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The Essential Role of Alpha Lipoic Acid on the Cardiovasculer System in Rabbits Subjected to Methandienone Adimnstration

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ABSTRACT

Introduction: Chemically, lipoic acid (LA) is (R)-5-(1,2-dithiolan-3-yl)pentanoic acid, and it is a naturally occurring substance (1-3). Lipoic acid has an amphipathic feature and is primarily a lipophilic molecule.

Objective: The aim of the current study was to examine the preventive effects of ALA on certain biological cardiac markers and histology of heart and aorta in rabbits.

Subjects and methods: A total of 20 rabbits were divided into four groups: Rabbits in *Group 1* (G1) were given distilled water. The rabbits in *Group 2* (G2) were given alpha lipoic acid orally**.** *Group 3* (G3) rabbits received Methandienone. *Group 4* (G4) rabbits received Methandienone (0.35mg/kg.B.wt) and alpha lipoic acid (10Mg/kg B.wt) orally. **Results:** The results reveal that supplemented groups (G2 and G4) 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 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. On the other hand, there was clear necrosis of cardiac muscle fibers with fatty dispassion, 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.

Conclusions: ALA has a protection on cardiovascular system.

Keywords: Methandienone, Alpha lipoic acid, cardiovascular system, Experimental study, Al-Rasheed University College.

INTRODUCTION

Chemically, lipoic acid (LA) is (R)-5-(1,2-dithiolan-3-yl)pentanoic acid, and it is a naturally occurring substance **[1,2].**

Due to the carboxylic acid group connected to the ring structure, lipoic acid has an amphipathic feature and is primarily a lipophilic molecule. Human organs with substantial metabolic activity, such as the liver, heart, and kidney, which have high quantities of this acid, produce LA. Both the cytosol and cellular membranes contain a lot of LA. LA may pass the blood-brain barrier, is quickly absorbed from the digestive tract, and has no negative side effects **[3].**

Due to the asymmetric carbon atom in LA, there are two potential optical isomers (R and S). Only the R-isomer is produced endogenously. LA has two thiol groups that can be reduced or oxidized (DHLA) included LA and DHLA **[4].**

The purpose of the current study was to determine how oral supplementation with ALA affected certain cardiovascular system-related parameters. Because LA stimulates the nuclear factor and peroxisome proliferator-activated receptors (PPAR) activation cascade and has implications for the regulation of carbohydrate and lipid metabolism **[5,6],** it was chosen as a potential target for future research.

Synthetic testosterone derivatives, or AASs, are anabolic androgenic steroids. It has been used for years to treat osteoporosis, severe anemia, burns, short stature, and other conditions **[1].** The third class of AAS employed in the recent work, methandienone, also known as dianabol on the marketplace, is distinguished by the addition of an alkyl group to its 17 carbon atoms **[7].** AASs are more commonly used to grow muscle bulk and maintain a state of intense exercise than for the euphoria they cause.

Additionally, androgenic steroids alter the release of stress hormone and endorphins, as well as the hypothalamic-pituitary-adrenal (HPA) axis, which may enhance the reinforcing effects of exercise **[2].**

After being exposed to stressful situations, the adrenal axis becomes activated, triggering a cascade [3] of hormonal secretions that alter bodily functions and behavior in order to preserve homeostasis (4After experiencing stress, the pituitary gland's proopiomelanocortin (POMC) gene is expressed more, which stimulates the release of corticotropin releasing hormone (CRH), which in turn boosts the production of peptides such beta endorphin and adrenocorticotropic hormone (ACTH) **[8].**

The stress response is then triggered by ACTH activating the adrenal gland, which is followed, at least in part, by beta endorphin blocking CRH release **[9].** A chemical with morphine-like effects called beta endorphin is released by the pituitary and hypothalamus. In response to pain, intense activity, and despair, it is released in large amounts **[4].**

The aim of the current study was to examine the preventive effects of ALA on certain biological cardiac markers and histology of heart and aorta in rabbits.

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SUBJECTS AND METHODS

From December 2019 to March 2020, 20 female rabbits, ranging in age from 7-8 weeks and weight from 820 to 1050 g, were employed in this investigation. These animals were maintained in an air-conditioned chamber at 20 to 25 degrees Celsius with a 12-hour photoperiod every day [10]. Throughout the study period, fresh water and a pellet diet were provided.

Alpha lipoic acid dose: Alpha lipoic acid, which can be found in thiotacid tablets from EVA Pharma-Egypt in doses ranging from 300 mg to 1800 mg, was taken every day. Each rabbit received a dose of 10 mg/kg B.wt., [13] and administered by gastric gavage every day for 60 days, 30 minutes before meals.

Blood samples assortment: At the end of the time period, jugular vein blood drawn while fasting was used to separate serum following centrifugation at a speed of 3000 rpm for 20 minutes. Before usage, serum was kept at a deep freeze (-18C°).

Experimental Design: After recuperation and acclimatization, the animals were divided evenly into 4 groups; Group 1 (G1) received distilled water, and Group 2 (G2) received a daily dose of 10 mg/kg B.wt ALA through a stomach tube. Additionally, Group 3 (G3) Methandienone was given. Group 4 (G4) also received 0.35 mg/kg B.wt. of ALA and methandienone. The trial was conducted for 60 days.

Serum Parameters determination: Serum creatine phosphokinase (CPK) concentration was determined using the procedure described ***Lewandrowski et al., 2002***. Also, concentration of serum C-reactive protein (C-rp) was measured. ichromaTM was used to measure Serum Troponin levels [11].

Ethical Consideration*:*

This study was ethically approved by the Institutional Review Board of the College of Veterinary Medicine, Baghdad University. Each contributor in the study was informed about ethical considerations in animal experimental studies. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on animals.

Statistical Analysis

The collected data were introduced and statistically analyzed by utilizing SAS. Qualitative data were defined as numbers and percentages. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as means and standard deviation (SD), and one-way ANOVA and least significant differences (LSD) were used for comparison between groups. P value ≤0.05 was considered to be statistically significant.

RESULTS

The effect of ALA on biological cardiac markers (serum CPK, CRP and Troponin level) and heart index in rabbits.

Table 1 demonstrates a substantial (P ≤0.05) decline in CPK level in ALA-received groups (G2 and G4) in comparison to the control groups (G1 and G3), respectively.

CRP level is significantly (P ≤0.05) lower in G3 compared to the control group (G1).

Additionally, the two ALA-treated groups (G2 and G4) displayed significantly (P ≤0.05) lower serum CRP levels than the D.W-treated groups (G1 and G3).

However, serum troponin levels in G3 group are significantly (P ≤0.05) lower than those in the intact group (G1).

The troponin level is also significantly (P ≤0.05) lower in ALA-treated groups (G2 and G4) than in the control groups (G1 and G3). The experiment's groups' heart indices were affected by ALA, and the Methandienone group (G3) showed a significantly (P ≤0.05) lower heart indices than the other groups.

Table (1): The protective role of ALA administration on some biolagical cardiac markers, heart index and histological study of the heart and aorta rabbits after 60 days of administration.

Groups / Parameter	G1 Intact rabbits Received D.W	G2 Intact rabbits Received ALA	G3 Methandienone rabbits Received D.W	G4 Methandienone rabbits Received ALA	LSD
CPK(IU/L)	412.60±3.52a	270.60±3.62c	317.40±0.24b	228.00±1.09d	7.77
CRP(mg/dl)	2.71±0.005a	0.66±0.007c	1.19±0.004b	0.24.00±0.003d	0.01
Troponin(ng/M)	1.92±0.008a	0.91±0.005c	1.29±0.003b	0.25±0.005d	0.01
Heart index (%)	0.43±0.002b	0.45±0.002a	0.35±0.002d	0.41±0.002c	0.007

*Values represent mean ± SE (n= 5 rabbits). Different Small letters denote a significant difference between groups (*P* ≤0.05).*

Histological Examination for the heart and aorta:

The longitudinal segment of the heart from healthy rabbits that had been given distilled water (G1) underwent microscopic examination and revealed no obvious pathological abnormalities. The heart's sections display healthy cardiac muscles with a spindle-shaped intercalated disc and thickening of the ventricular wall. Additionally, the rabbits' hearts were given oral administration of 10 mg/kg. B.w. ALA (G2) for 60 days and the histological examination of the aorta in this group revealed normal structure with some vacuoles in the sub intimae pointing to the normal typical histological section (with no obvious lesion) (**Figure 2**).

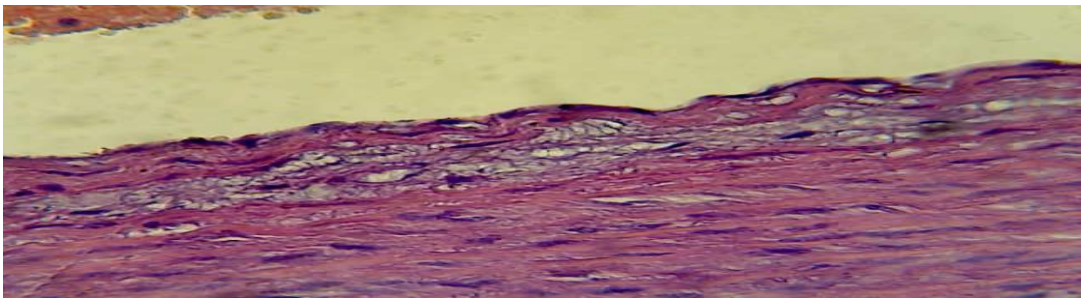

Figure (1): Section in the heart of ALA group shows no clear lesions (H & E stain 400X**).**

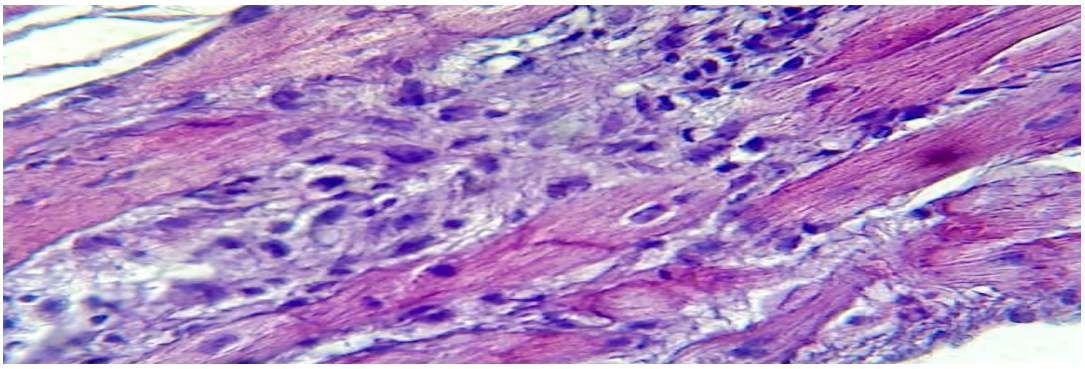

Figure (2): Section in the heart of ALA group shows no clear lesions (H & E stain 400X).

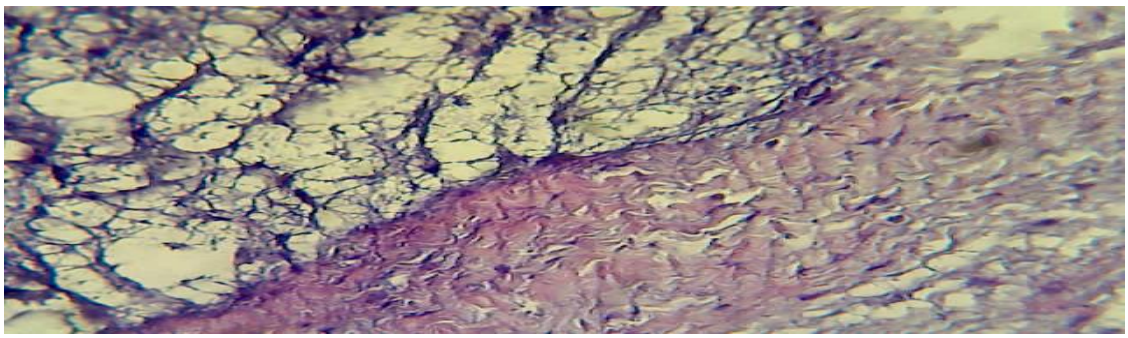

Figure (3): Section in the heart of Methandienone group shows necrotic cardiac muscle with vacuole of fatty droplets (H & E stain 40X).

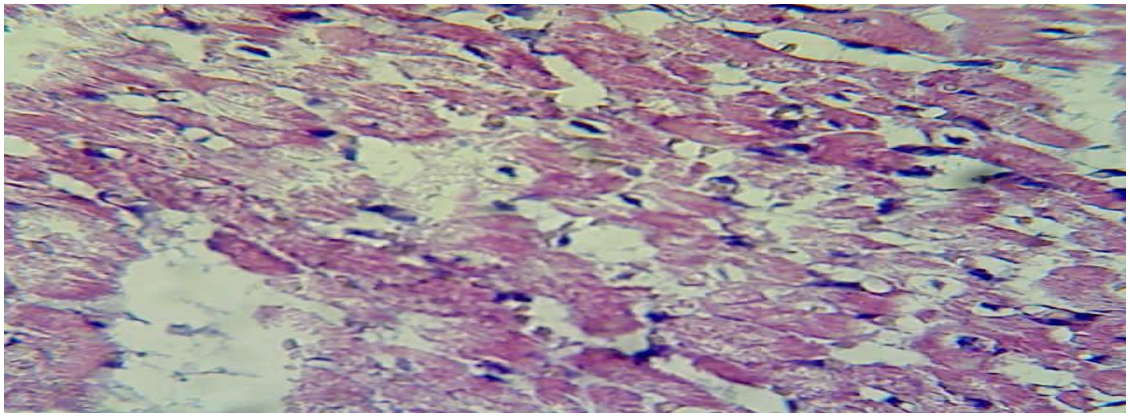

Figure (4): Section in the heart of Methandienone group shows fragment of cardiac muscle fiber with edema (H & E stain 40X)

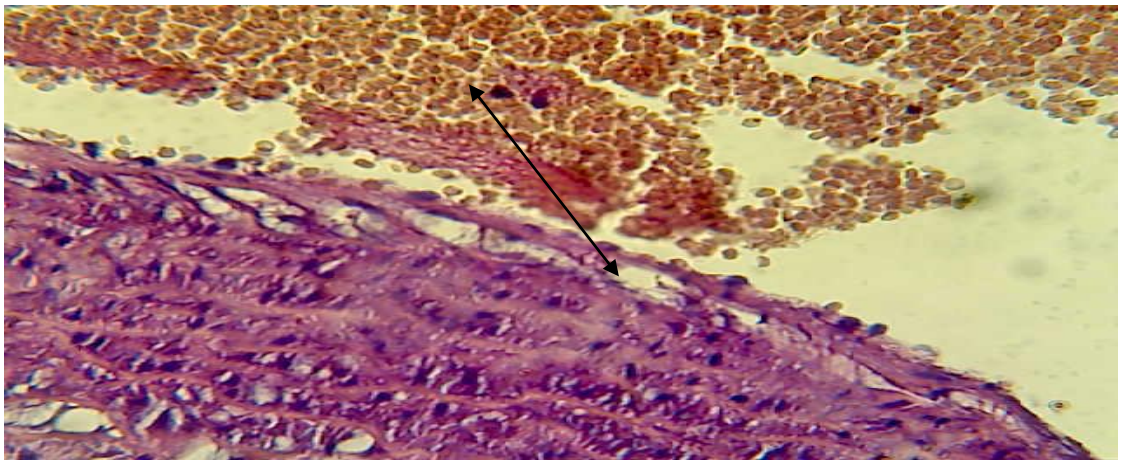

Figure (5): Section in the aorta of Methandienone group shows vacuolation in subintima (H & E stain 40X)**.**

DISCUSSION

Adenosine triphosphate (ATP) and creatine must be converted into phosphocreatine (PCR) and adenosine diphosphate (ADP), respectively, by an enzyme known as creatine phosphokinase (CPK) or phospho-creatine kinase (also known as CK) (ADP). Blood is clinically tested for CK as a sign of CK-rich tissue damage, such as in myocardial infarction (heart attack), muscular dystrophy, and acute kidney injury **(12).** In reaction to inflammation, the anular ring-shaped protein known as C-reactive protein (CRP) is discovered in plasma. In reaction to substances generated by adipocytes and microphages, the liver produces it **(13).**

In order to activate the complement system, it must bind to the lysophosphatidyl choline expressed on the surface of dead or dying cells or microorganisms. Because it was initially discovered in serum and reacted with the pneumococcus somatic "c" carbohydrate antigen, CRP was given that name **(14).** According to some experts, individuals with greater basal levels of CRP are more likely to develop diabetes, hypertension, and cardiovascular disease **(15).** The troponin complex consists of the troponin C, I, and T subunits. It is found on the thin (actin) myofibrillar filament of striated (skeletal and cardiac) muscle. After myocardial injury, cardiac troponin (CTn) was quickly released into the circulation from the myocytes' cystolic pool **(16).** However, lower left ventricular ejection fraction is linked to higher CTn in heart failure. Acute infarction, severe pulmonary embolism, and acute cardiac overload and heart failure were all diagnosed as its increase **(17).**

The results of the current study demonstrate a decrease in the serum levels of the cardiac markers CPK, CRP, and troponin in rabbits following ovariectomy surgery. The effect of ovariectomy on these indicators is not covered in the literature review that is currently available, but we can examine it based on the overall findings of this trial. Elevated levels of these proteins in the serum have been linked to cellular damage, tissue necrosis, and an increased risk for cardiovascular disease (CVD). Despite the fact that these markers were examined twice in the current study (after 30 and 60 days), the injury to the heart muscle may not have been significant enough to cause excessive protein secretion. On the other hand, when cell membranes become permeable or break, cytosolic enzymes that act as diagnostic markers seep out of the damaged tissue into the bloodstream, however this may not be sufficient to cause leakage. The cardiac damage was significantly lessened when ALA was given to the intact and ovariectomized rabbits at a level of 10mg/kg B.wt. This is related to lowering serum cholesterol levels and reducing oxidative stress in heart tissues. However, one of the most crucial advantages The current study demonstrates a considerable increase in the protective antioxidant glutathione level in the ALA-administered group [(18)]. connected to the repair of heart injury. Therefore, the heart experiences less oxidative stress in the ALA supplemented group due to decreased ROS and advance glycation end product (AGEP), which is demonstrated by a decline in the blood level of CPK. ALA aids in the conversion of glucose into energy, which plays a crucial part in the creation of energy in cell mitochondria. Additionally, ALA aids patients with peripheral neuropathy by reducing their pain **(19).** This might account for the lower CRP in the group receiving ALA supplements in the current study. Additionally, the antioxidant activity of ALA and its protective effects in the When rabbits are supplemented with lipoic acid, their heart indices rise, which is mostly because these groups' body weights significantly decreased in response to ALA administration. This outcome supports a prior finding **(19).** They discovered that giving mice on a high-fat diet ALA supplements reduced their body weight growth by roughly 40% compared to the control group. ALA has also been referred to as an anti-obesity medication for polycystic ovary ladies **(20).**

The results of the histological analysis of these groups show that the cardiac muscles have normal structure (without lesions), normal spindle shape, normal intercalated disc, and normal aorta. All of ALA's effects are connected to its antioxidant properties. Normally, it is compounded. This is supported by the

findings of the current study, which also point to the protective role of lipoic acid in the cardiovascular system by reducing or preventing the harm caused by low estrogen levels.

CONCLUSION

The result of the current study could prove that ALA has a protection on cardiovascular system by improving serum CPK, CPR, troponin levels as well as heart and aorta tissues.

Conflict of interest: Nil.
Sources of funding: Nil.
Author contribution: Author contributed equally in the study.

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