EFFICACY OF L-CARNITINE AGAINST MESTEROLONE INDUCED TOXICITY ON THE PANCREAS OF ADULT ALBINO RATS

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ABSTRACT

Background: The anabolic steroid drugs usage increased dramatically among the young athletes, resulting in many toxic effects, especially, acute pancreatitis and cardiovascular toxicity. L- carnitine (LC) supplementation in different models has anti-inflammatory and antioxidant roles. **Aim of the work**: to examine the ameliorative role of LC against mesterolone toxic effect on pancreas in adult albino rats. **Material and method:** Thirty-six rats were randomly assigned into 4 groups: Group I (control) 18 rats were equally presented into 3 subgroups; group Ia (no treatment), group Ib (0.5 mL corn oil), Ic (1 mL distilled water), Group II (LC) 6 rats received 350 mg/kg/day, group III (mesterolone) 6 rats were given 2.14 mg/kg/day and group IV (combined; LC and mesterolone) 6 rats received the same doses as mentioned above. All treatments were received by oral gavage for 4 weeks. Finally, rats were sacrificed; serum obtained for measuring malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), amylase and lipase levels, and blood samples for fasting blood glucose (FBG). Pancreatic tissues were prepared for histopathological, immunohistochemical and morphometric analysis of tumor necrosis factor receptor -associated factor 6 (TRAF6) and heme oxygenase-1 (HO-1). **Results:** Mesterolone caused redox imbalance, functional and structural damage of pancreatic tissue and increased TRAF6 and HO-1 immune-expression. In the combined group, LC co-administration mitigated redox imbalance, improved functional and structural damage of pancreatic tissue and decreased TRAF6 and HO-1 immune-expression. **Conclusion:** Co-administration of LC provides a protective role against mesterolone induced pancreatic toxicity through antioxidant and anti-inflammatory effects.

Keywords: *Mesterolone, Pancreas, L-carnitine, HO-1, TRAF6.*

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INTRODUCTION

Over the previous years, anabolic-androgenic steroids use has increased dramatically among athletes for enhancing their performance. The widespread use of these drugs by young athletes without any control is worrisome ***(Hallensleben et al., 2020)***.

Mesterolone (17-beta-hydroxy-1 alpha-methyl-5 alpha-androstan-3-one) is one of the frequently prescribed androgenic agonists as hormonal treatment in hypogonadism. It contains a distinctive structural configuration, which is different from other anabolic-androgenic steroids as it does not enter ring aromatization to be converted into estrogen. Accordingly it does not have estrogenic adverse actions. Furthermore, mesterolone was active through oral administration ***(Asfour et al., 2021)***. Misuse of these drugs is not exclusive to top athletes; it also has spread among many naive athletes. The abuse of androgenic steroids carries significant side effects on physiological, psychiatric and physical levels ***(Habibpoor Karimabadi et al., 2017)***.

Among numerous approved toxic effects of anabolic steroids, acute pancreatitis, cardiovascular toxicity and infertility have been mainly reported in recent years ***(Liane and Magee, 2016)***. Among possible causes of acute pancreatitis, it was found that biliary problems/gallstones and alcohol consumption represent 80% of causes. Other causes include

infections, trauma, and other causes. Drug-induced pancreatitis accounts for 0.5%-2% of all cases. The diagnosis of steroid-induced pancreatitis is intriguing, requiring careful review of medical history and exclusion of other possible causes ***(Atallah et al., 2020)***.

The mechanism that involved in induction of pancreatitis by using anabolic steroid is idiopathic; however, it could be due to the disturbed metabolism of lipid and calcium; another mechanism assumed that steroids could result in obstruction of some ductules within pancreatic tissue that in turn causes high viscosity of its secretions and pancreatic damage ***(Balani and Grendell, 2008)***.

Moreover, Pathogenesis of acute pancreatitis involves a cascade of redox imbalance and inflammatory events as pancreatic β-cells are exceptionally vulnerable to products of lipid peroxidation overload ***(Masoumi-Ardakani et al., 2020)***. Oxygen free radicals could result in pancreatic cell necrosis by oxidation of its bimolecular components of fatty acids, amino acids and nucleic acids. Moreover, it involved in initiating the cascade of pro inflammatory signaling ***(Leung and Chan 2009)***.

Furthermore, anabolic steroids increase the lipase enzyme activity, which accelerates lipolysis and boosts mitochondrial oxidation and ATP synthesis from long-chain fatty acids, resulting in the formation of reactive oxygen species and lipid peroxidation resulting in pancreatic changes ***(Hassan et al., 2023)***.

L-carnitine (LC) is an endogenous amino acid formed in the brain, kidney and liver of many mammals from two essential amino acids, methionine and lysine. It is a well-known antioxidant which actively participates in the transfer of fatty acids and the production of Acetyl CoA in the mitochondrial matrix, which is then oxidized to provide the energy required for metabolism ***(Masoumi-Ardakani et al., 2020)***.

Instead of directly scavenging free radicals or reducing their generation, LC, presumably shields tissues from oxidative stress by maintaining cell membrane stability and increasing their resistance to free radicals, enhancing the ability of phospholipid bilayer to heal from oxidative stress damage ***(Ibrahim et al., 2019)***.

The favorable effects of LC supplementation have been documented in human and animal models, as well as, it was found that it has positive antioxidant and anti-inflammatory role in variable toxicities ***(Samir et al., 2018)***. Additionally, LC has metabolic and biological role as a promising management for insulin resistance and diabetes ***(Sadighara et al., 2017; Masoumi-Ardakani et al., 2020)*** reported that LC intake has protecting effects counter to statin-induced pancreatic toxicity. LC role as a protecting therapy against pancreatitis has been investigated by many studies ***(Arafa et al. 2009; Xiang et al. 2013; Karakahya et al. 2016)***. Moreover, ***Kraft et al. (2012)*** detected the favorable impact of administration of LC in progressive cancer pancreas in a clinical trial.

THE AIM OF THE WORK

The aim of this study was to investigate the ameliorative role of LC against mesterolone toxic effect on pancreas in rat model.

MATERIAL AND METHODS

Chemicals

- Mesterolone: Obtained as a 25 mg Proviron tablet. There are two strips of ten white tablets in each pack. 25 mg of mesterolone is contained in each tablet. Made by Bayer Weimar Gm bH & Co KG, 99427-Weimar, Germany.
- L-Carnitine capsules: each contains 350 mg LC from Sigma Aldrich Co. branch in Cairo, Egypt.
- Corn oil is a commercially available oily solution used as a Mesterolone vehicle agent.

Animals

This research was executed in the animal house of Zagazig University. It included 36 adult albino rats, weighing 150-200 gm. They were kept in standard stainless-steel cages under standard environmental circumstances. They were given water and regular food. Before the study began, they were housed in appropriate settings for two weeks to allow them to adjust.

Ethical Considerations: The protocol of the experimental study was approved and the animals were treated according to the guidelines established by the Institutional Animal care and Use Committee of Zagazig University *(ZU-IACUC/3/F/326/2023).*

Experimental design:
Rats were divided into 4 groups.
Group I (control group): 18 rats
Equally and randomly divided into 3 subgroups (Ia, Ib, Ic,):
Group Ia (negative control): Rats took only tap water and regular diet for 4 weeks to estimate the basic parameters.
Group Ib (corn oil): Each rat received 0.5 mL corn oil (mesterolone solvent) through oral gavage daily for 4 weeks.
Group Ic (distilled water): Each rat was given distilled water (1 mL/day) (the solvent of LC) by oral gavage daily for 4 weeks.
Group II (LC group): 6 rats
L-carnitine was orally gavaged (350 mg/kg/day) **(*Abd-Elrazek and Ahmed-Farid, 2018)***, dissolved in distilled water daily for 4 weeks.
Group III (Mesterolone group): 6 rats
Mesterolone was orally gavaged (2.14 mg/kg) (**Yusuf et al., 2020**) dissolved in 0.5 ml corn oil daily for 4 weeks.
Group IV (combined group): 6 rats
This group was given LC and mesterolone at the same mentioned above doses daily for 4 weeks. (LC treatment was given two hours before mesterolone treatment).
Specimen collection
After 4 weeks, 24 hours after the past dose, all animals were anaesthetized by pentobarbital (50 mg /kg) through intra-peritoneal injection. A venous blood sample (3-5 mL) was obtained from the retro orbital plexus by a capillary tube. Every blood specimen was allocated into two parts. One was put into sterile tubes containing sodium fluoride to prevent glycolysis for analyzing fasting blood glucose. The other part was ejected into non-heparinized glass tubes and allowed to clot for 30 min. at 25 ºC, then the serum was separated by centrifugation (3000x g for 10 min at 4 °C) and kept at -20 °C for measuring serum levels of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), amylase and lipase. The biochemical analysis was performed at the central lab of the Biochemistry Department, Faculty of Medicine, Zagazig University. After that, animals were euthanized and pancreases were collected and splashed by normal saline fluid then by ice-cold 50 mmol/L sodium phosphate buffered saline (PBS) (100 mmol/L Na_2HPO4/NaH_2PO_4, pH 7.4) and 0.1 mmol/ L EDTA for removal of any blood clots and cells. Then fixed in formalin solution (10%) for twenty four hours before histopathological and immuno-histochemical investigations were done.
Biochemical examination:
1-**Detection of oxidative stress biomarkers:**

- **Measurement of Malondialdehyde (MDA)**
  Biodiagnostic kits (Egypt) were used to measure serum MDA in accordance with the ***De Leon and Borges (2020)*** method. A pink trimethylene complex is created when MDA and thiobarbituric acid (TBA) mix. The unit of MDA activity was nmol/ml.
- **Measurement of Reduced Glutathione (GSH) enzyme**
  Enzyme-linked Immunosorbent Assay ELISA, or double-sandwich enzyme-linked immunosorbent assay, was used. To produce a yellow composite, the method relies on using glutathione to reduce 5,5dithiobis (2-nitrobenzoic acid) (DTNB) nm (colorimetric techniques). At 405 nm, the lowered chromogen's absorbance may be observed, and it is directly correlated with GSH content ***(Tietze, 1969)***.
- **Measurement of Superoxide dismutase (SOD)**
  Superoxide radical (O2-) transition to H2O2 is facilitated by the SOD enzyme. Its activity was measured using the xanthine oxidase process, which produces O2−. When this radical combines with I.N.T [2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride], red formazan dye is produced SOD activity is linked to the suppression of this red dye production. The inhibition of 2, 2-Azino-di-[3-ethylbenzthiazoline sulphonate radical's blue-green color (ABTS) production measurement at 600 nm is the basis for TAS quantification ***(Shahouzehi et al., 2018)***.

2-Evaluation of amylase and lipase activity:
Klein and Foremann (1980) method was used to measure the serum amylase activity. On the other hand, lipase activity was

determined using the technique of ***Lott et al. (1986)***.

3- Measurement of fasting blood glucose level (FBG):

After overnight fasting, blood samples were collected to decrease variation in blood sugar ***(Ayala et al., 2010)***. Rats were sedated with ether to decrease anxiety ***(Akbarzadeh et al., 2007)***. FBG was done by using oxidase technique ***(Tietz, 1995)***.

Histo-pathological examination: All steps of histo-pathological, immune histochemical and morphometric examination were done at imaging unit, Anatomy and Embryology Department, Zagazig University, Egypt.

Hematoxylin & Eosin stain (H&E):

Histopathological studies: pancreas specimens were extracted from animals (6 specimen of each group) and after being fixed in 10% neutral bufered formalin, paraffin sections were prepared and sliced into 5um thick sections then mounted on glass slides. Following ***Bancroft and Gamble's (2008)*** instructions, sections were first deparafinized by treating them with xylene before being stained with hematoxylin and eosin (H&E).

Immunohistochemical staining of anti-Tumor necrosis factor receptor -associated factor 6 (TRAF6) and anti heme oxygenase-1 (HO-1):

Sequential slices of 4 µm thickness were dipped in 10 mM citrate bufer and exposed to heat for thirty minutes in hot water bath. All sections were left to cool for about 20 min. after that, they were washed by tap water. Every section was exposed to 3% hydrogen peroxide in methanol for about fifteen minutes to block endogenous peroxidase activity. Normal serum of goat acquired from the Vectastain Universal Elite ABC kit (Vector Laboratories) was used in order to prevent non defnite protein binding. TRAF6 antibody (1:200) (Abcam, USA) was used as primary antibodies and slides were kept with it at 4 ◦C all through the night and the same anti-HO-1 rabbit polyclonal antibody (Stressgen Biotechnologies, Canada) for 2.5 hours and finally, with HRP (horseradish peroxidase)-conjugated secondary antibodies (Zhongshan Goldernbridge Biotechnology Co., Beijing, China) for one hour at room ordinary temperature. To get brown color, 3,3-diaminobenzidine (DAB) fluid was used. The control was acquired by incubation of sections without primary antibody. Consequently, 0.1% hematoxylin was used to counterstain the sections for five minutes ***(Cote et al. 1993; Shen et al. 2020; Luo et al. 2021)***.

Morphometric analysis:

Six rats per group underwent morphometric analysis using the Image J IHC Profler plugin for Image J software.

The proportion of positive expression (brown-stained areas) was calculated after 400x magnification photos of anti-TRAF6 and anti-HO-1 immunostained slides were taken. For morphometric analysis, five pictures of each animal's non-overlapping fields were released.

Statistical Analysis:

IBM SPSS Statistics for Windows was used for data analysis ***(Version 25; IBM Corp., Armonk, NY, USA, 2017)***. Mean±SD was used for presentation of data. The data was evaluated using one-way analysis of variance (ANOVA) then; the least significant difference test was performed for comparisons between several groups. P values <0.05 were deemed statistically significant.

RESULTS

Mortality and General Observations: The administered dose of mesterolone caused no deaths during the experiment.

Biochemical results

The results displayed no statistically dramatic change among subgroups of control (Ia, Ib, Ic) regarding all assessed parameter. So, the negative control one was used a reference value for comparison with other treated groups.

Mesterolone (groupIII) notably ($P<0.01$) elevated MDA level and decreased GSH and SOD activity in comparison with control group. In the combined group (group IV), MDA level was significantly improved, GSH and SOD activity was increased in comparison with mesterolone (group III) but still not reached the control level (group I) **(Table 1)**.

Regarding pancreatic function (amylase, lipase and fasting blood glucose), mesterolone

(groupIII) showed a highly dramatic elevation in amylase, lipase and FBG levels compared to control (groupI). In the combined group (group IV), LC co-treatment showed a notable improvement in amylase,and lipase serum levels and FBG in comparison with mesterolone alone. However, still not reached the control level **(Table 1)**.

Histopathological results

Histopathological examination of H&E stained specimens of pancreatic tissues from groupI (control groups) as well as group II (LC) revealed normal architecture as exocrine portion of pancreas appeared with lobes separeted with narrow septa, having pyramidal cells with basal nuclei along densely packed acini and large duct lined with cuboidal cells.

The endocrinal portion represented by Islets of Langerhans appeared lightly stained with large cells, β cells are located in the center and have pale nuclei, while α cells are located on the periphery and appear as oval cells with black nuclei. Numerous capillaries are surrounded by these secretory cells **(Figure 1a, b)**.

The pancreatic islets of Langerhans in group III (mesterolone) displayed noticeable vacuolations both inside and between their secretory cells, which gave the appearance of being disorganized.

Additionally, several major blood vessels within the Islets of Langerhans and between acini that appeared congested with small number of inflammatory cells in between them. Bile ducts appeared dilated , filled with hyaline material and lined with vacuolated cells. The septa appeared wide **(Figure 1 c, d)**.

While group IV (LC and mesterolone), pancreas revealed restoration of islets of Langerhans, but still show marked vacuolations and congestion within them **(Figure 1e)**.

Immunohistochemical study

Immunohistochemical staining

- By using anti- TRAF6 antibody, the group I (control groups) as well as groupII (LC) showed minimal expression in anti TRAF 6 immunestained slides **(Figure 2a, b)**.
  In contrast, anti TRAF 6 immunopositive cells (appear with brown cytoplasm) in group III (mesterolone) islets of Langerhans that was significantly higher than control **(Figure 2c)**. The immune positivity showed apparent decrease for little extent in group IV (combined group) **(Figure 2d)**.
- In slides stained by anti HO-1 immunestaining, The group I control groups and group II (LC) showed little positive expression in anti HO-1 **(Figure 3a, b)**.
  In contrast, anti HO-1 immunopositive cells in group III (mesterolone) was markedly higher than control (**Figure 3c)**. Area percent of anti HO-1 positive expression in group IV (LC and mesterolone) was less than group III (mesterolone) but still more than control **(Figure 3d)**.

Morphometric results

Regarding morphometric analysis of anti-TRAF6 immune staining, the area percentage of its expression in pancreatic tissue of group III (mesterolone) was significantly higher compared to the control group. Furthermore, a notable decline in these earlier factors were detected in combined group if compared with group III (mesterolone) but still more than control group **(Figure 2)**.

There was a significant increase in anti-HO-1 immune-staining, expression in pancreas of group III (mesterolone) in comparison with the group I control group. Additionally, a significant decrease in these previous parameters were detected in group IV combined group if compared with group III (mesterolone) but still more than control group **(Figure 3)**.

Table (1): Statistical comparison among control, l-carnitine, mesterolone and combined groups regarding serum levels of MDA, GSH, SOD amylase, lipase, FBG, using ANOVA test.

Parameters	group I (control)	group II (L-carnitine)	group III (Mesterolone)	group IV (combined)	F	p-value
	Mean± SD					
MDA (nmol/mL)	11.75±0.75	10.08±1.96$^{\#}$	53.33±10.80^{a}	16.16±3.31^{*b}	76.341	<0.001**
GSH (nmol/mL)	163.88±5.53	166.66±6.71$^{\#}$	86.63±8.82^{a}	153.16±5.84^{*b}	182.193	<0.001**
SOD (U/Ml)	4.50±0.23	4.750±0.50$^{\#}$	1.07±0.20^{a}	3.35±0.58^{*b}	98.129	<0.001**
Amylase (U/L)	28.17±4.53	27.67±1.75$^{\#}$	216.67±42.74^{a}	50.50±6.53^{*b}	105.531	<0.001**
Lipase (U/L)	24.50±3.01	23.67±3.14$^{\#}$	198.67±33.74^{a}	35.17±3.25^{*b}	150.553	<0.001**
FBG (mg/dl)	91.50±9.66	86.83±7.65$^{\#}$	344.16±32.62^{a}	126.33±9.43^{*b}	276.270	<0.001**

*Data was expressed as mean± standard deviation (SD); number of rats in each group equals 6; **: statistically highly significant (p<0.001), #: statistically not significant (p>0.05) compared to control group, *: statistically significant (p<0.05) compared to control group, a: statistically highly significant (p<0.001) compared to control group; b: statistically highly significant (p<0.001) compared to Mesterolone group; SOD: superoxide dismutase, MDA: Malondehyldhde; GSH: reduced glutathione.*

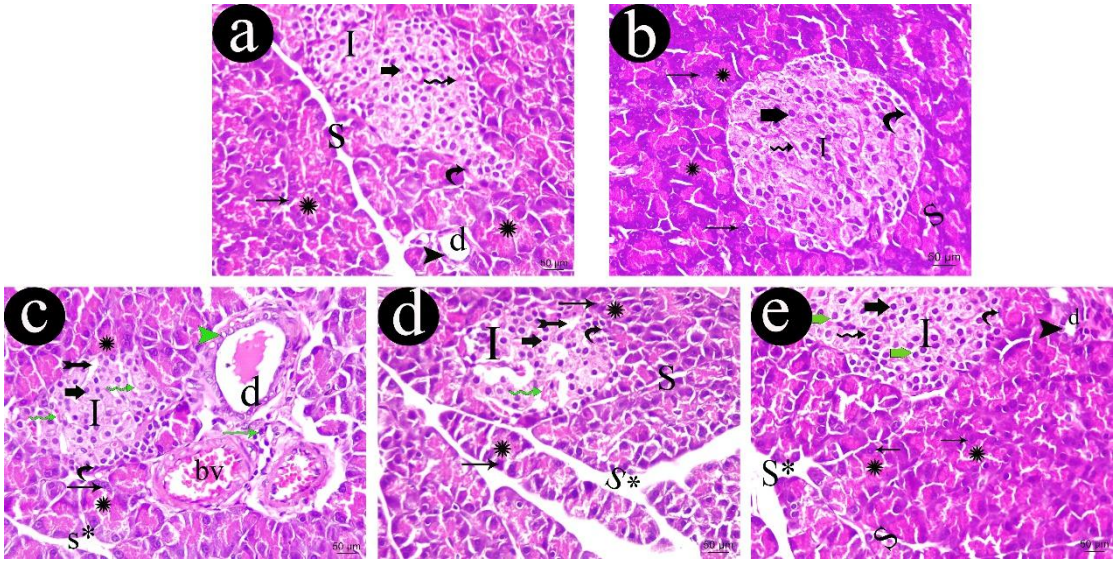

Figure (1): *Photomicrographs of pancreatic sections in different experimental groups, (**a**) control group, (**b**) group II both groups appear with normal architecture as having pyramidal cells with basal nuclei (arrow) along densely packed acini (asterisk) with narrow septa in between them (S) and large duct (d) lined with cuboidal cells (arrow head). The endocrinal portion represented by Islets of Langerhans (I) appeared lightly stained with large cells, β cells are located in the center and have pale nuclei(thick arrow) , while α cells are located on the periphery and are oval cells with black nuclei(curved arrow) . Numerous capillaries are surrounded by these secretory cells (zigzag arrow). (c and d) group III ; The pancreatic islets of Langerhans display noticeable vacuolations (tailed arrow) both inside and between their secretory cells, several congested blood capillaries within the Islets of Langerhans and between acini (green zigzag arrow) and a small number of inflammatory cells (green arrow) appear . Bile ducts appeared dilated, filled with hyaline material and lined with vacuolated cells (green arrow head). The septa appeared wide (S*). (**e**) group IV ; pancreas reveal restoration of islets of Langerhans (I), but still show marked vacuolation and congestion within them.(green zigzag arrow), (thick arrow) β cells,(curved arrow) α cells, (arrow head) cuboidal cells, (asterisk) acini, (arrow) basal nuclei ,(tailed arrow) represent vacuolation, (S) narrow septa and (S*) dilated septa. H&E, scale bar 50 µm.*

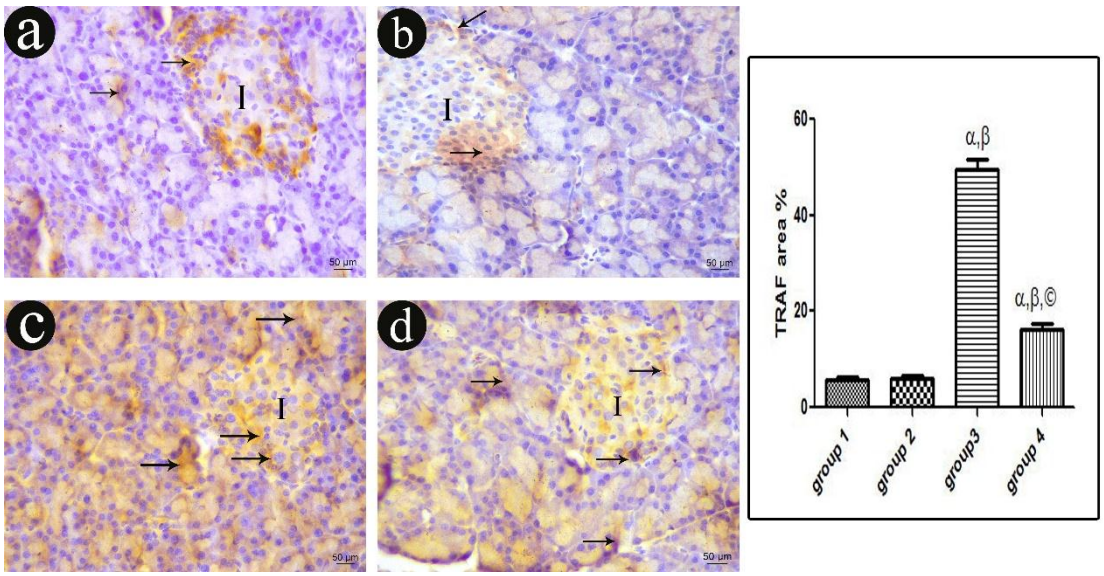

Figure (2): *Photomicrographs showing the distribution of anti TRAF 6 positive cells within pancreatic sections in different experimental groups by anti TRAF 6 immunostaining. (a) control group; (b) group II ; (c) group III; (d) group IV . (I) Islets of Langerhans, (arrow) positive cells .Scale bar; 50 µm.*
Bar chart: *showing statistical assessment of the area percentage of positive reaction to anti TRAF5 in different studied groups . The values are presented as mean ± SD. P value<0.05 was considered significant. (1-αP value<0.05 versus control group) 2- β P value<0.05 versus group II .3-© P value<0.05 versus group III.*

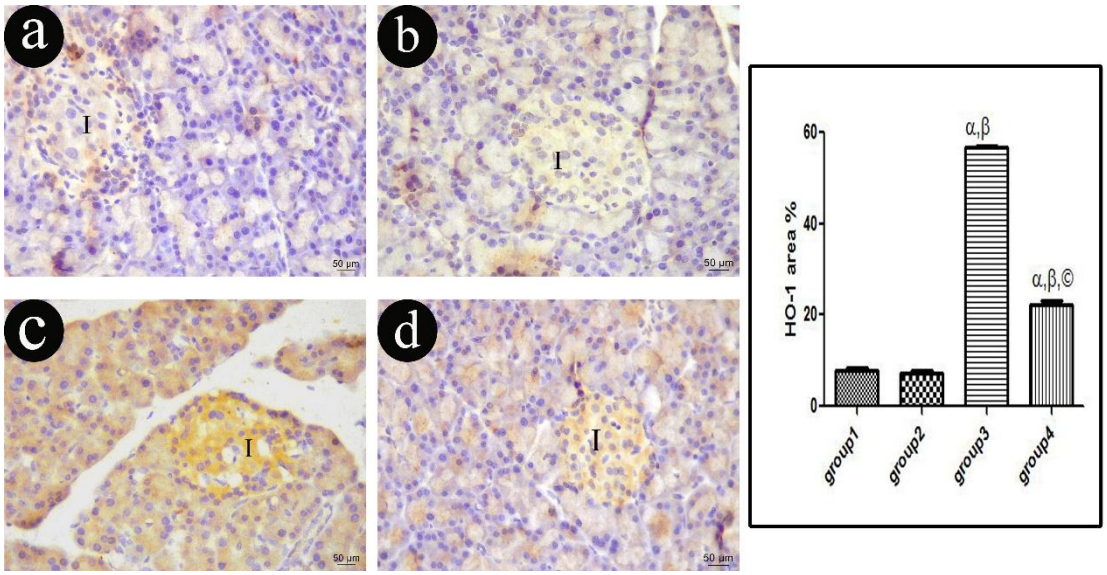

Figure (3): *Photomicrographs showing the distribution of anti HO-1 positive cells within pancreatic sections in different experimental groups by anti HO-1 immunostaining. (a) control group; (b) group II; (c) group III; (d) group IV. (I) Islets of Langerhans, .Scale bar; 50 µm.*
Bar chart: *showing statistical assessment of the area percentage of positive reaction to anti HO-1 in different studied groups . The values are presented as mean ± SD. P value<0.05 was considered significant. (1-αP value<0.05 versus control group) 2- β P value<0.05 versus group II .3-© P value<0.05 versus group IV.*

DISCUSSION

Anabolic steroid treatment is accompanied with numerous detrimental side effects such as acute pancreatitis, cardiovascular toxicity and infertility ***(Thanage et al., 2019)***. The lack of understanding of their harmful grave effects can affect athletes' physical and mental health. The alarming increase in the unregulated use of these substances by bodybuilders can result in a variety of organ-specific pathologies, including Acute Pancreatitis.

L-carnitine is an effective ancillary that can protect the pancreas from numerous threats ***(Sadighara et al., 2017)***. It is essential in the long-chain fatty acids transportation across the mitochondrial membrane especially in the cells that need high energy. Moreover, it has many protective properties against oxidation, inflammation and apoptosis ***(Mansour et al., 2021)***.

This research aimed to examine the possible ameliorating role of LC in reducing pancreatic oxidative overload, inflammation and apoptosis induced by mesterolone.

This work showed a notable elevation in fasting blood glucose, serum amylase, lipase and MDA levels in group III (mesterolone) versus group I (control group), although GSH, SOD level was significantly lowered

Treatment with LC in group IV combined group caused a decrease in level of FBG, serum amylase, lipase and MDA with increase in GSH, SOD level although, that increase remained less than the control.

In the same context, ***Sun et al. (2019)*** and ***Ataallah et al. (2020)*** reported a relationship between using steroid and development of acute pancreatitis. Among AP patients, the incidence rate of hyperglycemia increased. As there is a close correlation between glucose level and inflammation in AP, which could aggravate the disease by enhancing the inflammatory process. High-level glucose can be considered as one of the main markers for determining the progression of AP in clinical practice ***(Schaffler et al., 2007)***.

Moreover, ***Erdemli et al. (2021)*** approved that in AP; there was an elevation of glucose and MDA levels while SOD, GSH levels decreased; however, these parameters improved upon LC administration represented in reduced blood glucose, MDA and increased SOD, glutathione (GSH) ***(Yaribeygi et al., 2019; Masoumi-Ardakani et al., 2020)***.

Matull et al. (2006) and ***Rosenfeld et al. (2011)*** reported that acute pancreatitis developed because of anabolic androgenic steroid overuse and lead to an increase in the level of amylase and lipase enzymes, which were released from pancreatic acinar cells**.**

In the same context, ***Ibrahim et al. (2019)*** showed a significant up regulation of serum amylase and MDA level in AP while GSH level was significantly declined. During AP, amylase is liberated from pancreatic acini, so its high level in the serum is considered as confirmation of the diagnosis. But treatment with LC decreased serum amylase and MDA level, while increased GSH level.

Furthermore, ***Hasan et al. (2015)*** denoted that LC affects the activity of amylase and lipase enzymes through its potent antioxidant actions, accordingly maintenance of cell membranes of zymogen granules against reactive oxygen species and enhancing the restoration of phospholipid bilayer function.

The biochemical results were confirmed by histological finding, as pancreatic specimen of group III (mesterolone group) appeared with marked vacuolations and disorganization of Islets of Langerhans cells. Moreover, there was marked congestion,inflamatory infiltrations and bile ducts appeared dilated, filled with hyaline material and lined with vacuolated cells with wide septa in between. the same findings were reported by ***Iovanna (2009)*** and ***Ibrahim et al. (2019)*** as they described damage of the exocrine pancreas in acute pancreatitis leading to severe necrosis of acinar and ductal cells with massive edema and cellular infiltration.

On adminstration of LC along with mesterolone, pancreatic tissue revealed restoration of islets of Langerhans, but still with marked vacuolations and congestion within them.

In accordance with our results Also, LC therapy significantly reduced the total pancreatic histopathological changes. This may be explained by its anti-inflammatory, anti-oxidant and ROS scavenging actions. Also, ***Masoumi-Ardakani et al. (2020)*** confirmed that LC decreased the degree of

cell loss and necrotic changes in acute pancreatitis.

TRAF6 is an important protein in the tumor necrosis factor receptor-associated protein group, that is included in a wide array of inflammatory changes and cell death by regulating diverse signaling pathways ***(Yang et al., 2009)***.

The current immunohistochemical study revealed marked positive expression of TRAF6 and HO-1 in mesterolone group in comparison to control group. the positive expression showed apparent decrease on co-adminstration of LC along with mesterolone compared to mesterolone alone. These results were supported by morphometric analysis.

Wei et al. (2022) reported that TRAF6 has been included in AP and the related pulmonary tissue damage through the TLR4/NF-κB signaling pathway , these findings correlated with our result concerning the same pathway of anabolic steroid drugs causing the cascade of oxidative stress which leads to AP and also reported by ***Masoumi-Ardakani et al. (2020)***.

Hasan et al. (2015) demonstrated that, the dramatic elevation in the TRAF6 expression was linked to the activity of ROS and adminstration of LC decreased their expression via inhibition of TLR4/NF-κB signaling pathway.

Concerning HO-1 expression, our results were consistent with prior experiments done by ***Yang et al. (2014)*** and ***Zhang et al. (2017)*** who stated that, increased positive expression of HO-1 was evident in acute pancreatitis as a part of the stress response to inflammation and oxidative damage.

Lee et al. (2014) showed that LC adminstriation reduce oxidative stress, inflammatory cytokines and reduce pancreatic cell deaths which in turn improve the severity of AP. And our result showed reduction in the expression of HO-1 in the combined group IV compared to the mesterlone group III due to the decrease of the intenisty of AP. But still HO-1 level increased in the combined group in relation to control group as a result of LC intake as showed in the study of ***Salama et al. (2021)***.

CONCLUSION

Sub-acute intake of mesterolone has toxic effects on pancreas as reflected by impaired pancreatic function and structure, oxidative stress-mediated damage via increased lipid peroxidation and decreased GSH and SOD, and inflammation through increased immune expression of TRAF6 and HO-1 in treated rats. LC through its beneficial antioxidant and anti-inflammatory effect ameliorated mesterolone-induced functional and structural damage in pancreas of albino rats.

RECOMMENDATIONS

Mesterolone use in young athletes should be restricted, also, a simple test as fasting blood glucose may be routinely performed for body builders who are under effect of mesterolone to identify its toxicity, LC can be used as a supplement for prevention of mesterolone-induced pancreatic toxicity. Potential use of LC in toxicity of other anabolic-androgenic steroids needs further studies to be clarified.

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REFERENCES

1. **Abd-Elrazek, A. M. and Ahmed-Farid, O. A. H. (2018):** Protective effect of L-carnitine and L-arginine against busulfan-induced oligospermia in adult rat. *Androl., 50(1): e12806.*
2. **Akbarzadeh, A.; Norouzian, D.; Mehrabi, M. R. et al. (2007):** Induction of diabetes by streptozotocin in rats. India. *J. Clin. Biochem., 22: 60-64.*
3. **Arafa, H. M., Hemeida, R. A., Hassan, M. I. et al. (2009):** Acetyl-l-Carnitine Ameliorates Caerulein-induced Acute Pancreatitis in Rats. *Basic Clinical pharmacol. Toxicol., 105(1): 30-36.*
4. **Asfour, H. A.; Shaqoura, E. I.; Said, R. S. et al. (2021):** Differential response of oxidative and glycolytic skeletal muscle fibers to mesterolone. *Sci. Rep., 11(1): 1-9.*

5. **Ataallah, B.; Abdulrahman, M.; Al-Zakhari, R. et al. (2020):** Steroid-induced pancreatitis: a challenging diagnosis. *Cureus, 12(7): 1-3 e8939.*
6. **Ayala, J. E.; Samuel, V. T.; Morton, G. J. et al. (2010):** Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. *Dis. Models Mech., 3(9-10): 525-534.*
7. **Balani, A. R. and Grendell, J. H. (2008):** Drug-induced pancreatitis: Incidence, management and prevention. *Drug safety, 31(10): 823-837.*
8. **Bancroft, J. D. and Gamble, M. (2008):** Theory and practice of histological techniques, *Elsevier health sciences.*
9. **Cote A.; da Silva R. and Cuello A. (1993):** Current protocols for light microscopy immunocytochemistry. In: Cuello AC, ed. Immunohistochemistry II. Chichester: *John Wiley and Sons, pp. 147–168.*
10. **De Leon, J. A. D. and Borges, C. R. (2020):** Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *J. Visualized Exp. JoVE, (159): 10-3791.*
11. **Erdemli, Z.; Altinoz, E.; Erdemli, M. E. et al. (2021):** Ameliorative effects of crocin on tartrazine dye–induced pancreatic adverse effects: a biochemical and histological study. *Environ. Sci. Poll. Res., 28: 2209-2218.*
12. **Habibpoor Karimabadi, F.; Abbasi Daloii, A.; Abdi, A. et al. (2017):** Structural changes of cardiac tissue in response to L-carnitine supplementation during endurance training in Wistar male rats toxicated by steroid anabolic hormone. *Hormozgan Med.J., 21(2): 86-95.*
13. **Hallensleben, N. D.; Umans, D. S.; Bouwense, S. A. et al. (2020):** The diagnostic work-up and outcomes of 'presumed'idiopathic acute pancreatitis: a post-hoc analysis of a multicentre observational cohort. *United Euro. Gastroenterol. J., 8(3):340-350.*
14. **Hasan, M. I.; Bakr, A. G. and Shalkami, A. G. S. (2015):** Modulation of L-arginine-induced acute pancreatitis by meloxicam and/or L-carnitine in rats. *Int. J. Basic Clin. Pharmacol., 4:1247-1253.*
15. **Hassan, D. A. E.; Ghaleb, S. S.; Zaki, A. R. et al. (2023):** The toxic effects of anabolic steroids nandrolone decanoate on cardiac and skeletal muscles with the potential ameliorative effects of silymarin and fenugreek seeds extract in adult male albino rats. *BMC Pharmacol. Toxicol., 24(1):17.*
16. **Ibrahim, W.; Ghada, F. S. and Abdallah, M. (2019):** Therapeutic effect of L-carnitine on acute pancreatitis induced by L-arginine in rats: possible role of beclin gene and inducible nitric oxide synthase. *Med. J. Cairo Univers., 87(6): 1793-1803.*
17. **Iovanna J. L. (2009):** Cellular Stress in Acute Pancreatitis. *Pancreatol., 9-24.*
18. **Leung, P. S. and Chan, Y. C. (2009):** Role of oxidative stress in pancreatic inflammation. *Antiox. Redox Sig., 11(1): 135-166.*
19. **Karakahya, M.; Gül, M.; Işık, S. et al. (2016):** The Histopathologic effects of L-Carnitine in a sodium Taurocholate–induced severe pancreatitis model. *Int. Surg., 101(5-6): 241-248.*
20. **Klein, B. and Foreman, J. A. (1980):** Amylolysis of a chromogenic substrate, Cibachron Blue F3GA-amylose: Kinetics and mechanism. *Clin Chem.,26(2):250-253.*
21. **Kraft, M.; Kraft, K.; Gärtner, S. et al. (2012):** L-Carnitine-supplementation in advanced pancreatic cancer: A randomized multicenter trial. *Nutr. J., 11: 1-6.*
22. **Lee, B. J.; Lin, J. S.; Lin, Y. C. et al. (2014):** Effects of L-carnitine supplementation on oxidative stress and antioxidant enzymes activities in patients with coronary artery disease: a randomized, placebocontrolled trial. *Nutr. J., 13:79.* Doi:10. 1186/1475-2891-13-79
23. **Liane, B. J. and Magee, C. (2016):** Guerilla warfare on the pancreas? A case of acute pancreatitis from a supplement known to contain anabolic-androgenic steroids. *Milit.Med., 181(10): e1395-e1397.*
24. **Lott, J. A.; Patel, S. T.; Sawhney, A. K. et al. (1986):** Assays of serum lipase: analytical and clinical considerations. *Clin. Chem., 32(7): 1290-1302.*
25. **Luo, W.; Chen, X.; Ye, L. et al. (2021):** Kaempferol attenuates streptozotocin-induced diabetic nephropathy by downregulating TRAF6 expression: The role of TRAF6 in diabetic nephropathy. *J. Ethnopharmacol., 268:113553.*
26. **Maheshwari, R. and Subramanian, R. M. (2016):** Severe acute pancreatitis and necrotizing pancreatitis. *Crit. Care Clin., 32 (2): 279–290.*
27. **Mansour, H. H.; Ibrahim, A. B. and Omran, M. M. (2021):** Effect of L-Carnitine on cardiotoxicity and apoptosis induced by imatinib through PDGF/PPARγ/MAPK pathways. Arch. *Biochem. Biophys. J.,704: 108866.*

28.**Masoumi-Ardakani, Y.; Aminizadeh, S.; Fallah, H. et al. (2020):** L-carnitine different doses affect serum and pancreas tissue Antioxidative defense and histopathology in STZ-induced diabetic rats. *Biol., 75(9): 1415-1423.*

29.**Matull W. R.; Pereira S. P. and O'Donohue J.W. (2006):** Biochemical markers of acute pancreatitis. *J. Clin. Pathol.,59 (4):340-344*

30.**Rosenfeld, G. A.; Chang, A.; Poulin, M. et al. (2011):** Cholestatic jaundice, acute kidney injury and acute pancreatitis secondary to the recreational use of methandrostenolone: a case report. *J. Med. Case Rep., 5: 1-5.*

31.**Sadighara, M.; Joktaji, J. P.; Hajhashemi, V. et al. (2017):** Protective effects of coenzyme Q10 and L-carnitine against statin-induced pancreatic mitochondrial toxicity in rats. Res. *Pharmaceut. Sci., 12(6): 434-443.*

32.**Salama, A.; Fayed, H. M. and Elgohary, R. (2021):** L-carnitine alleviated acute lung injuries induced by potassium dichromate in rats: involvement of Nrf2/HO-1 signaling pathway. *Heliyon, 7: 6.*

33.**Samir, S. M., Abbas, A. M., Safwat, S. M. et al. (2018):** Effect of L-carnitine on diabetes-induced changes of skeletal muscles in rats. *J. Basic Clinical Physiol. Pharmacol., 29(1): 47-59.*

34.**Schaffler, A.; Landfried, K.; Volk, M. et al. (2007):** Potential of adipocytokines in predicting peripancreatic necrosis and severity in acute pancreatitis: Pilot study. *J. Gastroenterol. Hepatol. 22:326–334.*

35.**Shah, A. P.; Mourad, M. M. and Bramhall, S. R. (2018):** Acute pancreatitis: current perspectives on diagnosis and management. *J. Inflamm. Res. 11: 77–85.*

36.**Shahouzehi, B.; Shabani, M.; Shahrokhi, Net al. (2018):** Effects of Pistacia atlantica resin oil on the level of VEGF, hydroxyproline, antioxidant and wound healing activity in STZ-induced diabetic rats. *Ukrain. Biochem. J., 90(1): 34-41.*

37.**Shen, Z.; Yang, C.; Zhu, P. et al. (2020):** Protective effects of syringin against oxidative stress and inflammation in diabetic pregnant rats via TLR4/ MyD88/NF-κB signaling pathway. *Biomed. Pharmacother., 131: 110681.*

38.**Sun, Y. F.; Song, Y.; Liu, C. S. et al. (2019):** Correlation between the glucose level and the development of acute pancreatitis. *Saudi J. Biol. Sci., 26(2): 427-430.*

39.**Tietze F. (1969):** Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. *Anal. Biochem., 27 (3): 502-522.*

40.**Tietz, N. W. (1995):** Clinical guide to laboratory tests. In Clinical guide to laboratory tests, *Philadelphia, PA: Saunders, pp. 1096.*

41.**Thanage, R.; Chandnani, S.; Zanwar, V. et al. (2019):** Unusual case of simultaneous acute hepatitis and acute pancreatitis in a bodybuilder. *Topiwala Natl. Med. Coll. Gastroenterol. Pancreatol. Liver Disorder, 7(1):1-3.* doi:10.15226/2374-815X/7/1/00 1137.

42.**Wei, B.; Wu, Q.; Yang, X. et al. (2022):** Effect of TRAF6 in acute pancreatitis-induced intestinal barrier injury via TLR4/NF-κB signal pathway. *Tissue and Cell, (76*): 101792.

43.**Xiang, Y.; Piao, S. G.; Zou, H. B. et al. (2013):** L-carnitine protects against cyclosporine-induced pancreatic and renal injury in rats. *Transplant. Proceed.,45(8): 3127-3134.*

44.**Yang, W. L.; Wang, J.; Chan, C. H. et al. (2009):** The E3 ligase TRAF6 regulates Akt ubiquitination and activation. *Sci., 325 (5944):1134–1138*

45.**Yang, F.; Xie, J.; Wang, W. et al. (2014):** Regional arterial infusion with lipoxin A4 attenuates experimental severe acute pancreatitis. *PLoS One, 9(9): e108525.*

46.**Yaribeygi, H.; Noroozadeh, A.; Mohammadi, M.T. et al. (2019):** Crocin ımproves oxidative stress by potentiating ıntrinsic anti-oxidant defense systems in pancreatic cells during uncontrolled hyperglycemia. *J. Pharmacopunc., 22(2): 83-89.*

47. **Yusuf, M. K.; Ibegbu, A. O. and Alawa, J. N. (2020):** A Comparative Study of the Effects of Ethanolic Extract of Sesamum indicum Seeds and Mesterolone on Some Testicular Morphometry of Adult Male Wistar Rats Secondary to Ketoconazole-Induced Damage. *J. Clin. Med. Res., 1(1): 1-12.*

48. **Zhang, F. H.; Sun, Y. H.; Fan, K. L. et al. (2017)**: Protective effects of heme oxygenase-1 against severe acute pancreatitis via inhibition of tumor necrosis factor-α and augmentation of interleukin-10. *BMC Gastroenterol., 17(1-7):901–908.*