The protective effect of zinc

and its relationship with some hematological, biochemical, and histological parameters in adult male rats

Efecto protector del zinc y su relación con algunos parámetros hematológicos, bioquímicos e histológicos en ratas macho adultas

Ayoub Ali Hussein Al-Bayti Department of Medical Laboratory, College of Health and Medical Techniques-Kirkuk, Northern Technical University, Iraq. Email: ayoub.ali77@ntu.edu.iq
Fidan Fikrat Ahmed Department of Medical Laboratory, College of Health and Medical Techniques- Kirkuk, Northern Technical University, Iraq. Email: fidanfikrat1972@ntu.edu.iq
Sami Ibrahim Abdullah Department of Anatomy, College of Veterinary Medicine, University of Kirkuk, Iraq. Email: sami_ibrahim@uokirkuk.edu.iq
Ozdan Akram Ghareeb Department of Community Health Techniques, Kirkuk Technical Institute, Northern Technical University, Iraq. Email: ozdanakram@ntu.edu.iq
Kasim Sakran Abass Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Kirkuk, Kirkuk, Iraq. Email: Kasim_abass@uokirkuk.edu.iq
Received: 02/26/2021 Accepted: 05/15/2022 Published: 06/25/2022 DOI: http://doi.org/10.5281/zenodo.7221850

Abstract

The trace element zinc is essential for the construction and function of cellular proteins. The current study aimed to estimate the improved cause of zinc on the changes resulting from high concentrations of cholesterol. In this experiment, thirty male rats aged 3-4 months were randomly divided into three groups of ten rats each. Control group: the animals did not receive any treatment, cholesterol group: the animals received cholesterol at a concentration of 400 mg/kg mixed with diet for 4 weeks, while in the zinc group: rats received cholesterol at a concentration of 400 mg/kg for 4 weeks, then zinc was given 20 mg/kg for another 4 weeks. The study period was 56 days, after its completion, hematological and biochemical parameters were studied, in addition to the histological examination of the liver. The results showed that cholesterol treatment alone led to significant negative changes in hematological, biochemical and histological indices. Whereas zinc administration to experimental rats resulted in clear positive effects in those parameters. We concluded that the cholesterol-damaged rats improved after receiving zinc.

Keywords: Zinc, cholesterol, hematological parameters, liver.

Resumen

El oligoelemento zinc es esencial para la construcción y función de las proteínas celulares. El estudio actual tuvo como objetivo estimar la causa mejorada de zinc en los cambios resultantes de altas concentraciones de colesterol. En este experimento, treinta ratas macho de 3 a 4 meses de edad se dividieron aleatoriamente en tres grupos de diez ratas cada uno. Grupo control: los animales no recibieron ningún tratamiento, grupo colesterol: los animales recibieron colesterol en una concentración de 400 mg/kg mezclado con la dieta durante 4 semanas, mientras que en el grupo zinc: las ratas recibieron colesterol en una concentración de 400 mg/kg durante 4 semanas, luego se le administró zinc a 20 mg/kg durante otras 4 semanas. El periodo de estudio fue de 56 días, luego de su realización se estudiaron parámetros hematológicos y bioquímicos, además del examen histológico del hígado. Los resultados mostraron que el tratamiento del colesterol por sí solo condujo a cambios negativos significativos en los índices hematológicos, bioquímicos e histológicos. Mientras que la administración de zinc a ratas experimentales resultó en claros efectos positivos en esos parámetros. Llegamos a la conclusión de que las ratas dañadas por el colesterol mejoraron después de recibir zinc.

Palabras clave: Zinc, colesterol, parámetros hematológicos, hígado.

Introduction

Cholesterol can be defined as amphipathic lipids and thus can be considered an essential structural component of the outer layer of plasma lipoproteins[1]. It is found in tissues and in plasma combined with fatty acids along the chain as cholesteryl ester[2,3]. Cholesterol hemostasis is pivotal for appropriate cellular function and disorders of cholesterol level underlie a growing number of diseases such as heart diseases and cancers[4,5]. Recently, the consumption of unhealthy foods has increased, especially those related to the intake of fat, both in quality and quantity[6]. Numerous studies conducted on laboratory animals as well as humans have demonstrated that high intake of cholesterol and saturated fat causes an increase in oxidative stress effort, which determines a disturbance in the stability between antioxidant defenses and free oxygen radicals in tissues[7-10]. The ability of free radicals to cause damage to tissues and living cells increases in the absence of defense mechanisms against them, which are known as antioxidants[11,12]. Previous studies have also demonstrated the effect of supplemental antioxidants such as zinc in removing fat damage and oxidative stress[13]. The current experiment was designed to study the effect of high cholesterol level on many hematological and biochemical parameters in addition to the histological changes of the liver. In addition to evaluating the improved effect of zinc on those changes in male laboratory rats.

Materials and Method

Chemicals

Cholesterol product No. C8667, molecular formula: $C_{27}H_{46}O$, molecular weight: 386.7, manufactured by Sigma-Aldrich (USA), was used. As for zinc, high strength Holland & Barrett zinc tablets (UK) were consumed.

Rats and experiment design

Thirty adult male rats aged 3-4 months and weighing between 400 and 300 g were used in this research. They were housed in cages within a designated room with the appropriate conditions for animal husbandry, including food, temperature, lighting and ventilation. The animals were randomly distributed into 3 groups, each group containing 10 rats. Animals were conditioned for one week, and then treated with experiment-specific treatments for 56 days. The initial weights of the rats were taken, and then they were dosed with the diet as follows:

- Control group: Rats fed a normal diet.
- Cholesterol group: Rats were dosed with cholesterol mixed with a normal diet at a concentration of 400 mg/kg.
- Zinc group: They were fed a high-cholesterol diet of 400 mg/kg for 4 weeks, and then given zinc 20 mg/kg for an additional 4 weeks.

After the experiment ended the animals were fasted for 24 hours, the animals were sacrificed and 2-5 ml of blood was collected through cardiac puncture and placed in heparin-filled tubes for blood tests, the rest were placed in anticoagulant-free test tubes and left for 15 minutes in water baths at 37 ºC. The serum was then centrifuged at 3000 rpm and stored at -20°C in flat plastic tubes until special biochemical tests were performed. Then the liver was extracted and a part of it was placed in the prepared formalin solution at a concentration of 10% until it was treated histologically. The parameters that were evaluated included the following: complete blood picture, serum biochemical parameters such as triglycerides, HDL (high density lipoprotein) and LDL (low density lipoprotein).

Histological study

All slides be prepared using the procedure described by Carleton et al[14], in which liver tissue samples were collected, and the following steps were performed on tissue: processing, beginning with fixation, washing, dehydration, clearing, infiltrating, embedding, and finally trimming and sectioning. Then, tissues were stained with hematoxylin and eosin dye. Next, the slides were examined under an optical microscope paired with a German-made MOTIC digital camera, which was used for tissue imaging.

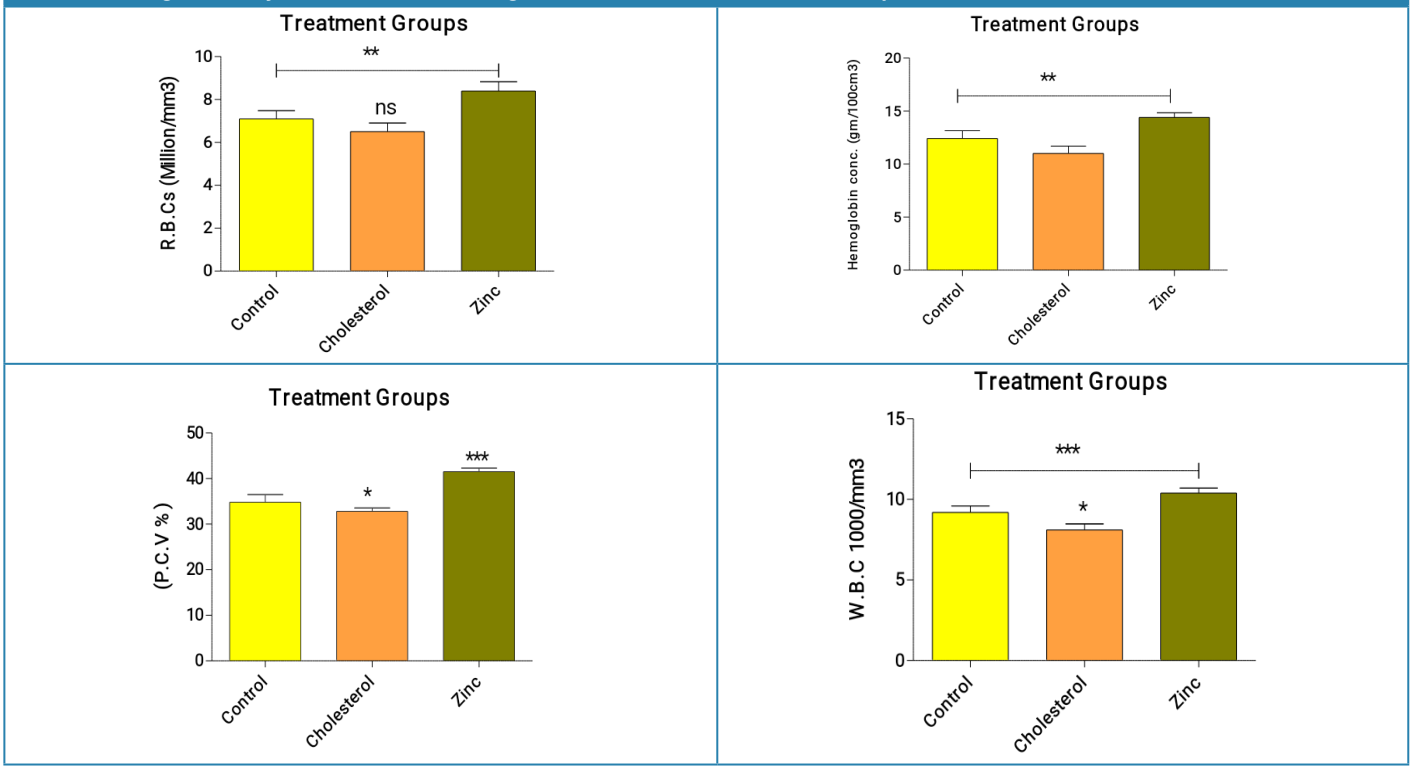
Statistical analysis

Graphs were performed in Prism v5.Ink software was used for data analysis. All data were expressed as mean ±standard deviation and one-way analysis of variance ANOVA followed by Duncan's test to find out the differences among the study groups with a significant difference at P. ≤ 0.05.

Results and discussion

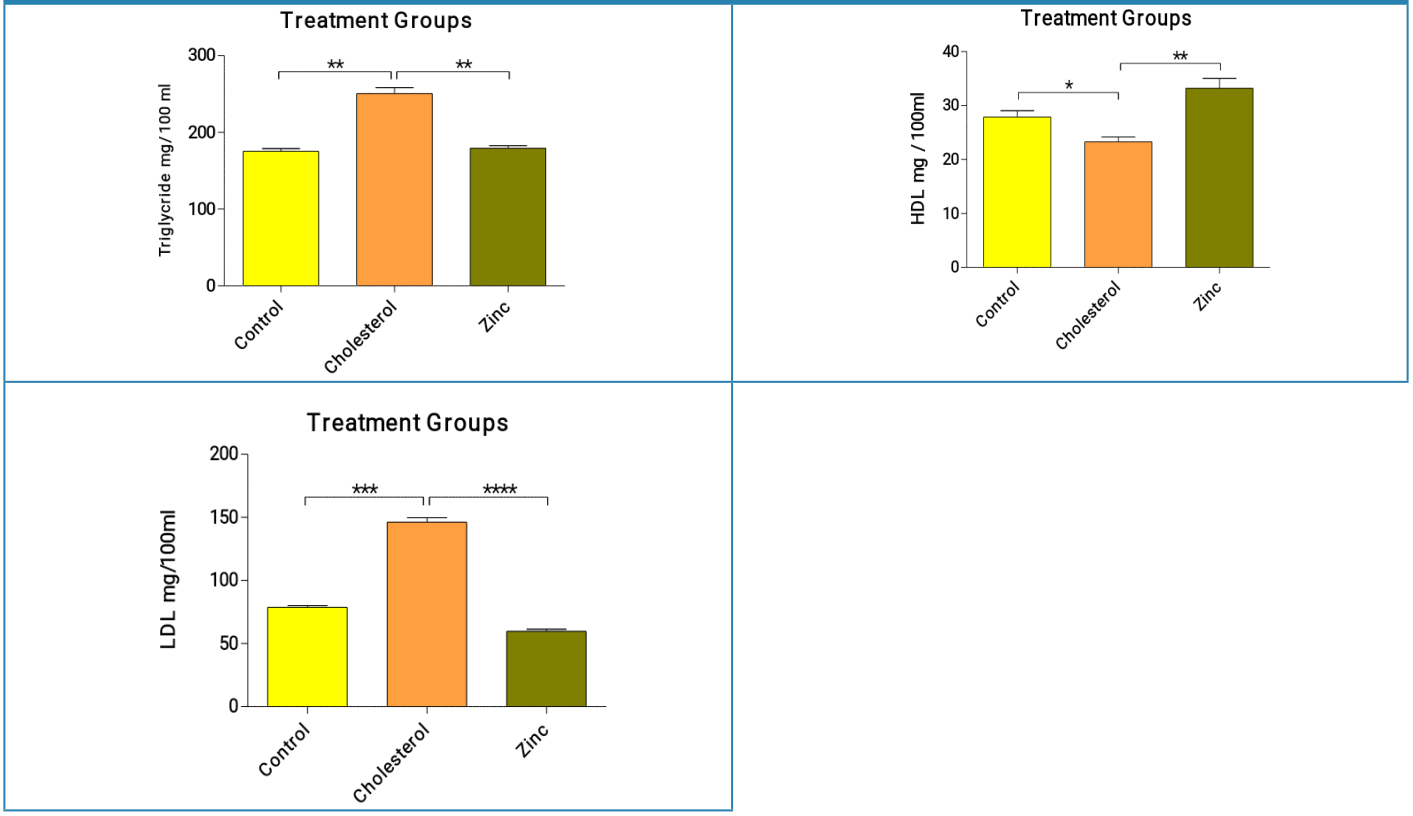
The results showed that treating rats with cholesterol led to a significant decrease at P≤0.05 in the count of W.B.C compared to the control rats, accompanied by a decrease in red blood cells and hemoglobin concentration, but not significantly. After they were dosed with zinc, a noticeable increase in these blood parameters was observed. As for the volume of packaged cells in cholesterol-treated rats, it was significantly lower compared to the control group, but after being treated with zinc, it increased clearly as shown in figure 1.

Figure 1: Effect of cholesterol and zinc on: a) RBCs b) HB c) P.C.V d) WBCs in blood of laboratory animals. The data were presented as mean ± SD. High small symbols*,,*** indicate significant intra-column differences at p < 0.05.**



Blood cells exposed to oxidative stress can sometimes undergo hemolysis, which caused deposits of Heinz bodies to form within the red blood cells that provoked their degradation and their number in the bloodstream[15,16]. This may explain what was done to rats treated with high doses of cholesterol. Also, a decrease in blood components may occur when their loss exceeds their production[17,18]. Zinc treatment improved negative changes in blood indicators, and the reason for this is due to its antioxidant properties, as it contains a group of elements that work to prevent the formation of free radicals, which enters the formation of superoxide dismutase, thus preventing oxidative stress[19].

Figure 2: Effect of cholesterol and zinc on: a) triglycerides b) HDL c) LDL in serum of laboratory animals. The data were presented as mean ± SD. High small symbols*,,*** indicate significant intra-column differences at p < 0.05.**



On the other hand, there was a clear trend to increase the concentration of triglycerides and LDL in the serum of cholesterol-dose rats compared to control rats. As for zinc-treated rats, a considerable decrease in their levels was demonstrated. On the contrary, a substantial drop in HDL concentration was detected when comparing the cholesterol-consuming group of animals to the control group. However, after receiving zinc, its level increased significantly, as shown in figure 2. This may be due to several explanations, including that oxidative stress leads to a decrease in the activity of the enzyme lipoprotein lipase responsible for collapse triglycerides or that active oxygen species leads to the inhibition of the enzyme triglyceride lipase accountable for breaking down triglycerides, leading to an increase in metabolism of dietary fats and increase their levels in the blood[20,21].In general, LDL is a prime carrier of cholesterol from the liver and into the tissues, it has elevated cholesterol content, and the observed increase in LDL level can be due to the increased malondialdehyde (MDA) level. Whereas MDA oxidizes low-density lipoproteins and diverts them to an oxidized form[22]. Low HDL cholesterol may be caused by liver damage, or by high levels of cholesterol, triglycerides, and LDL cholesterol, because HDL's function is to reverse cholesterol transfer from tissues to the liver. If concentrations of cholesterol and triglycerides increase in tissues and blood vessels, this presents an obstacle to reducing HDL's efficiency of cholesterol transport[23,24]. Our results in this study are consistent with several previous studies that showed zinc supplementation reduces LDL and triglycerides, as well as increases HDL levels[25, 26].

Figure 3: Liver section from the control group showing normal hepatic lobule consisting of normal hepatocytes, sinusoids and central vein (yellow arrows) (H&E, Scale Bar= 100µm).

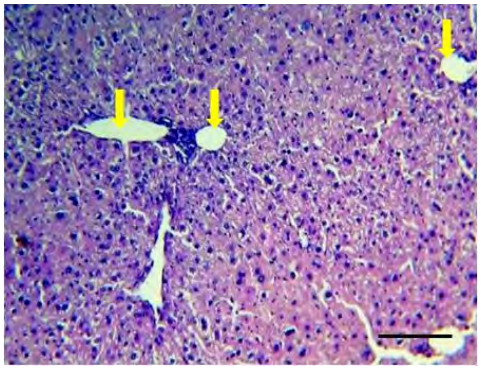


Figure 4: Liver section from the cholesterol group infiltration of mononuclear inflammatory cells mainly lymphocytes (yellow arrows) (H&E, Scale Bar= 50µm).

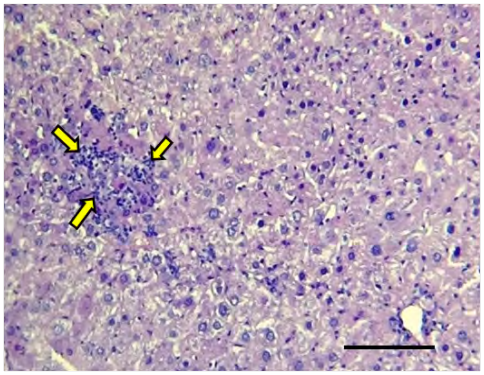


Figure 5: Histopathological examination of liver tissue showing an increase in the size of the hepatocytes showed fatty degeneration (yellow arrows) associated with narrowing of the sinusoids and containing many large fat vacuoles (red arrows) (H&E, Scale Bar= 25µm).

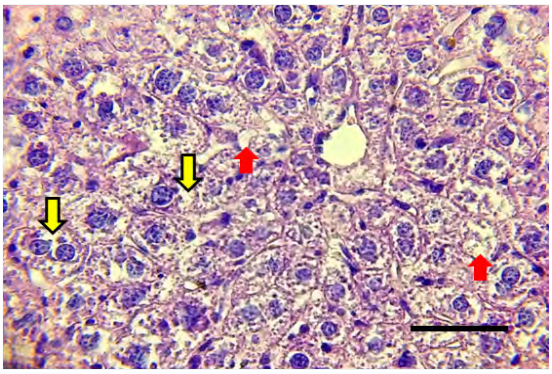


Figure 6: Liver section from the cholesterol group showing blood vessel congestion (yellow arrows), severe narrowing of sinusoids and marked nuclear pyknosis (red arrows), with pallor cytoplasm due to cytoplasmic vacuolation (blue arrows) (H&E, Scale Bar= 20µm).

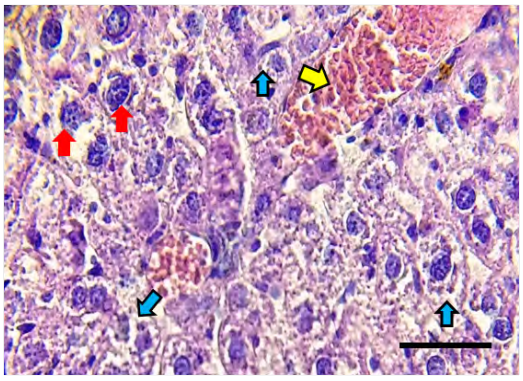


Figure 7: Liver section from the cholesterol group showing marked hepatocellular necrosis (yellow arrows) as indicated by the karyolysis (H&E, Scale Bar= 25µm).

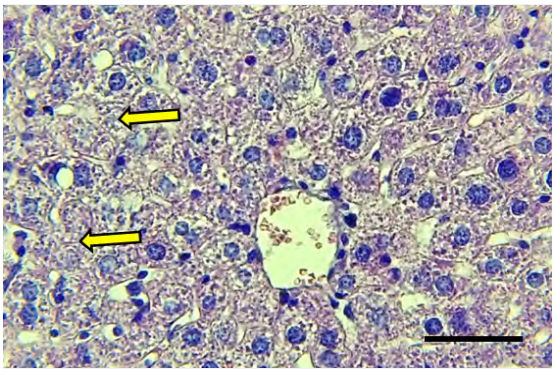
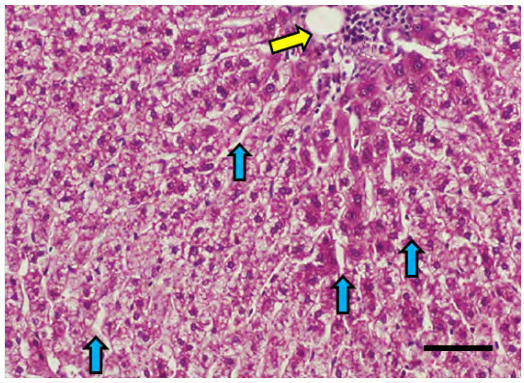


Figure 8: Liver section of the zinc-treated group showing few normal distributions of hepatocytes, central vein (yellow arrow) and the blood sinusoids (blue arrows) (H&E, Scale Bar= 50µm).



Regarding histological findings, a normal hepatic lobule consisting of normal hepatocytes, sinusoids and central vein was seen in the hepatic tissue of control rats (figure 3). As for the animals treated with cholesterol, they showed infiltration of inflammatory lymphocytes (figure 4), and an increase in the size of fatty degenerative liver cells (figure 5), in addition to seeing vascular congestion accompanied by a cytoplasmic vacuolation (figure 6). In addition, clear necrosis of hepatocytes (karyolysis) was also seen (figure 7). Whereas, zinc administration to rats had an obvious improvement effect in liver tissues, which seemed almost normal (figure 7).The liver has a high detoxification capacity, which is required for the metabolism and elimination of toxic chemicals, and exposure to toxic substances can cause harmful tissue changes[27,28]. Zinc treatment increases the activity of enzymatic antioxidants such as SOD, CAT, and A, as well as raising the level of effectiveness of non-enzymatic antioxidants such as GSH and MT and thus reduce oxidative stress on liver tissue[29,30].

Conclusions

Dosing cholesterol with high concentrations had a clear harmful effect on blood and liver tissues, as indicated by hematological, biochemical and histopathological indicators. However, upon post-treatment with zinc supplements, most of these adverse changes were evidently improved.

References

1 Luo J, Yang H, Song BL. Mechanisms and regulation of cholesterol homeostasis. Nature reviews Molecular cell biology. 2020 Apr;21(4):225-45.

2 Wang X, Wei L, Xiao J, Shan K, He Q, Huang F, Ge X, Gao X, Feng N, Chen YQ. Cholesterol and saturated fatty acids synergistically promote the malignant progression of prostate cancer. Neoplasia. 2022 Feb 1;24(2):86-97.

3 Wang X, Wei L, Xiao J, Shan K, He Q, Huang F, Ge X, Gao X, Feng N, Chen YQ. Cholesterol and saturated fatty acids synergistically promote the malignant progression of prostate cancer. Neoplasia. 2022 Feb 1;24(2):86-97.

4 Ooi KL, Vacy K, Boon WC. Fatty acids and beyond: Age and Alzheimer's disease related changes in lipids reveal the neuro-nutraceutical potential of lipids in cognition. Neurochemistry International. 2021 Oct 1;149:105143.

5 Riscal R, Skuli N, Simon MC. Even cancer cells watch their cholesterol!. Molecular cell. 2019 Oct 17;76(2):220-31.

6 Brouns F. Overweight and diabetes prevention: is a low-carbohydrate–high-fat diet recommendable?. European journal of nutrition. 2018 Jun;57(4):1301-12.

7 Pattanayak C, Bahinipati J, Srivastava V, Sarkar S. Evaluation of Hypolipidemic and Hepatoprotective Effects of Linum usitatissimum L. Seeds on Hypercholesterolemic Rats.

8 Evans EP, Scholten JT, Mzyk A, Reyes-San-Martin C, Llumbet AE, Hamoh T, Arts EG, Schirhagl R, Cantineau AE. Male subfertility and oxidative stress. Redox biology. 2021 Oct 1;46:102071.

9 Olaniyi KS, Akintayo CO, Oniyide AA, Omoaghe AO, Oyeleke MB, Fafure AA. Acetate supplementation restores testicular function by modulating Nrf2/PPAR-γ in high fat diet-induced obesity in Wistar rats. Journal of Diabetes & Metabolic Disorders. 2021 Dec;20(2):1685-96.

10 Macho-González A, Garcimartín A, López-Oliva ME, Bastida S, Benedí J, Ros G, Nieto G, Sánchez-Muniz FJ. Can meat and meat-products induce oxidative stress?. Antioxidants. 2020 Jul;9(7):638.

11 Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. European Journal of Medicinal Chemistry. 2021 Jan 1;209:112891.

12 Forman HJ, Zhang H. Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. Nature Reviews Drug Discovery. 2021 Sep;20(9):689-709.

13 Lorenzon dos Santos J, Schaan de Quadros A, Weschenfelder C, Bueno Garofallo S, Marcadenti A. Oxidative stress biomarkers, nut-related antioxidants, and cardiovascular disease. Nutrients. 2020 Mar;12(3):682.

14 Carleton HM, Drury RA, Wallington EA. Carleton's histological technique. Oxford University Press, USA; 1980.

15 Walski T, Drohomirecka A, Bujok J, Czerski A, Wąż G, Trochanowska-Pauk N, Gorczykowski M, Cichoń R, Komorowska M. Low-level light therapy protects red blood cells against oxidative stress and hemolysis during extracorporeal circulation. Frontiers in physiology. 2018 May 31;9:647.

16 Bissinger R, Bhuyan AA, Qadri SM, Lang F. Oxidative stress, eryptosis and anemia: a pivotal mechanistic nexus in systemic diseases. The FEBS journal. 2019 Mar 1;286(5):826-54.

17 Ghareeb OA, Sulaiman RR, Ibrahim SH. Impact of Silver Nanoparticles on Hematological Profiles and Hepatorenal Functions in Photosensitivity: In Vivo. Annals of the Romanian Society for Cell Biology. 2021 Apr 19:7448-59.

18 Mahmoud JH, Ghareeb OA, Mahmood YH. The Role of Garlic Oil in Improving Disturbances in Blood Parameters Caused by Zinc Oxide Nanoparticles, J. Med. Chem. Sci., 2022, 5(1) 76-81.

19 Salah H, Elkatory MR, Fattah MA. Novel zinc-polymer complex with antioxidant activity for industrial lubricating oil. Fuel. 2021 Dec 1;305:121536.

20 Ganjifrockwala FA, Joseph JT, George G. Decreased total antioxidant levels and increased oxidative stress in South African type 2 diabetes mellitus patients. Journal of Endocrinology, Metabolism and Diabetes of South Africa. 2017 Aug 15;22(2):21-5.

21 Ackerman D, Tumanov S, Qiu B, Michalopoulou E, Spata M, Azzam A, Xie H, Simon MC, Kamphorst JJ. Triglycerides promote lipid homeostasis during hypoxic stress by balancing fatty acid saturation. Cell reports. 2018 Sep 4;24(10):2596-605.

22- Khatana C, Saini NK, Chakrabarti S, Saini V, Sharma A, Saini RV, Saini AK. Mechanistic insights into the oxidized low-density lipoprotein-induced atherosclerosis. Oxidative Medicine and Cellular Longevity. 2020 Sep 15;2020.

23 Tanaka S, Couret D, Tran-Dinh A, Duranteau J, Montravers P, Schwendeman A, Meilhac O. High-density lipoproteins during sepsis: from bench to bedside. Critical Care. 2020 Dec;24(1):1-1.

24 Xiang AS, Kingwell BA. Rethinking good cholesterol: a clinicians' guide to understanding HDL. The Lancet Diabetes & Endocrinology. 2019 Jul 1;7(7):575-82.

25 Ranasinghe P, Wathurapatha WS, Ishara MH, Jayawardana R, Galappatthy P, Katulanda P, Constantine GR. Effects of zinc supplementation on serum lipids: a systematic review and meta-analysis. Nutrition & Metabolism. 2015;12(1):1-6.

26 Severo JS, Morais J, Beserra JB, de Farias LM, dos Santos LR, de Sousa Melo SR, do Nascimento Nogueira N, do Nascimento Marreiro D. Effect of zinc supplementation on lipid profile in obese people: A systematic review. Current Nutrition & Food Science. 2019 Oct 1;15(6):551-6.

27 Ghareeb OA, Mahmoud JH, Qader HS. Efficacy of Ganoderma lucidum in Reducing Liver Dysfunction Induced by Copper Oxide Nanoparticles. Journal of Research in Medical and Dental Science. 2021 Dec;9(12):14-7.

28 Ramadhan SA, Ghareeb OA. Toxicity of AgNPs upon Liver Function and Positive Role of Tinospora Cordifolia: In Vivo. Pakistan Journal of Medical and Health Sciences.2021; 15(6):2164-2166.

29 Prasad AS. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. Experimental gerontology. 2008 May 1;43(5):370-7.

30 Jan AU, Hadi F, Ditta A, Suleman M, Ullah M. Zinc-induced anti-oxidative defense and osmotic adjustments to enhance drought stress tolerance in sunflower (Helianthus annuus L.). Environmental and Experimental Botany. 2022 Jan 1;193:104682.