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ORIGINAL ARTICLE

Protective Effects of Methionine on some Physiological and Biochemical Parameters of Triton-Stressed Male Rats

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ABSTRACT

The aim of this study is to find out the effect of amino acid methionine 35 mg/kg of feed with the diet to minimize the harms caused by oxidative stress resulting from treatment with Triton at a dose of (300 ml/kg) on physiological and biochemical parameters. This study was conducted at the Technical Institute/Mosul for the period 1/12/2021 - 1/2/2022 to find out the protective effects of the amino acid methionine on some physiological and biochemical parameters of Triton-stressed white male laboratory rats, as their weight ranged from 180-220 g and their ages ranged between 12- 14 weeks. After confirming that they are free of diseases, with the help of a veterinarian. Some biochemical parameters were measured, namely, glucose, cholesterol T.C., triglycerides T.G, (LDL-c) low-density lipoprotein cholesterol, (VLDL-c) very low-density lipoprotein cholesterol, (HDL-c) high-density lipoprotein cholesterol, and the atherogenic index. In this study, were used 24 white male rats, distributed randomly into four groups of 6 rats/group. And it was as follows: Control group: was injected a 1 ml of distilled water to equalize the stress of holding the rats. Triton group: They were injected with Triton 300 ml/kg body weight of Triton. Methionine group: was given (35 mg/kg diet) of the amino acid methionine and triton methionine group: they were injected with triton (300 ml/kg body weight) and methionine amino acid at (35 mg/kg diet) concentration. Dosing process took place daily for a period of (8) weeks.The study showed a significant decrease at the probability value (P≤0.05) in the concentration of each of cholesterol, triglycerides, LDL-c, VLDL-c, the atherogenic index, and glucose, however, HDL-c levels were found to be higher in this group compared to the control group. Treatment with methionine also led to an improvement in some physiological and biochemical parameters of rats that were subjected to triton stress.

Keywords: methionine, triton, lipoproteins, atherogenic index.

INTRODUCTION

To regulate metabolic, physiological, and nervous balance to maintain proper nutritional status, as well as disease prevention. Mammals depend for their nutrition on many nutrients such as fatty acids, amino acids, minerals and vitamins in food (1).A great deal of recent research has been devoted to the chemistry of free radicals. Free radicals are unquestionably to blame for the oxidative damage that occurs to biomolecules like proteins, nucleic acids, or lipids in the nucleus and cell membrane components. A balance between free radicals and antioxidants must be maintained in order to be healthy(2). Thus, for the prevention and treatment of many diseases, such as diabetes, atherosclerosis, coronary artery disease, cancer, infections, liver diseases, cardiovascular diseases, cataracts, nephrotoxicity and neurodegenerative processes associated with aging, it becomes necessary to control processes oxidative stress(3).Excess free radicals are neutralized by enzymes and non-enzymatic antioxidants produced by the human body (with a few exceptions, such as glutathione, uric acid and uricinol), in order to maintain a redox balance that must be provided in the diet (4) .Amino acids are natural substances that play key roles in numerous vital biological processes, including metabolism, growth, and immunity (5). They are the building blocks of proteins and precursors to functional molecules (6,7) and amino acids have two types, there are essential amino acids and non-essential amino acids, with the latter being crucial for some growth processes and physiological processes (8).One of essential sulfur-containing amino acid is Methionine, and a precursor of succinyl-CoA, homocysteine, cysteine, creatine, and carnitine. In mammals, methionine can regulate metabolic processes, the innate immune system, and the functioning of the digestive system as reported in recent research it is also involved in lipid metabolism, glutathione biosynthesis and activation of endogenous antioxidant enzymes (such as methionine sulfoxide reductase A) to counteract oxidative stress (9) .Cardiovascular disease is a serious, life-threatening disease (10).Cardiovascular illnesses are thought to be the primary cause of morbidity and mortality in the near future as they account for one-third of all deaths worldwide (11).Cholesterol is an important and abundant component of the bloodstream and of the structure and function of the cell membrane, allowing cells to maintain the permeability and fluidity that are essential to all types of animal life (12).Cholesterol is required by the body to create cell membranes, some hormones, and substances that facilitate the breakdown of fats. Yet, having too much cholesterol raises one's risk of cardiovascular disease (13). Modern lifestyle contributes significantly to high blood cholesterol and cardiovascular disease due to lack of physical activity and a diet high in fat. Altered cholesterol composition in addition to impaired liver function is one of the predominant causes of hyperlipidemia in various diseases (14) . In most developing countries, the incidence of dyslipidemia is a major concern due to diet and other lifestyle changes (15). The cholesterol-lowering effect of dietary plants and many natural products has been well shown to be beneficial in lowering plasma cholesterol levels (16) . The mechanism of natural lipid-lowering products is either through inhibition of cholesterol absorption, inhibition of cholesterol synthesis, or antioxidant mechanisms (17) . Cardiovascular disease, atherosclerosis, cerebrovascular disease, stroke, and cerebrovascular disease are the results of hyperlipidemia and lipid disorders (18).Triton is one of the non-ionic detergents that is widely used to cause severe hyperlipidemia in laboratory animals, as it alters the biophysical properties of lipoproteins and then prevents their absorption from the blood, leading to an increase in their levels in the blood (18). Triton is used to induce hypercholesterolemia (19). Triton is a surfactant commonly used to cause hyperlipidemia in mice and male rats (20). Triton X-100 solution has been used successfully to induce hyperlipidemia in mice in previous studies and was chosen as the hyperlipidemia model because of its suitability and availability (21).

MATERIALS AND METHODS

The study was conducted on white male laboratory rats, after making sure that they were free from diseases, they were placed in cages made of iron specially prepared for this purpose, with six rats/group, whose weights ranged between (180-220) gm and their ages ranged between 12-14 weeks.In this study, Triton WR-1339 X-100 was used at a concentration of (300 ml/kg) by intraperitoneal injection as a single dose (23,24), to induce oxidative stress in the study animals. The essential amino acid methionine (35 mg/kg diet) was also used to observe its effect on changing the effects of oxidative stress in animals treated with Triton.The second group (triton group) and the fourth group (triton and methionine group) were injected with triton intraperitoneally at a single dose of (300 mg/kg body weight) to cause oxidative stress in them. The animals of the first and second groups were dosed

with distilled water daily to equalize the effect of holding and dose. The animals of the third and fourth groups were given methionine (35 mg/kg diet) daily for a period of (8) weeks.(5) ml of the blood of the study animals was taken for conducting biochemical examinations through the eye, where examinations of the level of glucose, cholesterol, triglycerides, LDL-c, VLDL-c, HDL-c, and atherogenic index. We used the modified Roeschlau's approach for calculating total cholesterol, the Wako method for calculating triglycerides, with revisions by McGowan et al. and Fossati et al., and the phosphotungstic acid method for calculating HDL cholesterol. We also utilized Friedwald's formula for calculating VLDL-c and LDL-c (25): Triglycerides/5 = VLDL T.LDL = Cho - (VLDL + HDL) .After collecting the results of the above-mentioned tests, a statistical analysis was conducted to find out the significant differences of the studied parameters within each group and to compare them with other groups.

RESULTS

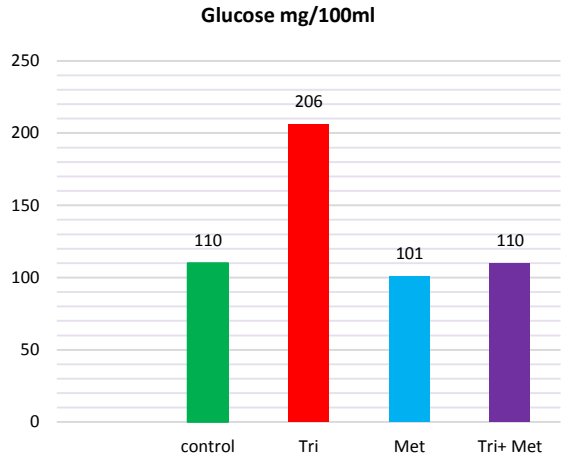

Figure 1: Effect of methionine on blood serum glucose concentration (mg/100ml) of white male rats.

Figure (1) shows that there was a significant decrease in blood glucose concentration of white male laboratory rats at a probability level (P≤0.05) for methionine group compared rest of the groups, where arithmetic mean was 101 ± 2.44 mg/100 ml, while arithmetic mean for control group and triton methionine group was 110 ± 1.41 mg/100 ml and 110 ± 1.67 mg/100 ml respectively, and arithmetic mean in triton group was 206 ± 2.45 mg/100 ml.

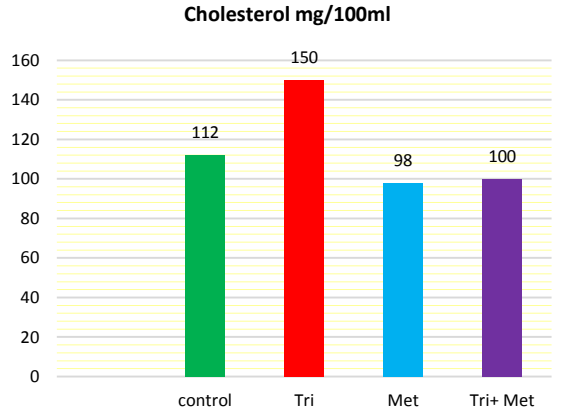

Figure 2: Effect of methionine on blood serum cholesterol concentration of white male rats (mg/100 ml).

A significant decrease in cholesterol concentration in blood serum of white male laboratory rats showed at figure (2) at probability level (P≤0.05) for methionine group, which had an arithmetic mean of 98.00 ± 2.90 mg/100 ml, and for triton group with methionine 100 ± 2.83 mg/100 ml, compared with other groups, the control's group arithmetic mean was 112 ± 2.45 mg/100 ml and for triton group 150 ± 2.61 mg/100 ml.

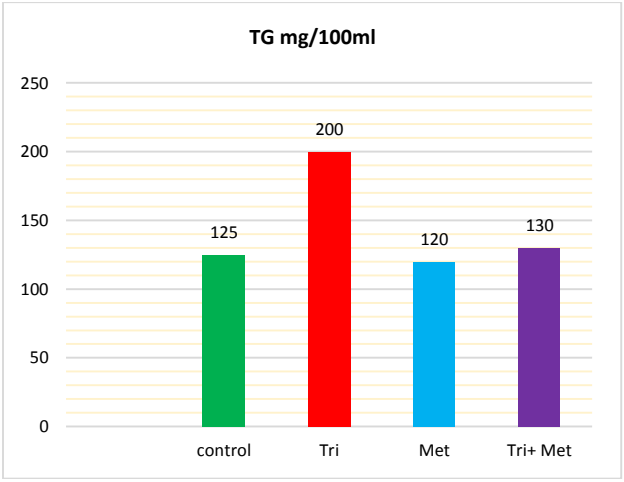

Figure 3: Effect of methionine on triglyceride concentration in the blood serum of white male rats (mg /100 ml).

Figure (3) showed a significant decrease in the concentration of triglycerides in the blood serum of white male laboratory rats of methionine group at a probability level (P≤0.05) with arithmetic mean of 120 ± 1.41 mg/100 ml as shown in the table, compared to other groups, whose arithmetic mean was as follows, control group 125 ± 1.79 mg/100 ml, triton with methionine 130 ± 2.45, triton group 200 ± 2.10 mg/100 ml.

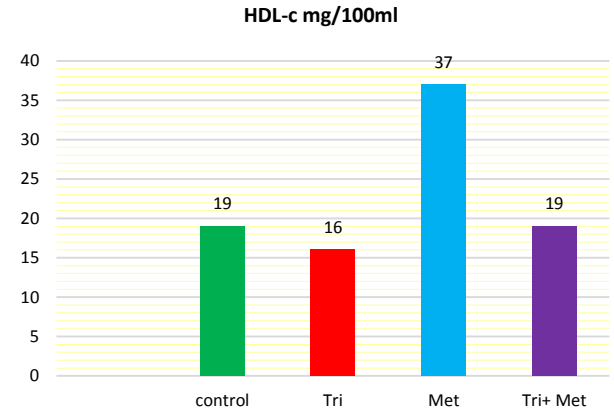

Figure 4: Effect of methionine on the concentration of HDL-c in the blood serum of white male rats (mg/100 ml).

Figure (4) shows a significant increase in high-density lipoprotein cholesterol concentration (HDL-c) in blood serum of white male laboratory rats treated with methionine, with an arithmetic mean of7.00 ± 2.83 mg/100 ml at a probability level (P≤0.05) compared to other groups, so the concentration of HDL-c in control group and triton group with methionine was 19.00 ± 1.79 mg/100 ml and 19.00 ± 3.16 mg/100 ml, respectively, and triton group was 16.00 ± 2.28 mg/100 ml.

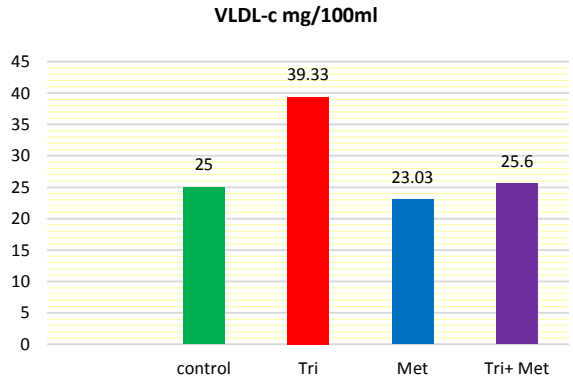

Figure 5: Effect of methionine on the concentration of low-density lipoprotein cholesterol in the blood serum of white male rats (mg/100 ml).

Figure (5) shows a significant decrease in the concentration of very low-density lipoprotein (VLDL-c) in blood serum of white male laboratory rats at a probability level (P≤0.05) for methionine group, where arithmetic mean was 23.03 ± 2.38 mg/100 ml compared to other groups, whose arithmetic mean was 25.00 ±0.36 mg/100ml for control group, 25.60±1.00 mg/100ml for triton with methionine group, and 39.33±1.69 mg/100ml for triton group.

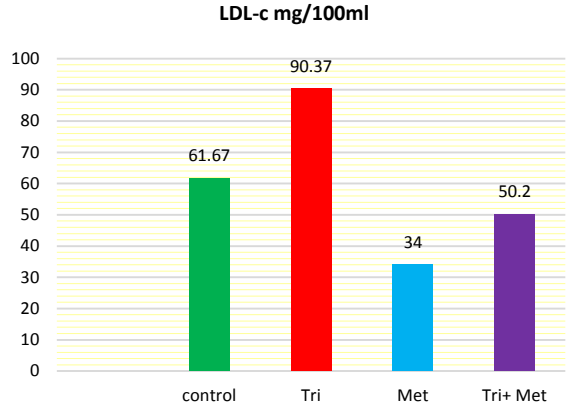

Figure 6: Effect of methionine on the concentration of low-density lipoproteins cholesterol in the blood serum of white male rats (mg/100 ml).

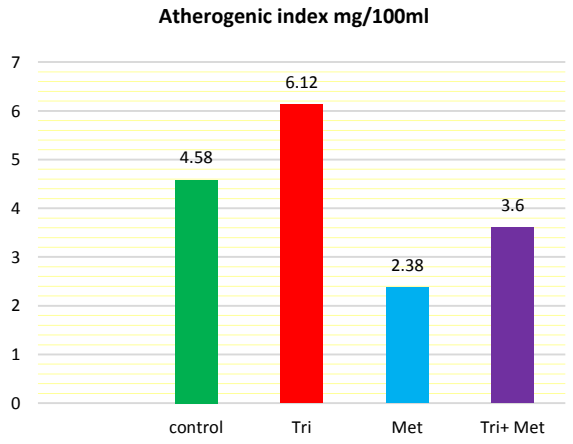

Figure 7: Effect of methionine on the concentration of atherogenic index in the blood serum of white male rats (mg/100ml).

Figure (6) also showed a significant decrease in the concentration of low-density lipoproteins cholesterol blood serum(LDL-c) of white male laboratory rats at a probability level (P≤0.05) for methionine group, and its arithmetic mean was 34.00 ± 8.40 mg/100 ml compared with the rest groups, where arithmetic mean for triton group with methionine it was 50.20 ± 1.29 mg/100 ml, for control group it was 61.67 ± 1.58 mg/100 ml and for triton group it was 90.37 ± 9.78 mg/100 ml.

The current study results, Figure (7), showed the atherogenic index in blood serum of white male laboratory rats decreased significantly for methionine group and triton methionine group at probability level (P≤0.05), as arithmetic mean was 2.38 ± 0.12 and 3.60 ± 0.35 mg/100 ml respectively compared with control group 4.58 ± 0.28 mg/100 ml and triton group, which had a arithmetic mean of 6.12 ± 0.31 mg/100 ml.

DISCUSSION

The high level of blood glucose in the Triton group is due to an increase in oxygen, this leads to an increase in reactive oxygen species, which causes a defect in the pancreatic beta cells function and disrupts the secretion of insulin as a result of attacking them (pancreatic beta cells) and stop the process of glycolysis and stimulate the process of glycogen degradation and the production of glucose from it (26,27). Reactive oxygen species lead to lipid peroxidation, RNA degradation, and inhibition of the synthesis of primary insulin, which affects insulin secretion in the required quantities to maintain blood glucose (28) . The rise in blood glucose may be due to the effect of oxidative stress on the hormone epinephrine, which increases the latter by stimulating the process of glycogenolysis in the liver and muscles from the level of blood glucose, as glucose-6-phosphite is formed from the transformation of glycogen in the liver by the enzyme glucose-6-phosphatase (29).The ability of methionine to lower the blood glucose level may be due to its ability to inhibit the secretion of the hormone corticosterone and thus inhibit the process of gluconeogenesis from non-sugar sources, which reduces tissue breakdown and increases the storage of glycogen in the body. Also, its ability to reduce the effect of oxidative stress by enhancing antioxidants activates the work of pancreatic beta cells and insulin secretion, thus reducing blood glucose levels (26).The ability of methionine to reduce VLDL-c, LDL-c, TG, T.Ch., is believed to be due to its ability to enhance antioxidants and their role in the cell and work to reduce the effect of oxidative stress, which activates the work of somatic cells, including pancreatic beta cells, and activates insulin secretion, which reduces the level of T.Ch., TG, indirectly in the blood (Oda. 2006). As for the ability of methionine to reduce oxidative stress and its effects, it may be due to its function as an antioxidant. The methionine when dealing with the oxidizing agent, it changes to methionine sulfoxide and therefore the methionine residues provide a high concentration of reactions that are traps for the oxidizing agents. In addition, it may occur reverse reduction of methionine by the enzyme Methionine sulfoxide reductase, which occurs catalytically alternating stimulation in the work of the antioxidant system within the body (30) .The reason for the decrease in the atherogenic index is due to the fact that the amino acid methionine has the advantage of its ability to lower total cholesterol and reduce the process of fat acidification

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