Effect of Cholesterol and /or Methionine on the Testis of Rats By

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Abstract

Aim of the work-Methionine is essential for maintaining proper growth and development in mammals. Also, cholesterol enriched diets significantly increase cholesterol level in body tissues. The present study aims to investigate the effects of dietary cholesterol and /or methionine on the histological and some histochemical changes in the testis and epididymis of the male rats. Material and methods- sixty adult male rats (5-7 weeks old) were used in this study and divided into 6 groups. Group 1 served as the control group (C). The following 5 treated groups administered the diets for four months. Group 2 treated with a diet enriched with 2% cholesterol, group 3 received 0.5% methionine, group 4 received 2% methionine, group 4 treated with 2% cholesterol and 0.5% methionine, group 5 received 2% cholesterol and 2% methionine. Results- Testes isolated from rats fed excessive amount of cholesterol showed disturbance in spermatogenesis and the epididymal structure with altered total protein, polysaccharides and collagen fibers. Dietary low doses of methionine with or without cholesterol improved spermatogenesis and architecture of the epididymis, but addition of high dose of methionine caused many drastic changes in the testis and the epididymis.Conclusion-Low dose of methionine (0.5%) showed ameliorative effect against adverse effects of cholesterol, but the high dose (2%) showed less protective effect. Key words: Methionine, Cholestrol, Histopathology, Histochemistry.

Introduction

Cholesterol triglycerides and are transported in the blood as lipoproteins. Plasma concentrations of cholesterol and triglyceride being a potential early indicator for hyperlipidemia and lipid metabolic disorders, since they reflect the lipid metabolism state in animals. The severity of hyperlipidemia varied between genders and appeared to be positively correlative with age (Hisao et al., 2007; Mori et al., 2009). The atherosclerosis is a disease of large and medium size muscular arteries: aorta and its major branches, coronary arteries and arteries of the legs (Gamble, 2006).

Elevated levels of LDL-cholesterol particles have been implicated in the

development of the atherosclerotic plaque, characterized by reduced receptor-mediated clearance, increased arterial wall retention and increased susceptibility to peroxidation (Holvoet, 2006). The author added that cardiovascular risk factors such as hyperlipidemia, hypertension and thrombosis contribute to the underlying atherosclerotic mechanisms of disease. promoting endothelial dysfunction, oxidative and pro-inflammatory stress pathways.

Lipoproteins are spherical structures that consist of a hydrophobic core containing lipids (i.e. triglycerides and/or cholesterol esters), and amphophilic (i.e. both hydrophobic and hydrophilic) outer layer of phospholipids, free cholesterol, and proteins that form a protective envelope surrounding the lipid core. Proteins that are part of the lipoproteins are known as apolipoproteins (or apoproteins) and play a significant role in lipid transport and metabolism (Haines, 2001; Schlenker and Williams, 2003; Johnson, 2005).

Because lipids are water-insoluble molecules, they cannot be transported in aqueous solutions, such as plasma. For that reason, lipids are transported in plasma as macromolecular complexes known as lipoproteins. Free fatty acids are transported bound to albumin and do not require incorporation into lipoproteins for transport (Bauer, 2004). The author added that lipoproteins contain one or a variety of apolipoproteins, which regulate their metabolic functions. Apolipoproteins are involved in several physiological functions of lipoproteins such as facilitation of lipid transport, maintenance of structural integrity and activation of certain enzymes that play key roles in lipid metabolism .

Lipoprotein lipase is an enzyme that is located on the luminal surface of the capillary endothelial cells, and hydrolyzes triglycerides within lipoproteins into free fatty acids, mono- and diglycerides, and glycerol (**Wang and Hartsuck, 1992**).

Low-density lipoproteins (LDL) transport cholesterol esters from the liver to rest of the body and are associated with apolipoprotein B and called the bad cholesterol, while high-density lipoprotein (HDL) is called the good cholesterol which transport cholesterol esters from organs back to the liver for degradation to bile acids (Hansen *et al.*, 2009).

Cholesterol is an essential component of all cell membranes and is a precursor for steroid hormones and bile acid biosynthesis. Triglyceride is central to the storage and transport of energy within the body (Chen et al., 2011). They also reported that lipoprotein metabolism can be classified into two cycles, one exogenous and one endogenous, both central on the liver. The exogenous pathway is associated with lipids from the diet, whereas the endogenous pathway is associated with metabolism of endogenously produced lipids. According to Andrew et al. (2010) postprandial serum triglyceride concentrations have recently been identified as a major, independent risk factor for future cardiovascular events.

Increased cholesterol causes atherosclerosis which is the most important artery-damaging disease and leading to death all over the world (De Winther et al., 2005). Hyperlipidemia in rats showed many dystrophic changes in liver tissue. These changes include: highly distorted and ruptured endothelial lining of the central vein and branches of the hepatic portal vein, increased lymphocytic infiltration in the portal area, haemolysed RBCs inside the blood vessels, increased Kupffer cells, distorted blood vessels and bile ducts with degenerated and vacuolated hepatocytes (El-Wahsh, 2011).

Methionine is a protein-based amino acid and lipotropic compound that helps with metabolism and breaks down fat. This amino acid has the ability to help keep the inside walls of the arteries naturally clear of accumulated fatty deposits. It can also help with chelation, which is the removal of heavy metals from the body to ensure that the liver, kidneys, and bladder remain healthy. This amino acid preserves artery function and maintains healthy nails, hair, and skin. Additionally, it is essential for muscle growth and energy. The human body does not naturally produce methionine, so humans can only get it by ingesting it. Foods containing it include: protein-rich foods like eggs, fish, and Brazil nuts, as well as cereal grains. People can also get it through a supplement or through intravenous (IV) therapy administered by a health care provider. Maintaining a sufficient level of methionine in the body helps ensure overall good health (Yen et al., 2002). Protective effect of methionine was studied by several authors (Miners et al., 1984; Neuvenon et al. 1985; Mocchegiani et al., 2008; Szentmihaltyi et al., 2009). They confirmed that methionine probably acted as hepatoprotective agent through generation of glutathione in the hepatocytes.

In **2009, Hemabarathy** *et al.*, noticed the hepatoprotective effect of methionine in paracetamol-exposed rats. Some common but significant side effects of methionine deficiency include: liver damage, edema, and brittle hair. Low levels can slow normal growth and development in children and in pregnant women may result in neural tube defects in infants, such as myelomeningocele or spina bifida. Deficiencies can also lead to severe mental disorders (**Yuanlong** *et al.*, **1996**), they added that maintaining a sufficient level of methionine in the body helps ensure overall good health.

The aim of this study is to investigate effects of dietary cholesterol and /or methionine on the histological and some histochemical changes in the testis and epididymis of the male rats and the possible protective role of methionine.

Material and methods

Sixty adult male rats (*Rattus albinus*) weighting 150±20 gm (5-7weeks old) were used in the present study. The experimental animals were fed on rodent diet and randomly divided into six groups, ten rats for each group. Group1 served as the control group (C). The following 5 treated groups administered the normal diets and supplementation of treatments for four months. Group 2 (CH) treated with a diet enriched with 2% cholesterol, group 3 (M1) received 0.5% methionine, group 4 (M2) received 2% methionine, group 5 (CH+M1) treated with 2% cholesterol and 0.5% methionine, group 6 (CH+M2) received 2% cholesterol and 2% methionine. Methionine and cholesterol were obtained from the Nile Company for Pharmaceutical and Chemical Industries.

The animals were dissected and the testes were immediately excised and fixed in 10% neutral formalin and Carnoy's fluid and processed routinely for making paraffin sections (5 μ). Sections were stained by haematoxylin and eosin stain according to the method of Drury and Wallington (1980), Mallory's trichrome stain for demonstrating collagen fibers (Pearse, 1977), polysaccharides (PAS+ve materials) were stained according to Pearse (1977) and mercuric bromophenol blue method for detecting total protein (Mazia et al., 1953).

Analysis of data: Analysis of data was performed using T test. Differences were considered significant at probability levels P< 0.05. **Image analysis:** The optical transparency (pixel) of PAS+ve materials and total protein were analyzed by using software Bel Microimage Analyzer Program ver. 2.3 for microscopy.

Results

Normal structure of the testis and epididymis of the control group is shown in figs. (1a,b) .Cholesterol treated group (Figs.2a,b) showed reduced cells of the spermatogenic layers, thickened walls of the blood vessels, highly dilated intertubular connective tissue, distorted Leydig cells which contained karyolytic nuclei and degenerated cytoplasm.This was accompanied with decreased number of mature sperms inside the epididymal tubules.

Methionine treatment with the low dose (M1) showed well developed testicular and epididymal architecture with highly increased number of sperms inside them (Figs.3a, b).High dose of methionine (M2) showed normal spermatogenesis and epididymal tubules, but stratified cuboidal cells were realized in their walls and immature sperms in their lumens.Edema was observed in the interstitial spaces between the seminiferous tubules.(Figs 4a,b).

Testis and epididymis of group (CH+M1) regained their normal appearance ,but highly dilated and congested blood vessels and increased connective tissue were detected (Figs.5a,b).

Faintly stained nuclei of cells of the spermatogenic layers and epididymal tubules with reduced number of mature sperms were realized in group (CH+M2) with highly dilated walls of the blood vessels which contained 2haemolysed blood cells (Figs.6a,b).Distorted Leydig cells contained vacuolated cytoplasm and faintly stained nuclei and they were surrounded by edema.Table (1) and histogram (1) revraling decreased mean diameter of the seminiferous tubules of groups (CH,CH + M1&CH + M2), where they reached $(141.216 \pm 11.595; 139.395 \pm 11.039 \&$ 142.361 ± 11.224) compared with the control group which reached 151.133 \pm 12.167.These values increased in groups (M1&M2), where they reached (158.125 \pm 14.151 & 170.844 ± 14.389) respectively. It is clear that this increase is more pronounced in group (M2). Normal distribution of collagen fibers was detected in figs. (7a,b),they are supporting cellular membranes of the spermatogenic layers Leydig cells, basement membranes and the connective tissue.

Figs. (8a,b) showed decreased collagen fibers in the seminiferous tubules and epididymal tubules of group (CH). Thin scattered collagen fibers were realized in the dilated inter tubular spaces of the testis and widened connective tissue areas of the epididymis with numerous fibrotic areas which were indicated by red bright stain affinity.Normal distribution of collagen fibers was detected in the testis and epididymis of group (M1) (Figs.9a,b). Group (M2) showed highly decreased collagen fibers in the seminiferous tubules and their basement membranes, walls of the epididymal tubules and in mature sperms, but they increased in the inter tubular spaces of the testis and widened connective tissue between the epididymal tubules with common fibrosis between them (10a,b).

Testis and epididymis of group (CH+M1)showed somewhat normal distribution of collagen fibers with decreased signs of fibrosis in between the epididymal tubules (Figs.11a, b) compared with those of groups (CH&M2).Reduced collagen fibers were detected in the testis and epididymis of group (CH+M2) with pronounced signs of fibrosis in the epididymal tubules(Figs.12a,b).

Figs.(13a,b) revealing normal distribution of PAS+ve materials in the testis (MOT = 19.263 ± 14.707) and epididymis (MOT = 46.158 ± 19.665) of the control group, where moderate stain affinity was detected in the basement membranes of the seminiferous tubules and Leydig cells with deeply stained heads of mature spermatozoa in the testis and epididymis [Table 2&Histogram 2]. Decreased stain affinity of PAS + ve materials is observed in the testis (MOT value=23.035±15.139) and epididymis (MOT value=28.860±17.651) of group (CH), but they increase in the thickened walls of the blood vessels (Figs.14a,b) and they are somewhat normal in the testis $(MOT value = 18.263 \pm 13.170)$ and increased in the epididymis (MOT value=58.491±15.218) of group (M1)with less stain affinity in the connective tissue and moderate stain affinity in the Leydig cells(Figs15a,b). Testis (MOT value = 11.421 ± 12.313) and epididymis (MOT value = 34.140 ± 16.424) of group(M2) showed decreased PAS +ve materials in testis and epididymis, but they were increased in the connective tissue areas and in wa6lls of the blood vessels of the epididymal tubules (Figs.16a, b).Deeply stained PAS+ve materials were noticed in the mature sperms of the epididymal tubules (MOT value=70.351±18.630) of group (CH+M1) and also in walls of the blood vessels of the testis with faintly stained connective tissue and testis(MOT value = 14.5 ± 13.394) (Figs.17a,b). Decreased stain affinity of PAS+ve materials is realized in the testis(MOT value =12.246

 \pm 13.535) with somewhat normal stain affinity in the epididymis (MOT value = 55.40 \pm 13.880) of group (CH+M2), but e8dema spaces acquired moderate stain affinity (Figs.18a,b). Figs. (19a,b) showed normal distribution of the total protein in the testis (MOT value = 116.464 \pm 16.736) and epididymis (MOT value = 160.968 \pm 12.103) of the control group[Table 3&Histogram 3]. Decreased stain affinity of the total protein was recognized in the testis (MOT value = 110.069 \pm 20.360) and epididymis(MOT value=105.666 \pm 37.588) of group (CH) (Figs.20a,b),but increased stain affinity was noticed in the testis (MOT values = 121.961 ± 16.557 , $139.594 \pm$ 17.101, 146.727 ± 19.558 respectively) and epididymis (MOT values = $175.036 \pm$ $18.605, 177.520 \pm 14.390, 172.764 \pm$ 17.024 respectively) of groups (M1,M 2 & CH + M1) with less stain affinity in the connective tissue (Figs.21a, b; 22a, b&23 a,b). Somewhat normal stain affinity of the total protein was realized in the testis (MOT value = 116.627 ± 16.521) and they increased in the epididymis (MOT value = 187.101 ± 12.103) of group (CH + M2) (Figs.24 a ,b) , testis (MOT = $116.464 \pm$ 16.736) and epididymis (MOT = 160.968) \pm 12.103) of the control group.



Plate (1):showing photomicrographs of the testes(a) and epididymis(b) Figs.1-3 (H & E, x 100). Figs.(1 a&b): group (C) testis and epididymis of the control group(C); a: showing the normal organization of spermatogenic cells of the seminiferous tubules , normal appearance of Leydig cells.b: normal mature spermatozoa within the epididymal tubules.

Figs.(2 a&b): group(CH) showing seminiferous tubules which contain immature spermatids with elongated head and very short tail, reduced cells of the spermatogenic layers and thickened wall of the blood vessel ,highly dilated inter tubular connective tissue with edema, presence of immature spermatozoa with many karyolytic nuclei in the distorted Leydig cells which show degenerated cytoplasm. b:decreased amount of mature spermatozoa within the epididymal tubules. Figs. (3 a&b): group (M1); a: showing increased cells of the spermatogenic layers and Leydig cells. .b: increased number of mature spermatozoa in the epididymal tubules with thin walls of them.



Plate(2): showing photomicrographs of the testis(a) and epididymis(b):Figs.(4-6) (H & E, x 100)Figs. (4 a, b): group (M2) showing well developed spermatogenic layers with normal architecture of Leydig cells. Notice edema in the interstitial spaces .Walls of the epididymal tubules contain stratified cuboidal cells with immature spermatozoa in their lumens.Figs. (5a,b): group(CH+M1)revealing normal spermatogenesis in the seminiferous tubules which contain crowded sperms in their lumens and inside the epididymal tubules, but highly dilated and congested blood vessels are demonstrated inside the testes and epididymis with increased connective tissue in the later. Figs. (6 a, b): group(CH+M2) showing numerous depleted nuclei in the different cells of the spermatogenic layers and in the cuboidal cells of the epididymis, reduced number of mature sperms in the seminiferous tubules and inside the epididymal tubules, highly dilated walls of the blood vessels which contain haemolysed RBCs and they are surrounded by edema, highly distorted Leydig cells with vacuolated cytoplasm and faintly stained nuclei .Notice: damaged walls of the blood vessel and epididymal walls .



Plate (3): photomicrographs of the testis (a) and epididymis(b) showing collagen distribution :Fig.(7-9) (Mallory's trichrome stain x 100).

Figs.(7a,b): group (C) showing normal distribution of collagen fibers.Collagen bundles are supporting membranes of the spermatogenic cells especially mature sperms,basement membranes,Leydig cells , walls of the epididymal tubules and in the connective tissue in between them.

Figs.(8a,b):group(CH) showing a decrease in collagen fibers in the seminiferous tubules and in the epididymal tubules .Thin scattered collagen fibers are distributed in the highly dilated interstitial spaces between the seminiferous tubules. Notice: red bright stain affinity which indicating fibrosis inside the epididymis.

Figs.(9 a,b):group(M1) showing somewhat normal appearance of collagen fibers in the testis and epididymis.



 $Plate(\ 4\)\ photomicrographs\ of\ the\ testis(a)\ and\ epididymis(b)\ showing\ collagen\ distribution: Figs.(\ 10-12)\ (Mallory's\ trichrome\ stain\ x\ 100)$

Figs.(10 a,b): group(M2) showing highly decreased collagen fibers inside the seminiferous tubules and their basement membranes, in walls of the epididymal tubules and in the mature spermatozoa inside them, but they were increased in the interstitial spaces between the seminiferous tubules and in the connective tissue in between the epididymal tubules with common fibrosis outside and inside them.

Figs.(11 a,b): group(CH+M1) showing somewhat normal distribution of collagen fibers in the seminiferous tubules and in the epididymis with reduced signs of fibrosis in between the epididymal tubules.

Figs.(12 a,b): group(CH+M2) showing highly reduced collagen fibers inside the seminiferous tubules and inside and outside the epididymal tubules .Notice that fibrosis was still detected inside and outside the epididymal tubules.



Plate(5): photomicrographs of the testis(a) and epididymis(b) showing PAS +ve materials. Figs.(13-15) (PAS reaction x 100)

Figs. (13a, b): group(C): showing normal distribution of PAS + ve materials in the testis of the control group .Notice: moderately stained basement membrane, Leydig cells and some spermatogenic cells especially spermatozoa which appear deeply stained inside the lumens of the epididymis.

Figs. (14a, b): group (CH): showing decreased stain affinity of PAS + ve materials in the seminiferous tubules of the testis and epididymis, but they increase in the thickened walls of blood vessels.

Figs. (15a, b): group (M1): showing deeply stained of PAS + ve materials in the spermatozoa inside the epididymis and the seminiferous tubules of the testis with less stained connective tissue in between the epididymal tubules. Normal stained basement membranes and the remnant of spermatogenic layers with moderately stained Leydig cells were observed.



Plate (6): photomicrographs of the testis(a) and epididymis(b) showing PAS +ve materials .Figs.(16-18) (PAS reaction x 100)

Figs. (16 a, b): group(M2) showing decreased stain affinity of PAS + ve materials in the cuboidal cells of the epididymal tubules and in the testis especially in the basement membranes of the seminiferous tubules with deeply stained walls of the blood vessels inside the testis and less stained widened areas of the connective tissue in the epididymal tubules.

Figs. (17 a, b): group (CH +M1) showing deeply stained PAS + ve materials in the mature sperms in the epididymis and also in the thickened walls of the blood vessels inside the testis with less stained connective tissue and seminiferous tubules.

Figs. (18 a, b): group(CH + M2) showing a slight decrease in PAS + ve materials in the testis with some what normal stain affinity in the epididymis, but edema spaces acquired moderate stain affinity.



Plate(7): photomicrographs of the testis(a) and epididymis(b) showing distribution of the total protein Figs.(19-21) (Mercuric bromophenol blue x 100)

Figs. (19a, b): group(C) showing normal distribution of total protein in the testis and epididymal tubules of the control group.

Figs. (20 a, b): group (CH) showing a marked decrease in stain affinity of total protein in the epididymis and testis.

Figs. (21 a, b): group(M1)): showing deeply stained total protein in the spermatozoa inside the epididymis and the seminiferous tubules of the testis with less stained connective tissue in between the epididymal tubules.



Plate(8): photomicrographs of the testis(a) and epididymis(b) showing distribution of the total protein Figs.(22-24) (Mercuric bromophenol blue x 100)

Figs. (22a, b): group (M2) showing increased stain affinity of the total protein in the testis and in the epididymal tubules.

Figs. (23 a, b): group (CH+M1) showing increased stain affinity of total protein in the mature sperms in the epididymis and the testis.

Figs. (24 a, b): group (CH+M2) showing somewhat normal stain affinity total protein in the testis, but they increase in the epididymis.

	CONT	СН	M1	M2	CH+M1	CH+M2
mean	151.133	141.216	158.125	170.844	139.395	142.361
$\pm SD$	±12.167	±11.595	± 14.151	± 14.389	±11.039	±11.224
%		-9.917	6.991	19.710	-11.7381	-8.772
T test		0.119007	0.216801	0.005349	0.018489	0.134859
р		non	non	sig	sig	non

Table (1): The mean diameter of the seminiferous tubules ($m \pm SD$) in the testes of control and treated groups, P< 0.05 is consedred significant.



Fig (1) : The averag diameter of the seminiferous tubules in the testis of control and treated groups.

Table (2) : revealing mean optical transparency (MOT \pm SD)values of PAS+ve materials in the testis and epididymis of the different groups. P<0.05 is considered significant.

Group	С		СН		M1		M2		CH+M1		CH+M2	
organ	Т	EPI	Т	EPI	Т	EPI	Т	EPI	Т	EPI	Т	EPI
mean ±SD	19.263 ±14.707	46.158 ±19.665	23.035 ±15.139	28.860 ±17.651	18.263 ±13.170	58.491 ±15.218	11.421 ±12.313	34.140 ±16.424	14.5 ±13.394	70.351 ±18.630	12.246 ±13.535	55.40 ±13.880
%			3.772	-17.298	12.33333	12.33333	-7.8421	-12.0175	19.263	24.193	-7.017	9.240
T test			0.239026	0.000299	0.414242	0.007116	0.03479	0.018021	0.128424	3.35932E-05	0.037622	0.02471159
sig			non	sig	non	sig	sig	sig	non	sig	sig	sig



Hist. (2): revealing mean optical transparency (MOT) values of PAS+ve materials in . the testis and epididymis of the different groups.

GROUP	cont		СН		M1		M2		CH+M1		CH+M1	
	Т	Epi										
mean	116.464	160.968	110.069	105.666	121.961	175.036	139.594	177.520	146.727	172.764	116.627	187.101
±SD	± 16.736	± 12.103	± 20.360	± 37.588	± 16.557	± 18.605	± 17.101	± 14.390	± 19.558	± 17.024	± 16.521	± 12.103
% of change			-6.394	-55.301	5.497	14.068	23.130	16.552	30.263	11.796	0.163	26.133
T test			0.006884	0.000371	0.200269	0.005562	0.00062	0.002675	0.000111	0.014673	0.492791	0.000395
р			sig	sig	non	sig	sig	sig	sig	sig	non	sig

Table (3): revealing mean optical transparency (MOT \pm SD) values of total protein in the testis and epididymis of the different groups. P<0.05 is considered significant.



Hist. (3): revealing mean optical transparency (MOT) values of total protein in the testis and epididymis of the different groups.

Discussion

Lipids are water-insoluble organic compounds, which are essential for many normal functions of living organisms. They are important components of cell membranes and they are used to store energy also they play a significant role as co-factors. hormones. enzyme and intracellular messengers (Rifai et al., 1999; Smith, 2006). There are many groups of lipids, the most important three of them are: fatty acids, sterols (mainly cholesterol), and acylglycerols (mainly triglycerides). Fatty acids are relatively simple lipids and are also important components of many other lipids. Cholesterol is the main sterol in animal tissues. Dietary intake is the major

source of cholesterol, but it can also be synthesized endogenously by the liver and other tissues. It plays a fundamental role in the central metabolic pathways, such as bile acid metabolism, steroid hormones and vitamin D synthesis.

Hyperlipidemia is an elevation of lipids in the blood stream and these lipids including: fats, fatty acids, cholesterol, cholesterol esters, phospholipids, and triglycerides. Coronary heart disease (CHD) is caused by the narrowing of the artery that supplies nutrients and oxygen to the heart. The main reason for this narrowing is atherosclerosis. There is a relationship between the elevated plasma lipids and the development of atherosclerotic plaques (Jain *et al.*, 2007). Low-density lipoproteins (LDL) transport cholesterol esters from the liver to rest of the body and is associated with apolipoprotein B 100 and called the bad cholesterol, while highdensity lipoprotein (HDL) is called the good cholesterol which transport cholesterol esters from organs back to the liver for degradation to bile acids (Hansen *et al.*, 2009).

In the present study, treatment of rats with cholesterol caused many deleterious changes in the testis and epididymis. These changes include: reduced cells of the spermatogenic layers, thickened walls of the blood vessels, highly dilated intertubular connective tissue with edema, distorted Leydig cells which contained karyolytic nuclei and degenerated cytoplasm. This was accompanied with decreased number of mature sperms inside the testis and epididymal tubules. Degenerative changes observed in this study post-cholesterol treatment may be due to hydroxyl radical formation. This opinion agrees with those of El-Wahsh (2011). Decreased collagen fibers were observed in the seminiferous tubules and in the epididymal tubules with common fibrosis inside the epididymis. Nuclear changes observed in the present study are in accordance with the results of Nayana and Janardhanan (2000), who stated that reactive oxygen species (ROS) such as superoxide anions (O₂), hydrogen peroxide (H₂O₂), hydroxyl radical (OH) and nitric oxide (NO) are directly or indirectly

involved in DNA damage leading to inflammation, mutagenic low grade dysfunction and endothelial decreased fibrinolysis with increased cardiovascular caused by hyperlipidemia. risk The changes histopathological which were observed in the present study are in accordance with those of Fried et al. (2001) who stated that hyperlipidemia plays a role in the pathogenesis of renal and hepatic diseases especially in walls of the blood vessels and this may drive the cascade of events that leads to impaired organ functions. Fibrosis observed in the present study was also realized by Antolin et al. (2009), who noticed a relation between obesity and different degrees of fibrosis.

Natural products and plants are highly in demand in developed as well as developing countries for primary health care because of their wide biological and medicinal activities, higher safety margins and lower cost (Palav and D' mello, 2006; Chattopadhyay and Bhattacharyya, 2007; El-Wahsh, 2011). Hyperlipidemia is known to enhance the risk of fatty liver disease (Festi et al., 2004) and carcinogenesis which is associated with hydroxyl radical formation. Decreased collagen fibers realized in the present study was also observed by El-Wahsh (2011) in the central vein of the hyperlipidemic liver, but they were increased in between hepatocytes. She also noticed a decrease in PAS+ve materials and total protein in the liver and kidney of hyperlipidemic rats. George et al. (2001) suggested that decreased synthesis of collagenolytic enzymes by the impaired hepatocytes might contribute to further accumulation of collagen.

Horn et al. (1985) declared that the presence of collagen in the presinusoidal spaces might affect the blood supply to liver cells and would reduce the exchange of metabolites, perhaps causing hepatocellular dysfunction and necrosis. Enzan et al. (1995) attributed a similar finding to the myofibroblast-like activation of cells present normally within the hepatic and renal parenchyma. George et al. (2001) suggested that decreased synthesis of collagenolytic enzymes by the impaired hepatocytes might contribute to further accumulation of collagen. Cholesterol treatment showed decreased stain affinity of PAS+ ve materials PAS and total protein in the testis and epididymis, but PAS+ ve materials increase in the thickened walls of blood vessels of the testis. Decreased protein content observed in the present study was also realized by El Banhawy et al. (1986), Eid and Al Dossary (2007) and El-Wahsh (2011) under physical and chemical factors.

According to El Banhawy *et al.* (1986) decreased protein content in the liver tissue may be due to increased action of lytic enzymes. In 2007, Eid and Al Dossary stated that the decrease in protein content in liver tissue may be due to the drastic effect on rough endoplasmic reticulum (RER), mitochondria and Golgi apparatus and increased lysosomes in the hepatocytes. Methionine treatments (0.5% increased cells of the & 2%) showed spermatogenic layers and Leydig cells, increased number of mature spermatozoa in the epididymal tubules with thin walls of them, but the higher dose showed edema in the interstitial spaces and walls of the epididymal tubules contained stratified cuboidal cells with immature spermatozoa in their lumens. This was accompanied with decreased collagen fibers in the testis and epididymis of both treatments, but in group (M2) they were increased in the interstitial spaces between the seminiferous tubules and in the connective tissue in between the epididymal tubules with common fibrosis outside and inside them.

Rousovan et al. (1992) declared that the increase in collagen fibers may be due to increased interstitial tissue and the white fibers under the effect of different factors, but Hassan et al. (1988) reported that increased collagen fibers may lead to increase the defense reaction against toxic materials. Methionine treatment (0.5%)showed deeply stained of PAS + ve materials in the spermatozoa inside the epididymis with less stained connective tissue in between the epididymal tubules and they are somewhat normal in the testis. Normal stained basement membranes and moderately stained Leydig cells were observed, while group (M2) showed decreased stain affinity of PAS + ve materials in the cuboidal cells of the epididymal tubules and in the testis especially in the basement membranes of the seminiferous tubules with deeply stained walls of the blood vessels inside the testis and less stained widened areas of the connective tissue in the epididymal tubules.Increased stain affinity of PAS + ve materials inside the testis and epididymal tubules may be due to increased cells of the spermatogenic layers especially crowded mature spermatozoa. Increased stain affinity of total protein in the testis and the epididymal tubules was observed post treatment with methionine (M1&M2). This may be due to increased cells of the spermatogenic layers, stratified cuboidal cells in the epididymal tubules or increased connective tissue. Ameliorative role of methionine against adverse effects of cholesterol is obvious in this study, where seminiferous tubules and epididymal tubules somewhat restored their normal architecture from the histological and histochemical point of view especially in group (CH+M1), but fibrosis and edema were still detected in group (CH+M2).

Together with cysteine, methionine is one of two sulfurcontaining proteingenic amino acids. Its derivative *S*-adenosyl methionine (SAM) serves as a methyl donor. Methionine is an intermediate in the biosynthesis of cysteine, carnitine, taurine, lecithin, phosphatidyl -choline, and other phospholipids. Improper conversion of methionine can lead to atherosclerosis (**Refsum** *et al* (1998). Antioxidant capacity of methionine was studied by **Coyan** *et al* (2010). The results of **Noori** *et al.* (2012) confirmed that dietary supplementation of methionine has growth performance benefits and influences the serum metabolite profile in broilers. Supplementation with 800 ppb methionine also seems to increase carcass yield in these chickens. In the present study, higher dose of methionine (2%) showed some adverse effects on the testis and epididymis. This result is in agreement with those of Gomez et al. (2009). They stated that increased methionine supplementation led to an increase in mitochondrial ROS generation in rat liver mitochondria, but not in rat heart. Antioxidative properties and radical scavenging activity may be the possible mechanisms by which methionine ameliorated cholesterol adverse effects. Animals are protected against ROS by these defense systems: (1) enzymatic antioxidant such as superoxide dismutase, glutathione peroxidase and catalase. (2) non-enzymatic antioxidant such as β -carotene, retinol, vitamin C, methionine and glutathione (Halliwell 1996; Refsum et al., 1998 ; Gomez et al., 2009).

Generally reactive oxygen species (ROS) produced by several agents play a key role in the pathological processes of a wide range of the body diseases. Thus, antioxidants are expected to decrease the adverse effects of these harmful agents, but with adjusted doses, dose rate and time of treatment. It is clear that the optimum level of required nutrients is better for human.

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الملخص العربي تأثيرالكوليستيرول و/أو الميثيونين على خصية الجرذان نهال على مصطفى أبو النجا جامعة الأزهر – كلية العلوم – قسم علم الحيوان_فرع البنات

المثيونين من الأحماض الأمينية الأساسية التي لها دور كبير في عملية النمو وتجدد الخلايا ، وعلى الجانب الآخر فإن زيادة نسبة الكوليستيرول في الوجبة الغذائية لها أضرارا عديدة على الأوعية الدموية وجميع أجزاء الجسم. وقد استهدف هذا البحث دراسة الآثار المترتبة على إضافة نسبة من كل من الكوليستيرول والميثيونين كل على حده أو مجتمعين في الوجبة الغذائية، وقد استخدم في هذا البحث60من الجرذان البيضاء تتراوح أعمارهم مابين 5-7 أسابيع قسموا إلى ست مجموعات تناولوا الوجبة الأساسية القياسية. المجموعة الأولى: الضابطة. المجموعة الثانية: تناولت% 2 كوليستيرول مضافة إلى الوجبة الأساسية. المجموعة الثالثة: 0.5% مثيونين مضافة إلى الوجبة الأساسية. المجموعة الرابعة: تناولت % 2 مثيونين. المجموعة الخامسة: تناولت %0.5 مثيونين بالإضافة إلى% 2 كوليستيرول. المجموعة السادسة: تناولت % 2 كوليستيرول بالإضافة إلى % 2 مثيونين، استمرت التغذية لمدة أربعة أشهر ثم تم الذبخ وفصل الخصية وتحضير قطاعات هستولوجية وهستوكيميائية حيث تم صبغ البروتين والكولاجين والكربوهيدرات. أوضحت النتائج تغيير ملحوظ في خصية الجرذان بعد إضافة الكوليستيرول إلى الوجبة الغذائية حيث قل قطر الأنابيب المنوبة مع ارتفاع طبقة الخلايا المبطنة لها و ظهور خلل في تكوبن الحيوانات المنوبة وتغير كمية الكربوهيدرات والبروتين والكولاجين. أوضحت الدراسة أن إضافة

المثيونين إلى الوجبة الغذائية قد ساعدت على تجدد خلايا الخصية مع تحسن ملحوظ في القياسات الهستوكيمائية خاصة عند إضافته بنسب بسيطة إلى الوجبة الغذائية.