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ALPHA LIPOIC ACID AGAINST THE ADVERSE ACTION OF MESTEROLONE ON CARDIAC AND RENAL SYSTEMS

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ABSTRACT : To investigate the potentially protective action of Alpha Lipoic acid on cardiac and renal system of rabbits subjected to Mesterolone administration, 20 female rabbits were subdivided in to four equal groups. G1-control, G2-received adaily oral dose of 0.65 mg/kg B,wt of Mesterolone, G3-supplemented with 10 mg/kg B.wt of Alpha Lipoic acid daily for 60 days, G4 received both Mesterolone and Alpha Lipoic acid. After 60 day blood was collected and serum was analyzed for some cardiac and renal biomarkers. B-endorphin level in serum of G2 was 95.6±0.84 compared with 54.6±0.93 for G3. There was significant decrease in serum thyroid stimulating hormone concentration in G2 with a normal restoration in G4. The level of creatine phosphokinase and troponin were highly elevated in G2 and decreased significantly in G3 and G4. Urea, creatinine, sodium and total protein were show a significant elevation in G2 in comparison with all other groups. It was concluded that Lipoic acid mechanism action as an antioxidant adverse the effect of Mesterolone by decreasing the release of endorphine and other cardio-renal damaging biomarkers.

Key words : B-endorphin, Cpk, troponin, urea, creatinine.

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INTRODUCTION

Anabolic-androgenic steroids (AAS), one of the most widely abused drugs by athletes and muscle builders with gain to improving their performance and muscle mass in recent times. Moreover, AAS offer inappreciable benefits to infertile and sub-fertile males, with possible deleterious effects on both human and animal physiology including sperm quality (Lukeman, 2018). AAS consists mainly of endogenous testosterone –T (natural) and their exogenous synthetic derivatives, which are produced or modified for enhanced anabolic activities and uses such as, to stimulate appetite and muscle growth, treating chronic wasting conditions, inducing male puberty, promoting protein synthesis and muscle growth. Besides, over 100 synthetic T-derivatives are available in the market today with different physiological impacts on the body, which includes anabolic effects on specific organs (for example, the muscles, bones, the heart and kidneys) with a little 5α-reductase activity (Allouh and Aldirawi, 2012). Lipoic acid (LA) also known as α-lipoic acid and alpha lipoic acid (ALA) and thioctic acid is an organosulfur compound (Teichert *et al*, 2003). α-lipoic acid (ALA, thioctic acid) is an organosulfur component produced from plants, animals and humans. It has various properties, among them great antioxidant potential and is widely used as a racemic drug for diabetic polyneuropathy-associated pain and paresthesia. Shakir and Khalil (2015) was concluded that alcoholic extract of dill seeds was increase in serum glutathione concentration (GSH) and effective in reducing the complications of diabetes mellitus such as nephropathy such as in ALA. Naturally, ALA is located in mitochondria, where it is used as a cofactor for pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase complexes (Salehi *et al*, 2019). These endorphin effects can be conceptualized as part of a larger adaptive response to the physical stress of exercise which engages the hypothalamic–pituitary–adrenal (HPA) axis to quickly mobilize energy stores to support intense physical activity. From of the glucocorticoids released during exercise, cortisol accounts for the majority of the activity. Cortisol stimulates lipolysis and decreases protein synthesis in muscle cells and stimulating the release of lipids. Acutely, resistance exercise leads to increase in ACTH and cortisol (Kraemer *et al*, 1999b). Recently, the AAS, methandienone has been shown to elevate TSH,

cortisole and endorphine concentration in serum of rabbits (Abed and Al-Azawi, 2020). Opioid modulating effects on various circulating factors implicated in blood pressure homeostasis. In fact, both activity of the sympathetic nervous system and release of atrial natriuretic factor (ANF) are affected by these peptides (Kienbaum *et al*, 2001) opioid system in essential hypertension. Sarcolemma of cardiomyocytes from hypertensive rats exhibits a higher concentration of opioid receptors (Zimlichman *et al*, 1996). Furthermore, opioid binding sites are up-regulated in the brain and spinal cord of spontaneously hypertensive rats (Kujirai *et al*, 1991). To date, little evidenceis available as to whether the increased plasma concentrations of endogenous opioid peptides in hypertensive patients simply represent a biological marker, or that these substances play a key role in regulating blood pressure; and opioid peptides have any effect on hormones with vasoactive properties in these patients. 12 endorphin induced a decrease in blood pressure, a decremental trend of plasma norepinephrine and endothelin-1, a plasma increase in ANF and an activation of the GH/IGF-I axis in all individuals (Noll *et al*, 1996).

MATERIALS AND METHODS

Animals and experimental design

We used twenty female adult rabbits (8-11 weeks old; body weight 700-1000 g) and was conducted at the University of Baghdad-Iraq from 1st August 2018 to 1st June 2019. The animals were divided into four equal groups as follows: G1 received 1 ml / kg of distal water orally, G2 received an oral dose of 0.65mg/ kg B.Wt of Mesterolone provided by bayerpharma (Germany). G3 was treated with 10 mg/Kg body weight of Alpha lipoicacide provided by Eva pharma company (Egypt). G4 received both Mesterolone and Alpha lipoicacide orally (0.65mg/ kg and 10mg/Kg, respectively). All animals received treatment every day for 60 days by oral administration. After the experiment, animals were anesthetized by double dose anesthesia and blood samples were taken for biochemical analysis.

Parameters determination

β-endorphin (pg/mL) in the serum was assayed using a commercially available ELISA Kit (Cat. No. E-EL-H0572, Elabscience Biotech Co., Ltd., China) according to the manufacturer's instructions.as described in appendix (III). The test uses a sandwich immunodetection method, such that the detector antibody in buffer binds to TSH in sample and antigen-antibody complexes are captured to another TSH antibody that has been immobilized on test strip as sample mixture migrates nitrocellulose matrix. Also creatinekinase is an enzyme located in skeletal, cardiac, smooth muscles and brain. Creatine kinase is found free in the cytoplasm of muscle cell and leaks from this cell when they are damaged. The principle of ichroma™ CK-MB uses a sandwich immunofluorescence assay. Fluorescence conjugated Anti-CK-MB in a detection buffer binds to CK-MB in a sample to form an antigen-antibody complex (Lewandrowski *et al*, 2002). On the otherhand,serum troponin detractionby ichroma™ Tn-I is based on a lateral flow immunoassay system using an antigen-antibody reaction with the fluorescence technology (Panteghini *et al*, 2004). The urea nitrogen assay is the modification of totally enzymatic procedure first described by Talke and Schubert (1965). The test is preformed as kinetic assay in which the initial rate of reaction is linear for a limited period of time. Urea in the sample is haydrolyzed by urease to ammonia and carbon dioxide. Creatinine is eliminated from blood by glomerular filtration. Reduced renal function results in an increased serum creatinine concentration. Measurement of serum creatinine is used to diagnose and monitor acute and chronic renal disease estimate glomerular filtrationrate. At an alkaline ph,creatinine in the sample react with picrate to form a creatinine-picrate complex. The rate of increase the absorption at 500nm due to formation of complex is directly proportional to the concentration of creatinine in the sample (Thomas, 1998). The sodium and the protein in the serum are precipitated with magnesium uranyl acetate. After separation by centrifugation the excess of uranyl ions in the supernatant react with thioglycolic acid forming a colored complex which absorbance varies inversely to the concentration of sodium in the sample (Trinder, 1951 and Maruna, 1958). Plasma protein derived primarily from synthesis in the liver, plasma cell, lymph node, spleen and bone marrow. In disease state both the total plasma protein levels and ratio of individual fractions may be dramatically altered from the normal value. The total protein assay used for quantitation of total protein in mammals serum or plasma (Alberghina *et al*, 2010).

RESULTS

Values represented in Table 1 shows serum B-Endorphin (β-EN) concentration of different groups. These results show a significant increase ($p \leq 0.05$) in serum β-Endorphin in (G2) as compared with all other groups. SerumThyroid stimulating hormon (TSH) in rabbits subjected toMesterolone administration showing a significant ($p \leq 0.05$) decrease in TSH level as compared to G1 and G4. These results show a significant increase ($P \leq 0.05$) of serum creatine phosphokinase (CPK) and Troponin (TNI) in rabbits received Mesterolone (G2) after 60 day of administration as compared with all other

Table 1 : The role of Mesterolone, ALA and both administration for 60 days on serum (β-EN, TSH, CPK, TNI, U, Cr, Na and T.protein) concentration in female rabbits.

Param. / G	β-EN (pg/mL)	TSH (mlu/l).	CPK IU/L	TNI (ng/M)	U (mmo/L)	Cr (µmol/L)	Na (g/L)	T.protein (g/L)
G1	54.6±0.9C	2.7±0.05A	3.4±0.01C	2.2±0.016B	52.8±0.32B	1.72±0.008B	135.3±0.72C	92.4±0.48B
G2	95.6±0.84A	2.3±0.07B	4.8±0.03A	3.4±0.005A	67.1±0.66A	3.7±0.01A	198.7±0.68A	191±1.7A
G3	54.6±0.93C	2.2±0.05B	3.2±0.06D	1.8±0.019C	48.8±0.31D	1.0±0.004D	97.2±0.52D	51.5±0.57D
G4	87.2±0.8B	2.7±0.07A	3.7±0.03B	1.60±0.01D	56.5±0.72C	1.6±0.009C	172.5±1.0B	85.5±0.41C
LSD	2.9	0.25	0.15	0.05	2.7	0.04	3.6	3.6

Values are presented as Means ± SE (n = 5 rabbits /group). The different capital letters refer significant differences between groups within one column at (P≤0.05).

groups. The results show that there is a significant increase (P≤0.05) in serum urea(U), creatnine(Cr) and Na of rabbits that received Mesterolone (G2) compared with all other groups. Moreover, there is significant decrease (P≤0.05) in serum urea, creatnine and Na concentration of ALA supplemented group (G3) in comparison to (G1, G2) and (G4). Serum T protein level in groups of animals that received Mesterolone, ALA and both. These results show a significant increase (P≤0.05). In serum T protein in (G2) after 60 days as compared with all other groups.

DISCUSSION

Endorphins contracted from endogenous morphine are endogenous opioid neuropeptides and peptide hormones in humans and other animals (IUPHAR/BPS Guide to, 2017). Endorphins may also produce a feeling of euphoria very similar to that produced by other opioids (Li *et al*, 2012). Other data suggest that opioids modulate renal function via central and sympathetic nervous system dependent and independent pathways. B-endorphin decreases in urine sodium excretion, without alterations in GFR or effective renal plasma flow (Kapusta and Obih, 1995). α-lipoic acid, an essential co-enzyme for energy production in mitochondria, demonstrates substantial antioxidant properties and an effect on whole-body physiology (Suzuki *et al*, 1992) has been used in several oxidative-stress models such as ischemia-reperfusion injury and neurodegenerative disorders (Müller *et al*, 2003). Effects of thyroid hormone on the heart are elicited by a number of genomic and nongenomic effects. Increase in thyroid hormone levels plays a role in transition from the fetal heart to adult phenotype of heart. The heart relies mainly on triiodothyronine (T_3) (Klein and Danzi, 2007). Mesterolone from the otherhand, have a direct anabolic effects include elevation in the creatine phosphokinase (CPK) (which convertescreatinekinase to creatinin and ATP) activity in skeletal muscle (Shiovitz *et al*, 2008). While in group received both Mesterolone and ALA there is a restoration in TSH level in rabbits, which could be explained by its protective role on cardiovascular system been studied by Tuncer *et al* (2016). Clinically, CK is assayed in blood as a marker of damage of CK-rich tissue such as in myocardial infarction (heart attack), muscular dystrophy, acute kidney injury (Schlattner *et al*, 2006). Mesterolon administration to rabbits of the present experiment raised concentration of CPK in serum significantly. One mechanisms of Mesterolone have direct and indirect anabolic effects include increases in the creatine phosphokinase activity in skeletal muscle, and increases in both circulating insulin-like growth factor (IGF)–1 (Arnold *et al*, 1996). We suggest that Supplementation of ALA alone and with mesterolon rabbits at a dose of 10mg/kg B.wt produced a significant amelioration in the heart damage markers which is represented by decreasing Cpk and troponin serum levels. This is associated with decreasing in the level of oxidative stress *i.e.* decreasing MDA with increasing GSH concentration. However, one of the most important benefits of ALA is directly related to its ability to restore and maintain glutathione levels. Therefore, there is reduced oxidative stress on the heart in ALA supplemented group with reduced ROS and AGAP make the heart to spend less amount of energy which is shown by decreasing CPK level in blood (Noeman *et al*, 2011). In addition, the use of AAS can increase the levels of serum creatinine, urine nitrogen and uric acid levels. Some studies also suggest that AAS can induce the formation and growth of tumors in the presence of other carcinogens (Maravelias *et al*, 2005). Other effect observed in AAS (Mesterolone) abusers was the retention of urea, creatinine and uric acid in blood, Urea production is increased when a greater number of amino acids are metabolized in the liver (Almukhtar *et al*, 2015). The susceptibility to these effects is primarily due to the high degree of filtration of potentially toxic products. For example, some reports have shown that long-term abuse of AAS is related with severe forms of focal segmental glomerulosclerosis (FSGS) (Herlitz *et al*, 2010). The

elevation of serum total protein in this study in group of animals received Mesterolone might be due to the changes in podocyte structure that occur during the development of nephropathy. The podocyte is an integral part of the filtration barrier, and changes in their structure have been observed in a broad range of proteinuric glomerular diseases. Loss of podocytes destroys the structure of the glomerular basement membrane. The foot processes of the podocytes may widen which results in a reduction in the ability of the podocytes to remain attached to the glomerular basement membrane. The consequent areas of bare glomerular basement membrane could result in glomerulosclerosis.this suggesting that oxidative stress may be one of the causes of the injury to podocytes (Siu *et al*, 2006). The FSGS is associated with hyperfiltration induced by; high protein diets; increased body mass; and/or reduced renal mass (Almukhtar *et al*, 2015). Another proposed mechanism seems likely to involve restoration of diminished activities of renal SOD, CAT, GSH peroxidase and GSH reductase and to suppress elevated lipid peroxidation (Moini *et al*, 2002). Another mechanism causing hypertension includes the elevation of the mineralocorticoid deoxycorticosterone, which may be caused by a testosterone-dependent decrease in cytochrome P450 which accompanied with mineralocorticoid excess, since steroid substrates pass through the biosynthetic pathway of aldosterone (Attard *et al*, 2008). This catabolic effect is normally evident following exhaustive exercise, when concentrations of glucocorticoids are high. This hypothesis is supported by the observation that several androgens are effective inhibitors of the binding of cortisol to the glucocorticoid receptor of skeletal muscle. Traditionally, the principal target organ for aldosterone was said to be the kidney. The mineralocorticoid receptor (MR) are found in high concentration in the renal distal nephron as well as other epithelial sites, such as the colon and ducts of sweat and salivary glands. However, MR have also been identified in non-epithelial sites, such as heart, brain, vascular smooth mu/scle, liver and peripheral blood leukocytes (Funder, 2005).

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