



Ameliorating the Cardiovascular Disorders after Ovariectomy By Alpha Lipoic Acid Supplementation

Hayder S. Rwayyih*, Tahani S.S. Al-Azawi

Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq.

Abstract | The present experiment was designed to study the protective role of ALA on some biological cardiac markers include creatinine phosphokinase, C- reactive protein and troponin and histology of heart and aorta in intact and ovariectomized rabbits. Twenty rabbits, aged seven to eight weeks, were employed in this investigation. The animals were split up evenly into the following four groups: The intact rabbits in Group One (G1) were given distilled water. The intact rabbits in Group Two (G2) were given an oral dose of alpha lipoic acid (10 mg/kg B.wt). The ovariectomized rabbits in Group Three (G3) were given distilled water. Group four (G4) Ovariectomized rabbits received alpha lipoic acid (10mg/kg B.wt) orally. The results reveal that supplemented groups show a significant decrease in CPK, CRP and troponin serum level. Moreover, there is an increase in heart index (0.45% and 0.41%) as compared with none supplemented intact and ovariectomized groups (0.43% and 0.35%) respectively. The histological sections of the heart from the supplemented groups show no clear lesions with normal spindle shape cardiac muscle and intercalated disc with normal aorta and epithelial cells. From the other hand, the ovariectomized non- supplemented rabbits show clear lesions in their heart and aorta. There was clear necrosis of cardiac muscle fibers with fatty change in endocardium or papillary muscles due to estrogen deficiency, irregular myocardial endothelial degeneration and congested blood vessels. The aorta of these groups has enlargement and thickening of endothelial cells with infiltration of fatty cells in tunica intima (The structure of intima changes in postmenopausal and it becomes multilayered due to migration of smooth muscle cells from the media to intima). In conclusions, the result of the current study could prove that Alpha lipoic acid has a protection on cardiovascular system in menopause estrogen deficiency and ovariectomized female rabbits. Alpha lipoic acid could fight the deleterious effect of menopause estrogen deficiency.

Keywords | Ovariectomy, Menopause, Estrogen deficiency Alpha lipoic acid, Cardiovascular system, Rabbits, Histology, Biological markers

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***Correspondence** | Hayder S. Rwayyih, Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq; **Email:** haider.s@comc.uobaghdad.edu.iq

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INTRODUCTION

Lipoic acid (LA) is a natural compound, chemically it is (R)-5-(1, 2-dithiolan-3-yl) pentanoic acid (Usacheva *et al.*, 2022). Lipoic acid is predominantly a lipophilic molecule with an amphipathic characteristic due to the carboxylic acid group attached to the ring structure. The

liver, heart, and kidneys have significant concentrations of LA, which is produced by human tissues with high prolonged metabolic activity. There is a lot of LA in the cytosol and cellular membranes. LA is easily absorbed from the digestive tract and does not have any notable side effects. Additionally, it is able to traverse the blood-brain barrier (Balhaj *et al.*, 2022). Due to the asymmetric

carbon atom in LA, there are two potential optical isomers (R and S). Endogenous synthesis is only present in the R-isomer. LA contains two thiol groups, which may be oxidized or reduced (DHLA). As with the thiol antioxidant- glutathione, Both LA and DHLA are easily soluble (Ghibu *et al.*, 2008). Endogenously produced LA is covalently linked to certain proteins that serve as cofactors for complexes of the enzyme mitochondrial dehydrogenase Cronan (2020). LA is well-known for being a coenzyme in multienzymatic complexes that catalyze the α -ketoacid decarboxylation process (Manda *et al.*, 2007). Both LA and DHLA are also responsible for the regeneration of active forms of other cellular antioxidants, including vitamin C and E (Attia *et al.*, 2020). LA stimulate nuclear factor β and peroxisome proliferator-activated receptors (PPAR) activation cascade, and implication in the regulation of carbohydrate and lipid metabolism (Pérez-López *et al.*, 2010). Gender differences in the risk of cardiovascular diseases are well recognized, with premenopausal female exhibiting a lower risk than age-matched male (Hurtado *et al.*, 2015). Estrogen is essential for cardiovascular health because, after menopause, there is no longer a benefit for women over men in terms of cardiovascular morbidity. Estrogens are known to have beneficial effects on the vascular wall. Fardoun *et al.* (2020) Through the activation of endothelial cell genes, such as endothelial nitric oxide generation, estrogen therapy improves endothelial dysfunction, a significant factor in the pathogenesis of cardiovascular disease (Xiang *et al.*, 2021). Based on the aforementioned insights, the current study aimed to examine the impact of oral ALA supplementation on certain cardiovascular system parameters in both intact and ovariectomized rabbits. The aim of these study is to study the protective role of ALA on the cardiovascular systems in intact and ovariectomized rabbits.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND MANAGEMENT

From December 2016 to March 2017, this experiment was conducted in the animal house at the University of Baghdad's College of Veterinary Medicine. Twenty female rabbits weighing between 820 and 1050 grams were employed in this study; their ages ranged from 7-8 weeks. These animals were housed in an air-conditioned room with a temperature between 20 and 25 degrees Celsius with a 12-hour photoperiod. Fresh water and pellet food were provided daily throughout the whole stay.

TECHNIQUE OF OVARIECTOMY OPERATION

Ten rabbits had ovariectomies on both ovaries in accordance with the protocol described by Baofeng *et al.* (2010). The ovaries of the remaining ten rabbits remained undamaged. The animals received intramuscular injections

of penicillin and streptomycin for five days while being housed individually and receiving post-operative care.

DOSAGE OF ALPHA LIPOIC ACID

Thiotacid tablets, also known as EVA Pharma-Egypt, are prescribed daily and contain 300–1800 mg of thioctic acid (also known as alpha lipoic acid). The dosage of 10 mg/kg B.wt. for each rabbit was determined in accordance with Al-Azawi and Rwayyih, (2017). It was administered by gastric gavage every day for 60 days, 30 minutes prior to meals.

COLLECTING OF BLOOD SAMPLES

After obtaining fasting blood from the jugular vein at the conclusion of the period, serum was separated via centrifugation at 3000 rpm for 20 minutes. Before being used, serum was kept at -18°C , or deep freeze (Santos *et al.*, 2019).

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 groups, one intact and one ovariectomized were received distilled water. The experiment was lasted for sixty days.

- Group One (G1): Intact rabbits received distilled water.
- Group Two (G2): Intact rabbits received LA, 10mg/Kg BW, orally.
- Group Three (G3): Ovariectomized rabbits received distilled water.
- Group Four (G4): Ovariectomized rabbits received LA, 10 mg/Kg BW, orally.

SERUM PARAMETERS DETERMINATION

Determination of serum creatine phosphokinase (CPK) concentration was carried out according to the method described by Ichroma, Serum C-reactive protein (C-r p) concentration was determined by ichromaTM which also used for serum Troponin level (Bains *et al.*, 2017).

ORGANS PREPARATION

At the end of the experiment, all animals were weighed and sacrificed by over dose anesthesia injection via jugular vein (Rwayyih and Khaleel, 2022). The heart and aorta were dissected and cleaned from the accessory contacted tissues. The heart was weighted and the heart index was calculated:

$$\left(\text{Heart Index (\%)} = \frac{\text{Heart Weight (g)}}{\text{Body Weight (g)}} \times 100 \right)$$

Heart and aorta were preserved in 10% formalin for histological examination.

STATISTICAL ANALYSIS

To determine whether there were any significant differences between the means, the data were subjected to a one-way ANOVA and the least significant differences (LSD) method (SAS SASSTAT, 2010).

RESULTS AND DISCUSSION

THE EFFECT OF ALA ON BIOLOGICAL CARDIAC MARKERS (SERUM CPK, CRP AND TROPONIN LEVEL) AND HEART INDEX IN INTACT AND OVARIECTOMIZED RABBITS

Table 1 shows a significant decrease in CPK level in intact and ovariectomized groups received ALA in comparison with other groups that don't receive it, respectively. Also, there is a significant decrease in CRP level in G3 as compared with control intact group. Moreover, the two groups received ALA showed significantly lower serum CRP as compared with those received D.W, respectively. On the other hand, serum troponin concentration in the ovariectomized rabbits reveals significantly less value than the intact group. AT the same time, there is a significant decrease in troponin level in groups received ALA in comparison with control groups. The effect of ALA on heart index of the groups in the experiment shows a significant decrease in heart index of the ovariectomized group in comparison with the intact. On the other hand, the values of both groups that received ALA are significantly higher than other groups that don't receive ALA, respectively.

THE EFFECT OF ALA ON THE HISTOLOGICAL SECTIONS OF HEART AND AORTA IN INTACT RABBITS

THE INTACT GROUP WITHOUT ALA SUPPLEMENTATION

Microscopic examination of the longitudinal section of the heart from intact rabbits received distilled water (G1) showed no significant pathological lesions. The sections of the heart show normal cardiac muscles with spindle shape and normal intercalated disc and ventricular wall thickening as in (Figure 1A). Moreover, the histological examination of the aorta of this group shows normal structure with some vacuoles in the sub intima (Figure 1B).

THE INTACT GROUP WITH ALA SUPPLEMENTATION

The normal typical histological section (with no clear lesion) of the heart of the rabbits administered orally with 10 mg/kg. B.wt ALA (G2) for 60 days was showed in (Figure 1C). Other sections in the heart as in (Figure 1D) show no mononuclear cell infiltration between cardiac muscle with normal spindle shape, cardiac cells and intercalated disc. The section of the aorta of the intact rabbits supplemented with ALA shows normal structure with no clear lesion as in (Figure 1E).

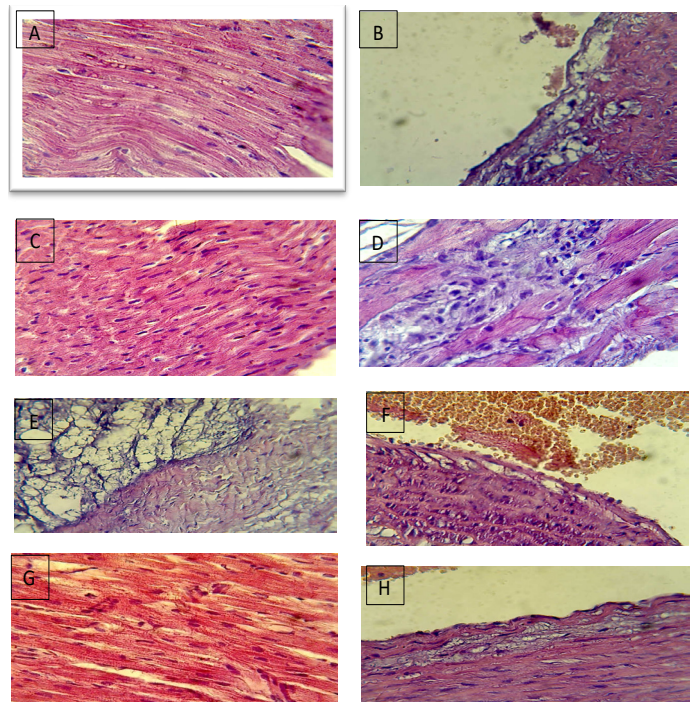


Figure 1: Section in the heart of I+D group shows no clear lesions (A). Section in aorta of I+D group shows vacuoles in subintima (B). Section in the heart of I+A group shows no clear lesions (C). Section in the heart of I+A group shows no mononuclear cells infiltration between cardiac muscle (D). Section in the heart of 0+D group shows necrotic cardiac (E). Section in the aorta of 0+D group shows vacuolation in subintima (F). Section in the heart of 0+A group shows no clear lesions (G). Section in the aorta of 0+A group shows no clear lesions (H).

Table 1: The protective role of ALA administration on some biological cardiac markers (serum CPK, CRP and Troponin level) and heart index in intact and ovariectomized rabbits after 60 days of administration.

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 CPK level (IU/L) LSD=7.7717	412.60 ± 3.52a	270.60 ± 3.62c	317.40±0.24b	228.00±1.09d
Serum CRP level (mg/dl) LSD= 0.0157	2.71 ± 0.005a	0.66±0.007c	1.19±0.004b	0.24±0.003d
Serum troponin level (ng/M) LSD=0.0188	1.92±0.008a	0.91±0.005c	1.29±0.003b	0.25±0.005d
Heart index (%) LSD=0.007	0.43± 0.002b	0.45±0.002a	0.35±0.002d	0.41±0.002c

*The values (n = 5 rabbits) represent the mean ± SE. A significant difference between groups is shown by different small letters (p<0.05).

THE EFFECT OF ALA ON THE HISTOLOGICAL SECTIONS OF HEART AND AORTA IN THE OVARIECTOMIZED RABBITS THE OVARIECTOMIZED GROUP WITHOUT ALA SUPPLEMENTATION

The histological section of the heart from ovariectomized rabbits received distilled water shows some clear changes. These changes are characterized by necrosis of cardiac muscle fibers with fatty deposition which appear clearly as round vacuoles of fatty droplets as in (Figure 1E). Other section in the heart of this group shows fragment of cardiac muscle fibers with severe edema. These sections also pointed to irregular myocardium endothelial degeneration and congested blood vessels. The aorta of this group of rabbits that are subjected to ovariectomy showed some pathological changes. This could be summarized by enlargement and thickening of endothelial cells and infiltration of fatty cells in tunica intima as in (Figure 1F). In addition, there is some vacuolation and degeneration in sub intima.

THE OVARIECTOMIZED GROUP WITH ALA SUPPLEMENTATION

Daily supplementation of 10 mg/kg. B.wt of ALA to the ovariectomized rabbits caused a regression of lesion in muscle fibers of the heart. Figure 1G Shows no clear lesions in the heart of this group as well as the figure shows normal spindle shape cardiac muscle and intercalated disc. The histological section of the aorta from this group shows no clear lesion as in (Figure 1H) which is clarified by normal epithelial cells with some proliferation of them.

EFFECT OF OVARIECTOMY ON BIOLOGICAL CARDIAC MARKERS (SERUM CPK, CRP AND TROPONIN) LEVEL

An enzyme called creatine phosphokinase (CPK), often referred to as phospho-creatine kinase or creatine kinase (CK), is required to use adenosine triphosphate (ATP) to convert creatine into phosphocreatine (PCR) and adenosine diphosphate (ADP). Clinically, CK is measured in blood as a sign of injury to CK-rich tissue, such as in acute kidney injury, muscular dystrophy, and myocardial infarction heart attack (Zrari and Mohammed, 2016). C-reactive protein (CRP) is an anular ring-shaped protein found in plasma in response to inflammation. It is synthesized by liver in response to factors released by microphages and adipocytes (Bains *et al.*, 2017). The troponin complex of three subunits troponin C, I and T. It is located on the myofibrillar thin (actin) filament of striated (cardiac and skeletal) muscle. It is the most sensitive and specific test than creatine kinase (CK) values for myocardial injury (MI). Following myocardial damage, cardiac troponin (CTn) released rapidly from the cystolic pool of the myocytes to the blood (Park *et al.*, 2017).

However, a lower left ventricular ejection fraction is linked

to higher CTn in heart failure. Its rise was linked to severe pulmonary embolism, acute infarction, acute cardiac overload, and acute heart failure (Harjola *et al.*, 2020). The findings of the present study show a depression in the serum cardiac markers (CPK, CRP and troponin) in rabbits after the surgical operation of ovariectomy.

EFFECT OF ALA ON BIOLOGICAL CARDIAC MARKERS (SERUM CPK, CRP AND TROPONIN) LEVEL

Supplementation of ALA to the intact and ovariectomized rabbits at a dose of 10mg/kg B. wt produced a significant amelioration in the heart damage (Al-Azawi and Rwayyih, 2017). The current study demonstrates a substantial increase in the protective antioxidant glutathione level in the group receiving ALA Mahdavi and Safa (2019), which is linked to the repair of heart damage. As a result, the heart experiences less oxidative stress in the ALA supplemented group due to decreased ROS and advanced glycation end product (AGEP), which causes the heart to expend less energy as evidenced by a decrease in the blood level of CPK (Salehi *et al.*, 2019). At the same time, ALA has an important role in energy production in cell mitochondria by helping turn glucose into energy. Moreover, ALA helps to reduce pain and thus used for patient with peripheral neuropathy (Nguyen and Takemoto, 2018).

EFFECT OF OVARIECTOMY ON HEART INDEX AND HISTOLOGICAL EXAMINATION OF THE HEART AND AORTA

The decline in the heart index of ovariectomized rabbits in the current study could be attributed either to a decrease in heart weight or an increase in body weight or both. However, the histological sections of the heart and aorta of this group reveal a pathological change represented by necrosis of cardiac muscle fibers with fatty deposition and myocardium endothelial degeneration. The aorta of this group shows an enlargement and thickening of endothelial cell with infiltration of fatty cells in tunica intima. Estrogen has a number of effects on cardiovascular system function and disease (Aryan *et al.*, 2020). Among these are the metabolism, insulin sensitivity, inflammatory response, vascular function, and cardiac myocyte survival that are all regulated by estrogen. On the other hand, it has been noted that the prevalence of obesity rises after menopause. This includes higher levels of intra-abdominal and total body fat when compared to premenopausal females. This disease promotes the development of atherosclerosis and is associated with a number of metabolic illnesses, including metabolic syndrome (Silveira *et al.*, 2022).

EFFECT OF ALA ON HEART INDEX AND HISTOLOGICAL EXAMINATION OF THE HEART AND AORTA

Lipoic acid supplementation to rabbits induces an increase in heart index which is mainly due to the significant

depression in body weight of these groups in response to ALA administration (Salehi *et al.*, 2019). The findings of the histological study of these groups reveal normal structure of cardiac muscles (without lesion) with normal spindle shape and intercalated disc with normal aorta. All these effects of ALA are related to its antioxidant effect. It is normally accruing compound synthesized in small amounts in plants and animal. In humans, ALA is synthesized in the mitochondria from octanoic acid. Lipoic acid has a protective role on the CVS summarized by neutralizing free radicals, preventing advance glycation end product (AGE0 formation and decreasing lipid peroxidation (Hu and Sun, 2020).

CONCLUSIONS AND RECOMMENDATIONS

From the results illustrated and discussed in this study, we could conclude: The ovariectomized rabbit's show some clinical signs show Dry skin and Vaginal dryness and show a significant increase in the biological cardiac indicators (CPK, CRP, troponin) in the ovariectomized rabbits. Alpha lipoic acid supplement provides a protective role in both intact and ovariectomized rabbits. This group has certain histological lesions as well as damage to the aorta and heart. When ALA was added to the serum of both intact and ovariectomized rabbits, the levels of biological cardiac markers such as CPK, CRP, and troponin were lowered. The cardioprotective activity of ALA is demonstrated by the full regeneration of aortic and cardiac lesions, as well as by similar clinical signs. Dry skin and atrophy or dryness in the vagina

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NOVELTY STATEMENT

The work is novel since it focuses on how ALA protects certain biological cardiac indicators and the histology of the aorta and heart in intact and ovariectomized rabbits. Lesions in the heart and aorta completely regenerate as a result of the cardioprotective activity of ALA.

AUTHOR'S CONTRIBUTION

Each of these writers made an equal contribution.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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