology, 1(4): 84–89 .
35. Wahba, H. M. A. and Ibrahim, T. A. A. (2013). Original Research Article Protective effect of flaxseed oil and vitamin E on potassium Bromate-induced oxidative stress in male rats. 2(9): 299–309
36. Aly, H. A., Mansour, A. M., Abo-Salem, O. M., Abd-Ellah, H. F., & Abdel-Naim, A. B. (2010). Potential testicular toxicity of sodium nitrate in adult rats. Food and chemical toxicology, 48(2), 572-578.
37. Oghbaei, H., Hamidian, G., Alipour, M. R., Alipour, S., & Keyhanmanesh, R. (2020). The effect of prolonged dietary sodium nitrate treatment on the hypothalamus-pituitary-gonadal axis and testicular structure and function in streptozotocin-induced diabetic male rats. Food & function, 11(3), 2451-2465
38. Haran, W., AL-Saeed, M. H., and AL-Masoudi, E. A. (2017). Study the effect of flax lignan extract of linum usitatissimum and conjugated estrogen on physiological parameters female rats. Bas. J. Vet. Res, 16(1): 2707.