

## EFFECTS OF SOME ANTIOXIDANT VITAMINS AND CHELATING AGENT ON BIOCHEMICAL ALTERATIONS IN HYPERCHOLESTEROLEMIC RATS.

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### ABSTRACT

A total number of sixty adult male Wistar rats were used in this study to investigate the protective effects of vitamin C (vit C), vit E and EDTA alone and their combinations on hypercholesterolemia through markers of lipid profile, thyroid function, some liver and oxidative enzymes. Rats were randomly divided into six groups ( $n = 10$ ). Group I: normal control, Group II: hypercholesterolemic rats, Groups III: received ethylenediaminetetraacetic acid (EDTA) (1gEDTA/kg 1g/Kg), group IV: received 1gml/Kg body weight of vit C, group V: received vit E 1g/Kg feed and Group IV received EDTA, vit C and vit E. The results of this study showed that ration supplemented with EDTA, VC and vit E either individual or in a

Combination group induced significant decrease ( $P < 0.05$ ) in total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels in hypercholesterolemic rats. Also, atherogenic index (AI), lipid peroxidation (MDA), thyroid stimulating hormone (TSH), the activities of serum aspartate and alanine aminotransferases (AST, ALT), alkaline phosphatase (ALP) gamma-glutamyltransferase (GGT) levels recorded significant decrease, but high-density lipoprotein (HDL), superoxide dismutase (SOD), reduced glutathione (GSH), serum triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>) exhibited significant increase. Moreover, the administration of EDTA to hypercholesterolemic rats (group III) causes significant decrease in serum calcium (Ca), phosphorus (P), magnesium (Mg) and iron with significant increase in total iron binding capacity (TIBC) in comparison with other groups. The overall result, the improvement being more in rats treated with mixture of EDTA, vit C and vit E. The present results suggested that

supplementation with EDTA, vit C and vit E attenuated most of the changes induced in hypercholesterolemic rats by feeding rats with cholesterol-rich diet owing to their observed anti-hyperlipidemic and antioxidant properties. In conclusion, the present study provides obvious evidence that both vit C, vit E and EDTA hold the promise of being useful hypocholesterolemic, and peroxidative agent and should be administered to the body continuously, targeted to the biological site susceptible to oxidative damage.

**KEY WORDS:** Hyperlipidemia oxidative stress, EDTA and antioxidant Vitamins.

## INTRODUCTION

Hyperlipidemia is characterized by elevated serum total cholesterol, low density, and very low-density lipoprotein and decreased high-density lipoprotein levels. Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease [1]. Blood cholesterol levels increase with free radical stress, elevation of blood cholesterol may be an indicator, not a cause, of excessive free radicals and increased risk of atherosclerosis and apoptosis. As cholesterol becomes oxidized, in the form of low-density lipoprotein (LDL) cholesterol, LDL receptor sites in the liver and elsewhere are altered, causing increased hepatic synthesis of endogenous cholesterol [2]. The above abnormalities of lipid metabolism associated with overt hypothyroidism predispose to the development of atherosclerotic coronary artery disease (CAD) [3]. Atherosclerosis is an emphatically serious condition where medium and large arteries become clogged up by fatty substances results in formation of plaques, the oxidized LDL is directly involved in the initiation of atherogenesis, and oxidative stress is also affected by thyroid function. Furthermore, endothelial and cardiac functions as well as atherosclerosis have been positively associated with thyroid hormone levels [4].

Reactive oxygen species (ROS) can cause nucleic acid mutation, protein oxidation and lipid peroxidation, contributing to the development of various diseases like atherosclerosis, inflammation, neurodegenerative diseases, cataract and cancer [5]. Antioxidants are the vitamins, minerals, enzymes, or other chemical compounds such as ethylene diaminetetraacetic acid (EDTA) that give up an electron to stop free radicals from causing oxidation. They play a key role in neutralizing the estimated thousands of oxidative hits in each cell suffers a day [6]. In other words, antioxidants are able to destroy free radicals in cells before they can attack DNA or cause lipids to oxidize. EDTA is a synthetic amino acid used orally and intravenously to cleanse, detoxify, and remove heavy metals from the body. EDTA

chelating therapy was approved for use by the Food and Drug Administration (FDA) in 1950. Physicians and alternative medicine practitioners also use EDTA to treat cardiovascular disease to improve circulation, remove plaque and improve oxygen flow to the brain since it was approved by the FDA in 1950 <sup>[7,8, 9]</sup>. Since generation of free radicals is the underlying mechanism responsible for lipid peroxidation, antioxidant vitamins C and E could possibly play important role of ameliorating some of these effects. Vitamin C, a known water soluble antioxidant is reported to reacts with peroxyl radicals formed in the cytoplasm before they reach the membrane <sup>[10]</sup> thereby preventing injury to the membrane; it also has a sparing effect on vitamin E <sup>[11]</sup>. While Vitamin E, a lipid soluble free radical scavenger is reported to protect the membrane from lipid peroxyl radical. Moreover, Vitamin C and E are important antioxidant, able of scavenging oxygen-derived free radicals, improved hyperlipidemia and decreased blood pressure <sup>[12]</sup> The present study aimed to investigate the protective effects of EDTA, vit C and vit E alone and/or their combinations on diet-induced hypercholesterolemia in rats on markers of lipid profile, thyroid function, some liver enzymes and oxidative stress.

## MATERIALS AND METHODS

**Animals:** Sixty adult male Wistar rats (140–180 g) obtained from animal house of the National Research Center (Cairo) were used in the present study. The animals were housed ten per cage, kept under suitable environmental conditions (temperature  $22 \pm 2$  °C; humidity  $55 \pm 4\%$ ) with a 12 h light/dark cycle and allowed free access to food and water *ad libitum*.

### Induction of Hypercholesterolemia

Hypercholesterolemia was induced by feeding 50 rats for four weeks with diet composed of essential diet (90.75%) cholesterol (1.5%), cholic acid (0.25%) and lard (7.5%) according to the method described by <sup>[13]</sup>.

### Experimental Design

Rats were randomly divided into six groups ( $n = 10$ ). Group I: (normal control) and Group II: (hypercholesterolemia) Groups III: received EDTA (1.0g EDTA/kg feed) Group IV: received 1g vitC/kg feed, Group V: received 1g vit E /kg feed and Group VI: concomitantly received a combination of EDTA, vit C and vit E as the same previous doses.

**Sampling:** At the end of the experiment (six weeks) blood samples were obtained from the orbital sinus and centrifuged at 3500 rpm for 15 minutes and serum was separated and kept at -20°C for further biochemical analysis, then animals were sacrificed and autopsied

immediately; liver specimen were taken, washed with saline and kept in liquid nitrogen in deep freezer at (-40°C) until estimation of lipid peroxidation <sup>[14]</sup>. Superoxide dismutase <sup>[15]</sup> as well as the estimation of reduced glutathione <sup>[16]</sup>.

### Biochemical Estimations

Total cholesterol was measured as described by <sup>[17]</sup>. Serum triglycerides according to <sup>[18]</sup>. HDL according to <sup>[19]</sup>. LDL-C and VLDL-C concentrations were calculated with Friedewald formula <sup>[20]</sup> Atherogenic index was calculated according to the formula adopted by. <sup>[21]</sup> as follows: total cholesterol /HDL and LDL/HDL. T<sub>3</sub>,T<sub>4</sub> and TSH hormones were analyzed by radioimmunoassay (RIA) method <sup>[22]</sup> Alanine and aspartate amino transaminase (ALT and AST) activities were determined according to <sup>[23]</sup>, serum GGT and alkaline phosphatase was determined according to <sup>[24,25]</sup> respectively, serum calcium, inorganic phosphorus and magnesium were measured as described by <sup>[26,27,28]</sup> respectively, iron and total iron binding capacity according to <sup>[29]</sup>.

**Statistical Analysis:** In the present study, all results were expressed as mean  $\pm$  S.E of the mean. Statistical package for the social Sciences (SPSS) program, version 14.0 was used to compare significance between groups, differences was considered significant when  $p < 0.05$  according to <sup>[30]</sup>.

## RESULTS AND DISCUSSION

**(Table1): Effect of EDTA, Vit C and Vit E And/ or Their Combinations on Serum Lipid Profile of Hypercholesterolemic Rats.**

Groups Parameters	Control	Hypercholesterolemia	EDTA	Vit C	Vit E	EDTA+C+E
TC (mg/dl)	178 $\pm$ 1.75	306 $\pm$ 1.69 <sup>a</sup>	216.0 $\pm$ 1.62 <sup>ab</sup>	228 $\pm$ 1.09 <sup>ab</sup>	221 $\pm$ 1.55 <sup>ab</sup>	188 $\pm$ 0.58 <sup>b</sup>
TG(mg/dl)	116 $\pm$ 1.95	259 $\pm$ 2.81 <sup>a</sup>	152.5 $\pm$ 1.35 <sup>ab</sup>	165.5 $\pm$ 1.11 <sup>ab</sup>	172 $\pm$ 1.0 <sup>ab</sup>	122 $\pm$ 1.64 <sup>b</sup>
HDL(mg/dl)	68 $\pm$ 1.68	41.3 $\pm$ 1.4 <sup>a</sup>	54.3 $\pm$ 0.86 <sup>ab</sup>	53.3 $\pm$ 0.88 <sup>ab</sup>	152. $\pm$ 1.71 <sup>ab</sup>	69.3 $\pm$ 1.2 <sup>b</sup>
LDL(mg/dl)	88.1 $\pm$ 0.6	214 $\pm$ 1.27 <sup>a</sup>	131.6 $\pm$ 1.02 <sup>ab</sup>	141.6 $\pm$ 1.57 <sup>ab</sup>	134.5 $\pm$ 1.7 <sup>ab</sup>	95 $\pm$ 1.11 <sup>b</sup>
VLDL(mg/dl)	23.1 $\pm$ 0.83	51.8 $\pm$ 0.76 <sup>a</sup>	30.50 $\pm$ 0.67 <sup>ab</sup>	33.1 $\pm$ 0.62 <sup>ab</sup>	34.4 $\pm$ 0.60 <sup>ab</sup>	24.6 $\pm$ 0.5 <sup>b</sup>
TCh/HDL	2.69 $\pm$ 0.03	7.4 $\pm$ 0.13 <sup>a</sup>	3.97 $\pm$ 0.02 <sup>ab</sup>	4.27 $\pm$ 0.68 <sup>ab</sup>	4.32 $\pm$ 0.06 <sup>ab</sup>	2.72 $\pm$ 0.4 <sup>b</sup>
LDL/HDL	1.35 $\pm$ 0.25	5.2 $\pm$ 0.33 <sup>a</sup>	2.42 $\pm$ 0.54 <sup>ab</sup>	2.66 $\pm$ 0.22 <sup>ab</sup>	2.58 $\pm$ 0.56 <sup>ab</sup>	1.37 $\pm$ 0.4 <sup>b</sup>

Data are expressed as means  $\pm$  S.E. (n = 10 in each group).

A: Significant change at  $p < 0.05$  with respect to control group I.

B: Significant change at  $p < 0.05$  with respect to hypercholesterolemic group II.

**(Table2): Effect of Edta, Vit C And E And/ or Their Combinationson Some Oxidative Stress Parameters In The Liver Tissue of Hypercholesterolemic Rats.**

Groups Parameters	Control	Hyperchole- sterolemia	EDTA	Vit C	Vit E	EDTA+C+E
MDA (nmol/g tissue)	34.3±1.16	82,4±1.09 <sup>a</sup>	39±0.14 <sup>b</sup>	37.8±0.67 <sup>b</sup>	36.7±.99 <sup>b</sup>	32.8±0.63 <sup>b</sup>
SOD(μmol/g tissue)	2.64±0.07	0.98±0.06 <sup>a</sup>	2.22±0.03 <sup>b</sup>	2.35±0.02 <sup>b</sup>	2.32±0.4 <sup>b</sup>	2.76±0.8 <sup>b</sup>
GSH(μmol/g tissue)	24.93±0.4 2	12.66±0.31 <sup>a</sup>	22.3±0.26 <sup>b</sup>	23.25±0.23 <sup>b</sup>	24.16±0.36 <sup>b</sup>	26.21±0.19 <sup>b</sup>

Data are expressed as means ± S.E. (n = 10 in each group)

a: Significant change at p< 0.05 with respect to control group I.

b: Significant change at p< 0.05 with respect to hypercholesterolemic group II.

**(Table3): Effect of EDTA, Vit C and Vit E And/ or Their Combinations on Thyroid Function of Hypercholesterolemic Rats.**

Groups Parameters	Control	Hyperchole- sterolemia	EDTA	Vit C	Vit E	EDTA+C+E
T <sub>3</sub> (ng/ml)	0.88±0.02	0.41±0.08 <sup>a</sup>	0.66±0.07 <sup>ab</sup>	0.72±0.06 <sup>ab</sup>	0.70±0.5 <sup>ab</sup>	0.84±0.01 <sup>b</sup>
T <sub>4</sub> (ng/ml)	9.43±0.13	4.07±0.1 <sup>a</sup>	7.55±0.15 <sup>ab</sup>	7.62±0.2 <sup>ab</sup>	7.53±0.17 <sup>ab</sup>	9.55±0.12 <sup>b</sup>
TSH(ng/ml)	4.07±0.11	7.92±0.15 <sup>a</sup>	5.21±0.07 <sup>ab</sup>	4.65±0.09 <sup>ab</sup>	4.81 ±0.08 <sup>ab</sup>	4.0±0.08 <sup>b</sup>

Data are expressed as means ± S.E. (n = 10 in each group).

a: Significant change at p< 0.05 with respect to control group I.

b: Significant change at p< 0.05 with respect to hypercholesterolemic group II.

**(Table4): Effect of EDTA Vit C and E Vit E And/ or Their Combinations Consumption on Liver Enzymes of Hypercholesterolemic Rats.**

Groups Parameters	Control	Hyperchole- sterolemia	EDTA	Vit C	Vit E	EDTA+C+E
ALT (IU/L)	14±0.70	39.0±0.88 <sup>a</sup>	18.9±1.08 <sup>b</sup>	17.5±0.48 <sup>b</sup>	16.6±0.5 <sup>b</sup>	12±0.54 <sup>b</sup>
AST (IU/L)	19.6±0.67	57.0±0.17 <sup>a</sup>	26.0±0.87 <sup>b</sup>	25.5±0.72 <sup>b</sup>	23±1.09 <sup>b</sup>	21±0.86 <sup>b</sup>
GGT (IU/L)	13.8±0.57	25.8±1.00 <sup>a</sup>	14.5±0.42 <sup>b</sup>	16.5±0.61 <sup>b</sup>	16.0 ±0.75 <sup>b</sup>	11.2±0.71 <sup>b</sup>
Al P (IU/L)	41±0.41	74.5±0.69 <sup>a</sup>	52.5±0.45 <sup>b</sup>	47.1±0.93 <sup>b</sup>	50±1.02 <sup>b</sup>	39.4±0.6 <sup>b</sup>

Data are expressed as means ± S.E. (n = 10 in each group).

a: Significant change at p< 0.05 with respect to control group I.

b: Significant change at p< 0.05 with respect to hypercholesterolemic group II.

**(Table5): Effect of EDTA Vit C and E Vit E And/ or Their Combinations Consumption on Minerals of Hypercholesterolemic Rats.**

Groups Parameters	Control	Hypercholesterolemia	EDTA	Vit C	Vit E	EDTA+C+E
Camg/dl	10.63±0.12	10.74±0.15 <sup>b</sup>	8.89±0.14 <sup>a</sup>	10.88±0.07 <sup>b</sup>	10.92±0.01 <sup>b</sup>	10.94±0.1 <sup>b</sup>
P mg/dl	5.45±0.55	5.11±1.07 <sup>b</sup>	3.21±1.03 <sup>a</sup>	5.26 ±0.77 <sup>b</sup>	5.31±0.53 <sup>b</sup>	5.33±0.44 <sup>b</sup>
Mg mg/dl	2.7±0.43	2.65±0.66 <sup>b</sup>	1.70±0.45 <sup>a</sup>	2.9±0.89 <sup>b</sup>	2.70 ±1.05 <sup>b</sup>	2.60±0.12 <sup>b</sup>
Iron ( µg/ dl)	28.1±0.71	24±0.9 <sup>b</sup>	15.6±0.76 <sup>a</sup>	24.8±0.81 <sup>b</sup>	25.8±1.03 <sup>b</sup>	25.1±0.95 <sup>b</sup>
TIBC ( µg/ dl)	116.5±1.31	123.4±1.49 <sup>b</sup>	183.2±1.46 <sup>a</sup>	121±0.98 <sup>b</sup>	120±0.9 <sup>b</sup>	119±0.58 <sup>b</sup>

*Data are expressed as means ± S.E. (n = 10 in each group).*

*a: Significant change at  $p < 0.05$  with respect to control group I.*

*b: Significant change at  $p < 0.05$  with respect to hypercholesterolemic group II.*

The quantity and quality of fats present in the ration play an important role in the regulation of the synthesis of cholesterol and triglycerides-rich lipoproteins, bile acid secretion, and intestinal output of cholesterol as well as its metabolites.

Disorders of lipid and lipoprotein metabolism *i.e.* dyslipidemia are traditional risk factors for atherosclerosis, These conditions may be manifested by: (1) chest pain with exertion (angina); (2) sensory loss due to reduced blood flow to the brain, (transient ischemic attacks temporary "mini-strokes"); or (3) pain in the calves after walking relatively short distances which is relieved by rest (intermittent claudication).

Data of the present study in table (1), revealed marked disturbance in lipid profile of rats fed with cholesterol-rich diet manifested by increased serum levels of TC, TG and LDL-c as well as atherogenic index parallel to a decrease in serum HDL-c level in comparison with the control. Similar results were reported by other investigators <sup>[31, 32, 33, 12]</sup>. This significant increase could be explained via involvement of two enzymes namely cholesterol ester hydrolase (CEH) and cholesterol ester synthetase (CES). These enzymes balance the cholesterol levels in the blood, with increased estrifying activity (when CEH: CES is lowered) cholesterol will be predominantly in its ester form(as in LDL) and can lead to accumulate leaving plasma LDL levels high, the present result is well documented by the study of <sup>[34]</sup>. It has been reported that cholesterol transport to extra hepatic tissues is primarily ensured by LDL while HDL has an important role in reversing the cholesterol transport process <sup>[35]</sup>. <sup>[36]</sup> demonstrated that hypercholesterolemia is accompanied by lipid deposition in the vessel resulting in foam cell, plaque formation and vascular calcification. Hypercholesterolemia is also associated with the production of oxidized LDL (oxLDL) which



is involved in endothelial injury, vascular calcification and increased aortic thickness<sup>[8]</sup>. Also, increasing atherogenic index in these result indicating risk of heart disease.<sup>[37]</sup> Have reported that changes in ratios of TC/HDL-C and LDL-C/HDL-C are better predictors of CHD risk.

On the other hand, hypercholesterolemic group treated with EDTA, vit E and vit C showed significant decrease in lipid profile in comparison with group II either individual or in a combination in the ration of hyperlipidemic rats<sup>[9,12, 33and 38]</sup> EDTA can improve lipid profile, atherosclerosis and cardiovascular diseases via their ability to chelate ectopic or metastatic calcium from atherosclerotic plaques also, inhibit cell-mediated LDL oxidation<sup>[39]</sup>. Vitamin C helps in the metabolism of TC, by increasing its elimination and thereby assisting lower blood TC<sup>[40]</sup>. Similarly, the decreases of TG concentrations observed during this experiment confirmed as well by the previous studies of<sup>[12,38,41]</sup>, these reduction can be explained by firstly, when rats were supplemented with ascorbate, the corticoid secretion was reduced and the lipoprotein and tissue lipases were consequently not stimulated. As a result, lipids and TC were not mobilised from tissues. Secondly, ascorbate is necessary for the transformation of TC to bile acids (most important pathway of cholesterol catabolism) simultaneous with increased fecal and liver bile acids through activation of 7 $\alpha$ -hydroxylase the limiting step of the TC catabolism in liver<sup>[42]</sup> Thirdly, ascorbate is required for carnitine synthesis, as carnitine improves beta-oxidation of lipids, leading to reduction of serum TG concentrations<sup>[42, 43]</sup> Moreover, Alpha-tocopherol (vit E) has been found to be the most abundant antioxidant in LDL particles by decreasing the susceptibility of LDL particles to oxidation, which suggests that alpha-tocopherol could prevent atherosclerotic lesions through the decrease of cholesterol concentrations and LDL oxidation. The reduction has been explained by elevated fecal excretion of cholesterol, impaired liver cholesterol uptake and increased plasma thyroxin levels<sup>[44]</sup>, However, Vit. E-induced decline in triglyceride concentrations, the reason might be due to a reduced synthesis of fatty acid<sup>[45]</sup>.

The present study showed significant increase in liver MDA levels and a significant reduction in liver SOD and GSH when compared with the normal control values table (2) as recorded by<sup>[31,33,39],[46]</sup>, stated that increased levels of MDA in the hyperlipidemic group attributed to increased reactive oxygen species (ROS) production and/ or deficiency of antioxidant defence system resulting decreased of SOD and GSH Activities. High level of cholesterol induces modification in lipid composition of cell membranes and the extracellular matrix to be more prone to free radical generation, overproduction of (ROS) plays a pivotal role in the oxidation of LDL molecules, which get accumulated in the layers of blood vessels, oxidized LDL may

affect many other aspects of arterial wall metabolism and thus contribute to the atherogenic process. Moreover, it has been found that oxidative stress causes mRNA expression levels of NADPH oxidase subunits increased, and mRNA expression levels and activities of antioxidant enzymes decreased<sup>[47]</sup>.

It is not surprising that the increased lipid peroxidation recorded in this study was completely reversed by antioxidant vitamins C or E and EDTA supplementation. Data presented in table 2 showed that, the supplementation of EDTA vit C, vit E either alone or combined resulted in a significant reduction ( $p < 0.05$ ) of liver MDA and a significant elevation of SOD and GSH when compared to the hypercholesterolemic group II. In this respect, there are published reports concordant with our results<sup>[9, 11, 33, 38, 48]</sup>

EDTA chelation therapy is effective for heart disease. It may help animals with atherosclerosis (hardening of the arteries) or peripheral vascular disease (decreased blood flow to the legs) by clearing clogged arteries and improving blood flow by preventing damaging molecules known as free radicals from injuring blood vessel walls and allowing plaque to build up.<sup>[8]</sup> suggested that traces of unbound metallic ions act as catalysts of uncontrolled proliferation of free radicals in tissues as calcium activates phospholipase-A<sub>2</sub> resulting in increased levels of arachidonic acid which in turn result in the production of more free radicals. EDTA can greatly reduce the excessive production of free radicals by binding those ionic metals, making them chemically inert or its ability to chelate calcium and rapidly removing them from the body<sup>[49]</sup>. Moreover EDTA was reported to inhibit cell-mediated LDL oxidation, and to chelate transitional metals which are important catalyst for lipid peroxidation, LDL oxidation and free radical formation<sup>[8]</sup>. Vit C is an efficient scavenger, or reducing antioxidant, capable of donating its electrons to ROS and eliminating them, because the ascorbyl radical is relatively stable, it makes ascorbate a powerful, important antioxidant. Furthermore, vit C prevents the prooxidant activity of vitamin E by decreasing the activity of  $\alpha$ -tocopheroxyl radical to  $\alpha$ -tocopherol thereby acting as a co-antioxidant and further contributing to increased total antioxidant status<sup>[50]</sup>. Supplementation of Alpha-tocopherol was shown to reduce plasma MDA levels significantly, this could be due to the fact that, vit E increased the level of SOD and GSH, these enzymes scavenge free radicals and prevent oxidative damage<sup>[51]</sup>, Alpha-tocopherol is lipid soluble and has been found to be the most abundant antioxidant in LDL particles, Alpha-tocopherol supplementation could decrease ROS and apoptosis in endothelial cells induced by OxLDL,



prevent atherosclerotic lesions through the decrease of LDL oxidation <sup>[47]</sup>. The efficiency of vitamin E against lipid peroxidation was apparent through the reduction of the susceptibility of erythrocytes to hydrogen peroxide-induced lipid peroxidation and a potent lipophilic agent that forms an important scavenger component of the cell membrane, it may protect the safety of the membrane by reducing the production of lipid peroxides <sup>[52]</sup>.

Thyroid function regulates a wide array of metabolic parameters, they induce the 3-hydroxy-3-methylglutarylcoenzyme A(HMG-CoA) reductase, which is the first step in cholesterol biosynthesis. Moreover, triiodothyronine (T3) upregulates LDL receptors by controlling the LDL receptorgene activation, also,T3 has been associated with protecting LDL from oxidation Thyroid hormones can influence HDL metabolism by increasing cholesteryl ester transfer protein (CETP) activity, which exchanges cholesteryl esters from HDL2 to the very low density lipoproteins (VLDL) and triglycerides <sup>[53]</sup>.

From table (3), it is clearly notable that rats fed on hyperlipedemic ration gp II had significant decreased ( $P < 0.05$ ) in serum T<sub>3</sub>, T<sub>4</sub> with significant increase in TSH in comparison with group I and group VI. These result come in accordance with <sup>[3]</sup> .<sup>[54]</sup>, mentioned that there was an association between lipid profile and thyroid function ,hypothyroid rats are accompanied by reduced activity of HMG-CoA reductase, with increased levels of total cholesterol (TC) and LDL This is due to the decreased LDL-receptors' activity, resulting in decreased catabolism of LDL. Moreover, a decrease in lipoprotein lipase activity with subsequent decreasing the clearance of triglycerides (TG) rich lipoproteins, so increased levels of TG and VLDL <sup>[55]</sup>.

Treatment hypercholesterolemic rats with EDTA, vit C or vit E causes gradual improvement in lipid profile which in turn causes gradual increase in thyroid function, While the uses of the combination EDTA vit C and vit E group VI strengthens the effect of each one which reflected in improvement of lipid profile, thyroid function, this may be due to interactive or additive effects of the numerous bioactive constituents found in these combination <sup>[56]</sup>

Results of ALT, AST, alkaline phosphatase, GGT are given in Table (4). The result revealed that serum ALT and AST activities increased significantly ( $P < 0.05$ ) in hypercholesterolemic rats group II in comparison with other groups; these results come in accordance with <sup>[2]</sup>. The significant changes in activities of these enzymes in blood serum indicate that tissue impairment caused by dyslipidemia may be led to adverse effect by increasing lipid

peroxidation which in turn produce damage to liver tissue so outflow of these enzymes from the liver cytosol to the blood stream which indicate that inability of liver to metabolize the ALT and AST <sup>[11]</sup>. While, the addition of EDTA, vit C or vit E either alone or in combination significantly decreased these enzymes activities due to their anti-oxidants activities as reported by <sup>[8, 41, 51 and 57]</sup>

The result recorded in table(5) pointed out that addition of EDTA to hypercholesterolemic rat group III causes significant decrease in serum Ca, P, Mg and Fe with significant increase in TIBC in comparison with other groups these results are in are inharmony with those established by <sup>[41, 49]</sup>. This finding may be referring to the properties of EDTA as a chelating agent to minerals <sup>[58],[59]</sup>, expressed that there was significantly increased in urinary losses of minerals following EDTA chelating therapy. While adding vit C and vit E to EDTA, (group VI) improves the EDTA application by decreasing depletion of serum minerals <sup>[41, 57]</sup>

## CONCLUSIONS

The present study revealed that supplementation with EDTA, vit C or vit E in hypercholesterolemic rats attenuated the alteration in the lipid profile, thyroid function and oxidative stress coupled by a compensatory increase in serum level of liver enzymes. Most of these changes owing to their observed anti-hyperlipidemic and antioxidant properties, but it seems that concomitant using of these substances with together strengthen the effect of each one. In conclusion, EDTA and antioxidant vitamins should be administered to the animals continuously, targeted to the biological site susceptible to oxidative damage.

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