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Protective role of alpha Lipoic acid on some parameters related to metabolic disorders in intact and ovariectomized rabbits

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Abstract

The present experiment was designed to study the protective role of ALA on some related metabolic disorder parameters in intact and ovariectomized rabbits. A total of twenty rabbits (7-8 weeks) have been used in this study. Ovariectomy was performed to ten of them and the other ten rabbits were left with intact ovaries. The animals were divided equally in to four groups as follows: Group one (I+D): intact rabbits received distilled water. Group two (I+A): intact rabbits received alpha lipoic acid orally (10 mg/kg B.wt). Group three (O+D): Ovariectomized rabbits received distilled water. Group four (O+A): Ovariectomized rabbits received alpha lipoic acid orally (10 mg/kg B.wt). The daily administration of ALA for 30 days caused a significant ($p \leq 0.05$) decrease in platelets number (143.8 and $132.0 \times 10^3/\text{mm}^3$ compared with 175.6 and $248.8 \times 10^3/\text{mm}^3$ in intact and Ovariectomized groups respectively), cholesterol, triglyceride, TSH and serum glucose concentration in both treated. Moreover, the result of this experiment also showed that ALA supplementation has a protective role concerning the increase in coagulation and bleeding time. There is also a significant ($p \leq 0.05$) increase in HDL (84.80 and 86.74 mg/dl compared with 82.34 and 80.38 in intact and Ovariectomized respectively), GSH and insulin concentration in groups received ALA in both intact and Ovariectomized rabbits. The results confirm the protection action of ALA in intact and ovariectomized rabbits against the effect of some aged metabolic disorder parameters.

Keywords: Alpha lipoic acid, ovariectomy, metabolic disorder

1. Introduction

Lipoic acid (LA) also known as α -lipoic acid and alpha lipoic acid (ALA) and thioctic acid is an organosulfur compound [1]. ALA is made in plants and animals normally, and is essential for aerobic metabolism. Lipoic acid is cofactor for at least five enzyme systems, two of these are important for many organisms to turn nutrients into energy [2]. Mitochondrial nutrients, such as coenzyme Q10 and α -lipoic acid, beside their acknowledged antioxidant activities, show interesting features in relation to their redox state and consequent biological activity [3]. Moreover, a recent study had reported that alpha lipoic acid (ALA)—a naturally occurring compound that is essential in energy metabolism—has been shown to exert significant anti-inflammatory, antioxidant, and neuroprotective effects in treatment of relapsing-remitting multiple sclerosis (RRMS). It is effective in reducing relapses and progression of disability [4]. In the last years, investigations in human and animal models have provided abundant evidence that age-dependent oxidative stress plays an important role in cardiovascular diseases. (Skg) [5] Studies indicate that antioxidants prevent development of many cardiovascular diseases and may even improve course of diseases, such as atherosclerosis, hypertension, ischemia reperfusion, or heart failure [6]. Alpha-lipoic acid administration has been shown to be effective in preventing or at least delaying pathological processes in various experimental models in which ROS have been implicated [7]. Gender differences in the risk of cardiovascular diseases are well recognized, with premenopausal female exhibiting a lower risk than age-matched male [8]. The advantage of female over male in cardiovascular morbidity disappears after menopause, suggesting that estrogen plays an important role in cardiovascular health. Estrogens are known to exert beneficial effects on the vascular wall [9]. Estrogen treatment improves endothelial dysfunction, a major contributor to the pathophysiology of cardiovascular disease, through upregulation of endothelial cell genes, such as endothelial nitric oxide synthases [10]. Therefore, in light of the above insights, present study was conducted to investigate the potential influence of oral supplementation with ALA on some aged related metabolic disorders in intact and ovariectomized rabbits

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2. Materials and Methods

2.1 Experimental animals and management

This experiment was carried out in the animal house at the College of Veterinary Medicine/University of Baghdad during the period from December/2016 till February/2017. Twenty female Rabbits were used in this study, their ages were ranged between 7-8 weeks, and their weight was around 820-1050 gram. These animals were kept under suitable environmental conditions of 20-25 °C in an air conditioned room and photoperiod of 12 hours daily [11]. Fresh water and pellet diet were offered all over the period.

2.2 Ovariectomy Operation technique

Ovariectomy for both ovaries was performed to ten rabbits according to the procedure by [12]. The other ten rabbits were left with intact ovaries. The animals were kept individually with post-operative care and penicillin streptomycin was injected intramuscularly for 5 days.

2.3 Alpha lipoic acid dose

Thiotacid tablets contain thioctic acid (alpha lipoic acid) EVA pharma-Egypt could be found as 300mg-1800mg and be taken daily. The dose for each rabbit (10mg/kg) B.wt. was calculated according to [13].

2.4 Blood samples collection

At the end of the period, fasting blood was obtained from jugular vein and serum was isolated after centrifugation at a speed of 3000 rpm for 20 minutes. Other sample of whole blood were also collected for estimation of thrombosis profile.

2.5 Experimental Design: After recovery and acclimatization of animals, they were divided equally into four groups, one group from the intact and ovariectomized were supplemented daily by stomach tube with 10 mg/kg B.wt ALA. The other two group, one intact and one ovariectomized were received distilled water.

2.6 Parameters determination

Blood platelets was counted manually according to the method described by [14] using hemocytometer chamber. The capillary tube method was used to determine the coagulation time and The filter paper method was used for bleeding time estimation [15]. However, enzymatic assay kits (Linear chemicals-Spain) was used for estimation cholesterol [16]. Triglycerides [17]. HDL-c [18]. Concentration in serum. Reduced glutathione (GSH) was determined according to [19]. The concentration of TSH in serum was measured using the sandwich immunodetection method [20]. Serum glucose concentration was enzymatically measured (enzymatic oxidation method) as described by [21]. The assay of serum insulin concentration was according to the competitive inhibition enzyme immunoassay technique Insulin resistance (IR) was determined using the homeostasis model assessment (HOMA1 and HOMA 2 IR) index according to [22].

2.7 Statistical analysis

All data were subjected to one -way ANOVA and least significant differences (LSD) to assess the significant differences among mean by using SAS [23].

3. Results and Discussion

3.1 The protective role of ALA on Thrombosis profile (Platelets count, Coagulation time and Bleeding time) in intact and ovariectomized rabbits.

Table -1- showed a significant ($p \leq 0.05$) increase in platelets

count in ovariectomized rabbits (G3). At the mean, time, this group show a significant ($p \leq 0.05$) decrease in coagulation and bleeding time in comparison with other groups. On the other hand, supplementation with ALA induced a significant ($p \leq 0.05$) decreased in platelets count with a non significant ($p \geq 0.05$) increase in coagulation time in both intact and ovariectomized rabbits (G2 and G4). Where as a significant ($p \leq 0.05$) longer bleeding time is evidenced in ovariectomized rabbits received ALA (G4) only. The results of increase platelets number in the current experiment is coincided with decreasing coagulation and bleeding time in the same group of ovariectomized rabbits. Loss of ovarian estrogen after ovariectomy had been reported to decrease the activity of nitric oxide synthase enzyme III as well as prostacyclin. These in turn will cause vasoconstriction of blood vessels which enhances platelets-vessel wall interactions and platelets aggregation contributing to increase response to injury which in turn would be associated with arterial occlusive diseases after menopause [24]. The decrease in platelets count in group supplemented with ALA may be associated with improvement of the endothelial function and production of nitric oxide synthase and prostacyclin [25]. This in turn will enhance blood flow and prevents the formation and precipitation of free radicals (FRs) and advances glucation end product (AGE) as well as prevents platelets aggregation due to the vasodilation of blood vessels. Groups received ALA also has longer coagulation and bleeding time. This may be attributed to the increase in blood fluidity due to the action of ALA to increase RBCs membrane elasticity. ALA inhibits the activation of nuclear factor Kappa B (NFkB) which is responsible for the precipitation of FRs on endothelium [26].

3.2 The protective role of ALA on Lipid profile (serum Cholesterol, Triglycerides and HDL-c concentration) in intact and ovariectomized rabbits

Concerning lipid profile, Table -2-reveals a higher significant ($p \leq 0.05$) increase in cholesterol and triglycerides concentration which is coincided with a significant ($p \leq 0.05$) decrease in serum HDL-c in ovariectomized group (G3). There is a significant ($p \leq 0.05$) decrease in serum cholesterol and triglycerides level accompanied by an increase in serum HDL-c concentration in intact and ovariectomized groups that received ALA (G2 and G4). There is growing concern regarding fat accumulation in tissues and organs in chronic diseases such as obesity, cardiovascular disorders, insulin resistance [27]. These diseases are commonly increased at menopause and in ovariectomized animals. However, hormonal replacement has been used to reduce the fat mass caused by Ovariectomy [28]. This is confirmed by the results of this study that ovariectomized rabbits showed higher serum cholesterol and triglyceride level which is coincided by decrease HDL concentration in serum. Whether, these changes in lipid profile and fat depot are associated with increase cholesterol synthesis or increase its absorption from the gut or decrease cholesterol uptake by tissues is not confirmed yet. However, ALA supplementation to rabbits fed on a high fat diet caused a decrease in lipid peroxidation, serum cholesterol, triglycerides, LDL-c and an increase in HDL-c [29]. Moreover, when ALA was administered intraperitoneally for 7 and 14 days to aged rats, shown to prevent the elevated lipid level [30]. This action of ALA might be related to the increase rate of lipolysis by increasing of plasma lipase activity at the same periods of decrease plasma triglyceride [31].

3.3 The protective role of ALA on some parameters related to oxidative and metabolic disorders (serum glutathione, TSH, and glucose concentration) in intact and ovariectomized rabbits.

Serum glutathione concentration is represented in table which (3) show a significant ($p \leq 0.05$) increase in ovariectomized group that received distal water (G3) as compared with intact one (G1). At the same time, ALA supplementation to group (G2 and G4) causes higher significant ($p \leq 0.05$) increase in comparison with the non supplemented rabbits (G1 and G3). Values representing TSH concentration in serum reveals a significant ($p \leq 0.05$) decrease in ovariectomized group (G3) compared with intact one (G1). Moreover, there is significant ($p \leq 0.05$) decrease in intact and ovariectomized ALA supplemented groups (G2 and G4) respectively. Concerning serum glucose level, this table shows a significant ($p \leq 0.05$) increase of glucose in ovariectomized group (G3), whereas ALA supplementation induces a significant ($p \leq 0.05$) decreases in both intact and ovariectomized groups (G2 and G4). The increase of GSH level in serum of the ovariectomized rabbits in the current study could be associated to its antioxidant action. Glutathione is one of the major endogenous antioxidants produced by cells and participating direct in neutralizing of free radical and ROS and also maintaining the exogenous antioxidants such as vitamins C and E in their active (reduced) forms [31]. On the other hand, estrogen deficiency has been related to some metabolic syndromes and CVD which characterized by increase ROS such as free radicals which lead to an increase in GSH synthesis. Although, there is no clear explanation concerning the effect of estrogen on GSH, but it could be related with the regulation of nitric oxide cycle [32]. Nitric oxide synthase (NOs) has been reported to be regulated and enhanced by Es and females have higher level of NOs than males. It is scientifically proven than ALA to increase cellular glutathione level [33] mainly by stimulating the enzyme gamma-glutamylcysteine ligase (GCL) which is involved in the synthesis of glutathione. ALA is also increase the cellular uptake of amino acid cysteine [34]. Moreover, ALA is vital in the cycle of returning glutathione from oxidized form back to its reduced (active) form. Hypothyroidism has been considered as cardiovascular risk factor in majority of studies, mainly because of its association with elevated serum total and LDL cholesterol [35]. This result documented the finding of the present study that ovariectomy induce a depression in TSH level as compared with intact rabbits. There is no available literature concerning the effect of ALA on thyroid hormone and TSH concentration in blood. Therefore, the decrease in TSH level in intact and ovariectomized rabbits supplemented with ALA could be explained by its protective role on cardiovascular system. ALA is found naturally within the mitochondria as essential cofactor for pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase which are important for energy production and improvement of ATP synthesis [36]. The metabolic syndrome (MS) is a combination of obesity, elevated blood cholesterol, triglyceride, LDL-c, low HDL-c, hypertension and impaired fasting glucose with

insulin resistance. Elevating blood glucose is coinciding with high formation of glycosylated end product the advance Glacation end product (AGEP) which is formed by reaction of proteins and sugar [37]. Moreover, AGEp increase the risk of CVD by oxidizing LDL and making blood vessels tough and inflexible. Furthermore, the glycosylated proteins are unable to bind to receptors on liver cell to signal the cessation of cholesterol manufacturing [38], intum will cause the body to continue the production of cholesterol. The depression of serum glucose concentration in rabbits supplemented with ALA is mainly attributed to its anti hyperglycemic action [39]. This action has been shown to be related with increasing glucose uptake in skeletal muscles [40].

3.4 The protective role of ALA on serum Insulin concentration and insulin resistance index (HOMA1-IR and HOMA2-IR) in intact and ovariectomized rabbits

The represented data in Table -4- show a non significant ($p \geq 0.05$) differences in serum insulin level between intact and ovariectomized rabbits. On the other hand, there is a significant ($p \leq 0.05$) increase of this level in group received ALA (G2 and G4) compared with other groups. The IR index (HOMA1 and HOMA2) significantly ($p \leq 0.05$) increased in ovariectomized group (G3 and G4) in comparison with intact groups (G1 and G2) respectively. The ovariectomized rabbits in the present study had shown an elevation of fasting glucose level beside other lipid disorders. This could be attributed to the effect of estrogen deficiency which increase insulin resistance (IR) by cells. Metabolic syndrome and insulin resistance are risk factors for type 2 diabetes which intum associated with CVD morbidity and mortality [41]. The first described model is HOMA1 – IR but the modified new model HOMA2 – IR is a more accurate representation of the metabolic process because it models the feedback relationship between insulin and glucose in the various organs in body [42]. The findings of the present study shown a significant increase in insulin level and HOMA1- IR and HOMA2-IR for the ovariectomized group. Moreover, in the current study both intact and ovariectomized groups which received ALA had an increase in serum insulin concentration. Whether this elevation in insulin level is due to direct effect of ALA on beta cells of Langerhans or indirect effect as a response for depressing glucose level is not clear yet. The effect of ALA on insulin sensitivity in type 2 diabetes mellitus had been studied [43]. It was reported that oral administration of ALA for 4 weeks, significantly improved glucose utilization, mainly by its effect on glucose transporter (GLUT1, GLUT4) to stimulate their opening. Moreover, ALA had been reported to stabilize the nuclear factor Kappa beta (NF- Kappa B) transcription factor in the body. Thus ALA can regulate a number of genes that are related to inflammation and cell cycle control which are involved in the pathology of diabetes, CVD, atherosclerosis and cancer [44]. From the finding of this study we could concluded that ALA supplementation at 10 mg/kg B.wt. has potential role in protection of some age related metabolic disorder in intact and ovariectomized animals.

Table 1: The Protective role of ALA on Thrombosis profile (Platelets count, Coagulation time and bleeding time) in intact and ovariectomized rabbits.

Group / Parameter	G1 Intact rabbits Received D.W	G2 Intact rabbits Received ALA	G3 Ovariectomized Rabbits Received D.W	G4 Ovariectomized Rabbits Received ALA
Platelets count (cell $\times 10^3/\text{mm}^3$) LSD=2.705	175.666 \pm 1.173b	143.800 \pm 0.663c	248.800 \pm 0.860a	132.000 \pm 0.836d
coagulation time (min) LSD=0.369	4.33 \pm 0.003a	4.58 \pm 0.003a	2.74 \pm 0.005b	3.04 \pm 0.24b
Bleeding time (min) LSD=0.020	1.86 \pm 0.008b	1.60 \pm 0.006c	1.11 \pm 0.004d	1.89 \pm 0.006a

Values represent mean \pm SE (n=5 rabbits).

Different Small letters denote a significant difference between groups ($p \leq 0.05$).

Table 2: The Protective role of ALA on Lipid profile (serum Cholesterol, Triglycerides and HDL-c concentration) in intact and ovariectomized rabbits

Group / Parameter	G1 Intact rabbits Received D.W	G2 Intact rabbits Received ALA	G3 Ovariectomized Rabbits Received D.W	G4 Ovariectomized Rabbits Received ALA
Serum Cho. Level (mg/dl) LSD=2.015	107.78±0.74d	70.76±0.76b	119.20±0.50a	89.38±0.36c
serum Triglyceride level (mg/dl) LSD=2.3737	81.34±0.41b	70.48±0.92c	114.00±1.00a	71.54±0.67c
Serum HDL-c level (mg/dl) LSD=1.053	82.34±0.41c	84.80±0.25b	80.38±0.48d	86.74±0.30a

Values represent mean ± SE (n=5 rabbits).

Different Small letters denote a significant difference between groups ($p \leq 0.05$).

Table 3: The protective role of ALA on some parameters related to oxidative and metabolic disorders (serum glutathione, TSH, and glucose concentration) in intact and ovariectomized rabbits.

Group/Parameter	G1 Intact rabbits Received D.W	G2 Intact rabbits Received ALA	G3 Ovariectomized Rabbits Received D.W	G4 Ovariectomized Rabbits Received ALA
Serum GSH level (mm) LSD=0.0709	2.53±0.02d	4.75±0.029a	3.15±0.02c	4.47±0.02b
Serum TSH level (mIU/L) LSD=0.0416	2.39±0.004a	1.49±0.01c	2.00±0.005b	1.47±0.02c
Serum Glucose Level (mg/dl) LSD=11.163	132.20±1.39b	120.80±0.37c	155.80±1.23a	130.20±1.01bc

Values represent mean ± SE (n=5 rabbits).

Different Small letters denote a significant difference between groups ($p \leq 0.05$).

Table 4: The Protective role of ALA on serum Insulin concentration and insulin resistance index (HOMA1-IR and HOMA2-IR) in intact and ovariectomized rabbits.

Group / Parameter	G1 Intact Rabbits Received D.W	G2 Intact rabbits Received ALA	G3 Ovariectomized Rabbits Received D.W	G4 Ovariectomized Rabbits Received ALA
Serum Insulin Level (mu/L) LSD=0.197	8.52±0.03c	11.70±0.04b	8.70±0.09c	15.90±0.07a
HOMA1- IR LSD=0.166	2.52±0.01d	3.82±0.03b	3.18±0.02c	5.05±0.10a
HOMA2- IR LSD=0.030	1.18±0.005d	1.65±0.006b	1.27±0.01c	2.21±0.01a

Values represent mean ± SE (n=5 rabbits).

Different Small letters denote a significant difference between groups ($p \leq 0.05$).

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