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RESEARCH ARTICLE

IMMUNO PROTECTIVE, ANTI-DIABETIC AND HISTOCHEMICAL-ANTI OXIDATIVE ACTIVITIES OF L-CARNITINE, AND CALF THYMUS EXTRACT IN AGED MALE MICE.

Hanem El-Gendy¹, Shima R. masoud², Seham S.Hadad³, Saber El-Hanbally¹ and Amra Abd El Latif⁴.

1. Department of Pharmacology, Faculty of Veterinary Medicine, University of Sadat City, Egypt.
2. Department of Physiology, Faculty of Veterinary Medicine, University of Sadat City, Egypt.
3. Department of Anatomy&Embryology, Faculty of Veterinary Medicine, University of Sadat City, Egypt.
4. Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

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Abstract

Background: The mechanisms behind of immunosenescence have remained largely unknown in elderly. Some studies are referred the cause to that, L-carnitine is essential nutrient factor which it is important in transporting of long chain fatty acids to mitochondrial matrix, a process essential for fatty acid oxidation and energy release. The immunobiological properties of a new formulation of the lipid thymus calf extract (CYTOIMMUNE ®) were determined.

Methods: In this study, the immunomodulating effect of L-carnitine and calf thymus extract were studied in aged male mice. Forty mice were divided into four groups, each group included ten aged male mice. Group I, each mice was injected intraperitoneal (I.P.) with normal saline for 7 successive days. Group II, each mice was injected I.P. with L-carnitine at dose 200 mg/kg b.wt. for 7 successive days. Group III, each mice was injected I.P. with calf thymus extract at dose 0.5 mg/kg b.wt. for 7 successive days. Group IV, each mice was injected I.P. with L-carnitine at dose 200 mg/kg b.wt. Plus calf thymus extract at dose 0.5 mg/kg b.wt. for 7 successive days. RBCs & WBCs count, PCV, differential leukocytic count, phagocytic activity, phagocytic index, total protein, globulin, albumin, interleukin2, ALT, AST & blood glucose were measured. Moreover, after slaughtering the animals, histological sections were taken from main internal organs (liver, spleen, kidney) to show internal changes of previous tissues and evaluated the protective and antioxidant properties by using the previous experimental preparations (L-carnitine at dose 200 mg/kg - b.wt., calf thymus extract at dose 0.5 mg/kg b.wt. and combination of them).

Results: The interaperiotoneal administration of L-carnitine at dose 200 mg/kg b.wt. calf thymus extract at dose 0.5 mg/kg b.wt. and combination between them. L-carnitine at dose 200 mg/kg b.wt. and calf thymus extract at dose 0.5 mg/kg b.wt. for 7 successive days had an improving effect on immune response, glucose level and hepatic marker enzymes as well as improving the histological architecture in the internal tissues (liver, kidney and spleen) and a significant increase in CAT (catalase enzyme), in liver and kidney. These results clearly

Corresponding Author. Seham S.Hadad

Address:-Department of Anatomy&Embryology, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

show the antioxidant and protective property of experimental preparations.

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Introduction:-

The immune system identified and eliminates pathogens with the induction of natural and adaptive immune responses (Colucci *et al.*, 2013). Several researchers studied the immunomodulating effect of different pharmaceutical agents, and natural substances. L-carnitine was discovered in muscle extracts & named the substance for the Latin word *carnis* flesh or meat (Karlic *et al.*, 2004). L-carnitine is a quaternary amine (4-N-trimethylammonium-3-hydroxybutyric acid) that is essential for the normal oxidation of long-chain fatty acids by mitochondria for β oxidation and ATP production in tissues (Edres *et al.*, 2018). It is synthesized in liver, kidney and brain from two essential amino acids; lysine and methionine.

L-carnitine has powerful exogenous anti-oxidant, (Thangasamy *et al.*, 2008), by preventing the formation of reactive oxygen species, scavenge free radicals and protect cells from peroxidative stress (Uysal *et al.*, 2003).

L-carnitine has anti-diabetic properties (Malik *et al.*, 2011 & Hajinezhad *et al.*, 2016), and possesses ability to induce immunomodulatory effects, as its supplementation stimulates protective immunity on vaccination through improve neutrophil and macrophage activity (Thangasamy *et al.*, 2008) so the defective of the peripheral blood lymphocytes of elderly age with acquired immune deficiency syndrome is improved by L-carnitine which has a hepatoprotective effect (Yapar *et al.*, 2007).

The thymus gland is the primary lymphoid organ that provide site of T cell production and activation, it represents a key organ of the immune system, (Haroun, 2018). With aging and after puberty, the thymus begins to shrink and involute and the production as well as development of T-cells reduce to a lower rate (Haroun, 2018 and Janeway *et al.*, 2001), therefore, the thymus extracts have been shown to modulate the development, maturation and activation of T cells, stimulate production of interleukin-2 (IL-2), their effect on the specialization and migration of T-lymphocytes throughout the body. The thymus also releases hormones that regulate immune function (Colic *et al.*, 2008). Pretreatment with the calf thymus extract increased the antioxidant enzymes in a dose-dependent manner (Ganie *et al.*, 2014).

Natural thymic peptides have been isolated from calf thymus by mild acid extraction. Pharmaceutical containing natural peptides, thymalin was put into practice as immune corrector. Thymalin and thymogen were used in persons with chronic pathology and immune dysfunction. The previous results indicated that thymic peptides participate in the regulating mechanisms of inflammatory processes as cytokine antagonists (Morozov and khavinson, 1997). The researcher reported that thymalin stimulated T cells while acting with immunoglobulins (Wilson, 1999). Thymalin is a polypeptide complex extracted from the thymus. It is approved for medical application as an immunomodulator by USSR Ministry of Health Order No. 1008 of 10.11.1982 ,Registration No. 8.1008.8 (khavinson and Morozov, 2003). Barboza *et al.* (2000) have described the use of thymus extract in therapy of different diseases in few animal species (dogs, rabbits and pigs). The thymus extract acts upon the production, maturation and activation of T lymphocytes and macrophages, and stimulates the maturation of immature thymocytes (T6 cells) to T3 cells in the bone marrow.

Thymosin, soluble hormone-like peptides produced by the thymus gland, can mediate immune and non-immune physiological processes and have gained interest in recent years as therapeutics in inflammatory and autoimmune diseases (Severa *et al.*, 2019). Thymalin and thymosin are the main components of thymus extract. After oral administration of crude thymus extract at dose 1 ml/chick significantly increased the total protein, globulin, albumin, T3, T4 and the body weight gain in chick of one-day-old. Thymus extract also increased total lymphocytic count (Abdel Fattah *et al.*, 1999).

Further investigations are required to elucidate role of L-carnitine and calf thymus extract in various immune functions and hence the present study was designed to evaluate the effect of L-carnitine and calf thymus extract on certain immunological functions and performance characteristics in aged male mice.

Materials & methods:-**Drugs****L-carnitine**

It was obtained as Pure white powder from Mepaco Company (Arab company for pharmaceutical & Medicinal plants) (Egypt).

Calf thymus extract

It was obtained from Biomediacompany for Biological and Veterinary Products, Egypt, under a trade name (CYTOIMMUNE ®). It is a liquid preparation, each bottle of 500 ml capacity of calf thymus gland extract. Main ingredients of this extract are natural thymic peptides, thymalin & polynucleotides.

Animals:

Forty adult male mice apparently healthy, two years old, with an average weight of 37-40 gm, were selected for this study. They were fed standard mice pellets and water *ad libitum*. They were left for one week before starting the experiment for acclimatization to laboratory conditions. The protocol of the study has been approved by the Ethical Committee of the College of Medicine/ Faculty of Veterinary Medicine, University of Sadat City, Egypt.

Experimental design:

The animals were divided into four groups (10 mice each) as the following:

Group I (Control):

Each mice in this group was injected intraperitoneal (I.P.) with normal saline (0.9 %) every day for 7 successive days.

Group II:

Each mice in this group was injected I.P. with the tested drug A (L-carnitine dissolved in normal saline) at dose 200mg/kg b.wt. Every day for 7 successive days.

Group III:

Each mice in this group was injected I.P. with the tested drug B (calf thymus extract in normal saline) at dose 0.5 mg/kg b.wt. Every day for 7 successive days.

Group IV:

Each mice in this group was injected I.P. with the tested drug A (L-carnitine dissolved in saline) at dose 200mg/kg b.wt. Plus the tested drug B (calf thymus extract dissolved in saline) at dose 0.5 mg/kg b.wt. Every day for 7 successive days.

Samples collection

Blood samples were collected from five randomly selected animals from each group. Blood samples were obtained from the retro-orbital sinus of the eye and each blood sample was divided into three portions. The first portion was collected in a small labeled dry and clean vial containing one drop of heparin as anticoagulant to determine the phagocytic activity and phagocytic index. The second portion of blood was collected in a small labeled dry and clean vial containing EDTA for hematological studies. The Third portion of blood was collected in plain clean dry and sterile centrifuge tubes and were allowed to coagulate and then centrifuged at 3000 rpm for 10 min. to separate sera for measurement of ALT , AST, immunoglobulins (Ig) and interleukin 2(IL2) .

Twenty-four hours after the last injection the mice were weighed and then the mice were sacrificed. Spleen from treated & untreated mice were removed & were weighed.

Specimens from internal organs (liver, spleen and kidney) of all studied mice groups were taken for histological and immune histochemistry examinations.

Hematological examinations

RBCs& WBCs count, PCV and differential leukocytic count were measured.

Biochemical analysis:

ALT, AST, blood glucose levels, immunoglobulins (Ig), phagocytic activity, phagocytic index and IL2 were measured.

Candida phagocytosis:

The Phagocytic activity of neutrophils performed according to the method described by **Wilkinson (1981)** as follow: In clean dry tube, the following aliquots were mixed; 100ul mice serum, 100ul heat killed *Candida albicans* (5×10^6 /ml) and 100ul of heparinized tested blood. The tube was mixed and then incubated at 37°C for 30 minutes, after which it was centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded leaving a droplet into which the sediment was re-suspended. Smear was prepared from the deposit, dried in air, fixed with methyl alcohol for about 3 min and stained with Giemsa stain for 30 min., the slides were washed well with water and dried in air, The slides were examined under oil immersion lens. Then one hundred neutrophils were examined and the number of neutrophils ingesting *Candida* was counted and expressed as percentage.

No of neutrophils ingesting *Candida*

Phagocytic activity % = ----- x100

Total number of neutrophils

Phagocytic index = Total no of ingested *Candida*/ Phagocytic activity %

Body weight and immune-related organs relative weight:

Just before killing at the end of experiment, final body weight of mice in all experimental groups was recorded. Upon being killed, the spleen was removed aseptically, weighed and their relative organ weights (ROW) were calculated according to **Aniagu et al. (2005)** using the following formula:

ROW = [Absolute organ weight (g) / body weight of mice on sacrifice day (g)] × 100.

Histological and immunohistochemical examinations:-**Histological examination:-**

Specimens of liver, spleen and kidney from control and treated mice were fixed in 10% (v/v) formalin in phosphate-buffered saline (PBS) were embedded in paraffin. Sections (4 μm thickness) were air-dried on slides. After deparaffinization they were stained by hematoxylin & eosin according to **Bancroft and Stevens (2008)**, and then examined under a light microscope.

For immunohistochemistry:-

Specimens from the liver, and kidney, were fixed in 10% (v/v) formalin in phosphate buffered saline (PBS). They were processed for paraffin sections (4 μm in thickness). Immunostaining for CAT was performed using monoclonal antibody to human CAT (Santa Cruz Biotechnology, Inc. Texas, USA). Antigen retrieval was performed by heating at 98 °C in 10 mMTrisodium citrate, pH 6.0, with 0.05% (v/v) Tween-20 for 15 min. Endogenous peroxidases were quenched using 0.3% (v/v) H₂O₂ in PBS for 10 min. Sections were incubated with the monoclonal anti-CAT overnight at 4 °C. The CAT antibody was diluted in PBS at the ratio of 1:100 PBS. Normal mouse IgG (Santa Cruz Biotechnology, Inc. Texas, USA) diluted in the same way was used for negative control staining. After washing by PBS sections were incubated with biotinylated anti-mouse IgG binding protein (1:200) for 1 h, followed by incubation with avidin-biotin complex (1:50) (Santa Cruz Biotechnology) for 1 h. The immunoreaction products were visualized using a reaction mixture of 0.02% (w/v) 3, 3'-diaminobenzidine-4HCl and 0.05% (v/v) H₂O₂ in 10 mMTris-buffered saline, counterstained with hematoxylin, and then examined under a light microscope.

Statistical analysis:

Data obtained in this study was statistically analyzed for variance (ANOVA) with confidence limits set as 95 % (significance at $p < 0.05$ probability level) described by **Duncan (1955)**. The results were reported as mean ± standard error "SE", multiple range tests should be performed to compare among different groups of experiment. Statistical analysis was performed using SPSS statistical package.

Results**Hematological analysis:**

The mean values of the RBCs, Hb and PCV were significantly higher in group II , group III, respectively than control group. This values were non significantly higher in group IV compared to control one as shown in **table 1**.

Table 1 illustrated that WBCs were significantly higher in group II compared to other groups. The mean values of neutrophils were significantly decreased in group III on comparison with other groups. Lymphocytes % were significantly higher in group III on comparison with other groups. In addition, Lymphocytes % were significantly higher in group II and group IV compared to control group as shown in **figure 1**. Moreover, neutrophil/lymphocyte (N/L) ratio was significantly lower in group III (0.32) compared to other groups and N/L ratio was significantly lower in group II (1.13) and group IV (1.58) in comparison with control group (3.11). Monocytes % were significantly higher in group IV on comparison with other groups. Eosinophils were significantly lower in group IV, group III and group II, respectively on comparison with control group. There is no changes in basophils between groups.

Biochemical findings:

Levels of AST, ALT and glucose in control, L-carnitine at dose 200 mg/kg b.wt., calf thymus extract at dose 0.5 mg/kg b.wt. and L-carnitine at dose 200 mg/kg b.wt. Plus calf thymus extract at dose 0.5 mg/kg b.wt. groups are shown in **table 2** and **figure 1**. According to these data, it was determined that levels of AST and ALT were significantly decreased at group II, group III and group IV on comparison with control group. A significant decrease in glucose levels were observed in group IV (52.73 mg/dl), group II (55.93 mg/dl), and group III (62.60 mg/dl), respectively on comparison with control group (70.37 mg/dl).

Phagocytic function is the primary mechanism through the immune system eradicates most of pathogens and infections. Phagocytic activity and phagocytic index were illustrated in **table 2** and **figure 1**. From this table we observed that there were significantly increased in phagocytic activity in group II (69%), group III (62%) and group IV (55.25%), respectively on comparison with control group (46.25%) and a significant increase in phagocytic index was observed in group II, group III and group IV on comparison with control group. Phagocytic activity and phagocytic index mainly higher in all treated groups than control one.

Total protein, albumin, globulin concentrations and A/G ratios are demonstrated in **table 2**. There is no significant changes in total protein concentrations between all groups. However, the mean values of globulin were significantly increased in group II, group III and group IV, respectively when compared with control group (group I). A/G ratios and albumin concentrations were significantly lower in group II, group III and group IV on comparison with control group (group I).

Interleukin 2 are shown in **table 2** and **figure 2**. The mean values of IL 2 were significantly increased in group II, group III and group IV, respectively on comparison with control group.

Relative spleen weight (RSW) was calculated and it was presented in **table 2**. There was significantly increased in RSW in group II, group IV and group III, respectively on comparison with control group.

Table 1:-Hematological parameters in control group, L-carnitine treated group, calf thymus extract treated group and calf thymus extract plus L-carnitine treated group in aged male mice. Values were expressed as (MV \pm SE). (n = 5).

(RBCs): red blood cells; (Hb): hemoglobin; (PCV): packed cell volume; (WBCs): white blood cells; (N/L ratio); Neutrophil/lymphocyte ratio; (n): Number of animals. Subgroups with different letters (a, b, c) indicates statistical significantly difference than other subgroups (P<0.05).

| Variables | Group I (control) | Group II | Group III | Group IV |
|-----------------------------|-----------------------|--------------------|----------------------|-----------------------|
| RBCs ($10^6/\mu\text{l}$) | 5.48 ± 0.73^c | 9.393 ± 0.28^a | 7.53 ± 0.33^b | 6.63 ± 0.26^{bc} |
| Hb(g/dl) | 10.13 ± 1.48^c | 16.68 ± 0.29^a | 13.35 ± 0.49^b | 11.45 ± 0.29^{bc} |
| PCV (%) | 30.38 ± 4.45^c | 49.73 ± 0.89^a | 40.05 ± 1.46^b | 34.35 ± 0.86^{bc} |
| WBCs ($10^3/\mu\text{l}$) | 11.55 ± 0.29^{ab} | 15.37 ± 2.38^a | 10.25 ± 0.66^b | 8.65 ± 0.26^b |
| Neutrophil (%) | 51.63 ± 1.14^a | 44.53 ± 3.69^a | 17.93 ± 4.84^b | 45.83 ± 2.01^a |
| Lymphocyte (%) | 16.89 ± 1.23^c | 40.85 ± 3.35^b | 63.18 ± 6.67^a | 29.20 ± 0.74^b |
| Monocyte (%) | 18.99 ± 0.84^{ab} | 9.55 ± 1.36^c | 14.65 ± 1.78^b | 21.13 ± 1.55^a |
| Eosinophil (%) | 11.00 ± 0.41^a | 4.00 ± 0.41^b | 3.25 ± 0.25^{bc} | 2.25 ± 0.25^c |
| Basophil (%) | 1.25 ± 0.25 | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.50 ± 0.29 |
| N/L ratio | 3.11 ± 0.25^a | 1.13 ± 0.18^b | 0.32 ± 0.10^c | 1.58 ± 0.11^b |

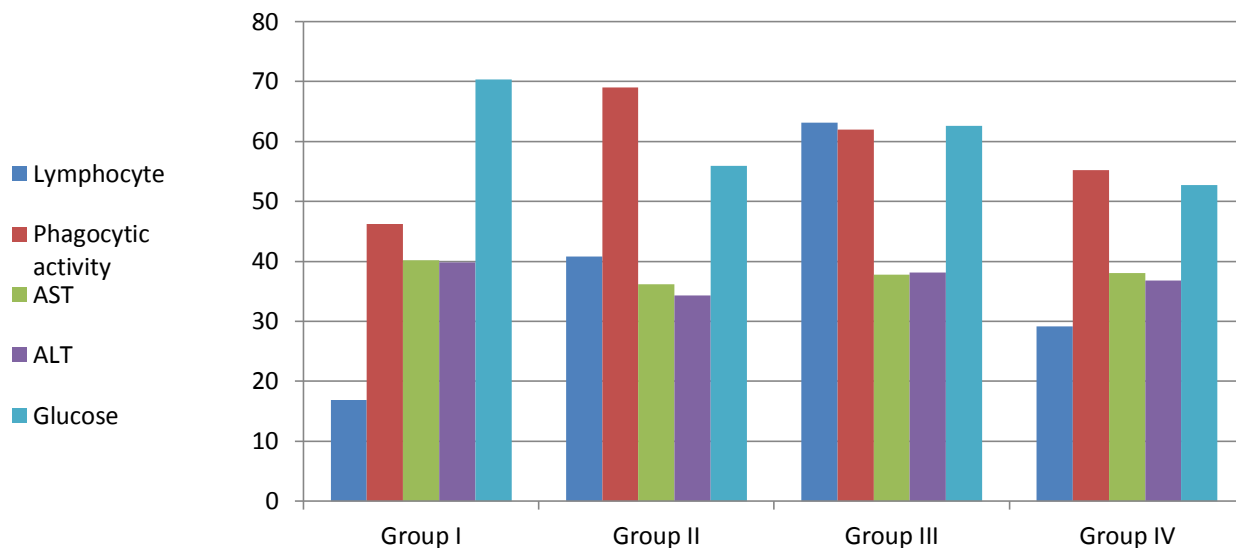


Figure 1:- Mean of the effect of L-carnitine at dose 200 mg/kg b.wt. , calf thymus extract at dose 0.5 mg/kg b.wt. and L-carnitine at dose 200 mg/kg b.wt. Plus calf thymus extract at dose 0.5 mg/kg b.wt. On lymphocyte %; phagocytic activity; AST; ALT and glucose level in aged male mice compared to control group. (n=5).

Table 2. Biochemical analysis in control and L-carnitine treated groups, calf thymus extract treated group and calf thymus extract plus L-carnitine treated group in aged male mice. Values were expressed as (MV \pm SE) (n = 5).

| Parameters | Group I (control) | Group II | Group III | Group IV |
|-------------------------|-------------------------------|--------------------------------|--------------------------------|-------------------------------|
| Phagocytic activity (%) | 46.25 \pm 0.25 ^d | 69.00 \pm 1.68 ^a | 62.00 \pm 1.08 ^b | 55.25 \pm 1.10 ^c |
| Phagocytic index | 2.74 \pm 0.09 ^b | 3.77 \pm 0.18 ^a | 3.93 \pm 0.19 ^a | 3.58 \pm 0.12 ^a |
| AST (U/l) | 40.02 \pm 0.52 ^a | 36.16 \pm 0.24 ^c | 37.82 \pm 0.45 ^b | 38.04 \pm 0.36 ^b |
| ALT (U/l) | 39.18 \pm 0.69 ^a | 34.33 \pm 0.63 ^c | 38.13 \pm 0.36 ^{ab} | 36.85 \pm 0.15 ^b |
| Glucose (mg/dl) | 70.37 \pm 3.38 ^a | 55.93 \pm 1.04 ^{bc} | 62.60 \pm 2.53 ^b | 52.73 \pm 0.81 ^c |
| Total Protein (gm/dl) | 5.62 \pm 0.01 | 5.50 \pm 0.17 | 5.63 \pm 0.02 | 5.54 \pm 0.07 |
| Albumin (gm/dl) | 2.55 \pm 0.05 ^a | 2.42 \pm 0.007 ^b | 2.43 \pm 0.005 ^b | 2.36 \pm 0.02 ^b |
| Globulin (gm/dl) | 3.07 \pm 0.05 ^b | 3.25 \pm 0.01 ^a | 3.21 \pm 0.02 ^a | 3.18 \pm 0.06 ^{ab} |
| A/G ratios | 0.84 \pm 0.03 ^a | 0.75 \pm 0.01 ^b | 0.76 \pm 0.01 ^b | 0.74 \pm 0.01 ^b |

| | | | | |
|--------------|---------------------------|---------------------------|---------------------------|---------------------------|
| IL2 (Pg/ ml) | 20.20 ± 1.04 ^d | 88.07 ± 5.66 ^a | 53.00 ± 2.16 ^b | 39.07 ± 1.74 ^c |
| RSW (%) | 0.74 ± 0.06 ^c | 2.57 ± 0.34 ^a | 1.31 ± 0.20 ^b | 2.11 ± 0.07 ^a |

(AST): Aspartate aminotransferase enzyme; (ALT): Alanine aminotransferase enzyme; (A/G ratios): Albumin/Globulin ratios; (IL2): Interleukin 2; (RSW): Relative spleen weight; (MV): Mean value; (SE): Standard error; (n) and Number of animals. Subgroups with different letters (a, b, c, d) indicate statistical significantly difference than other subgroups (P<0.05).

IL-2

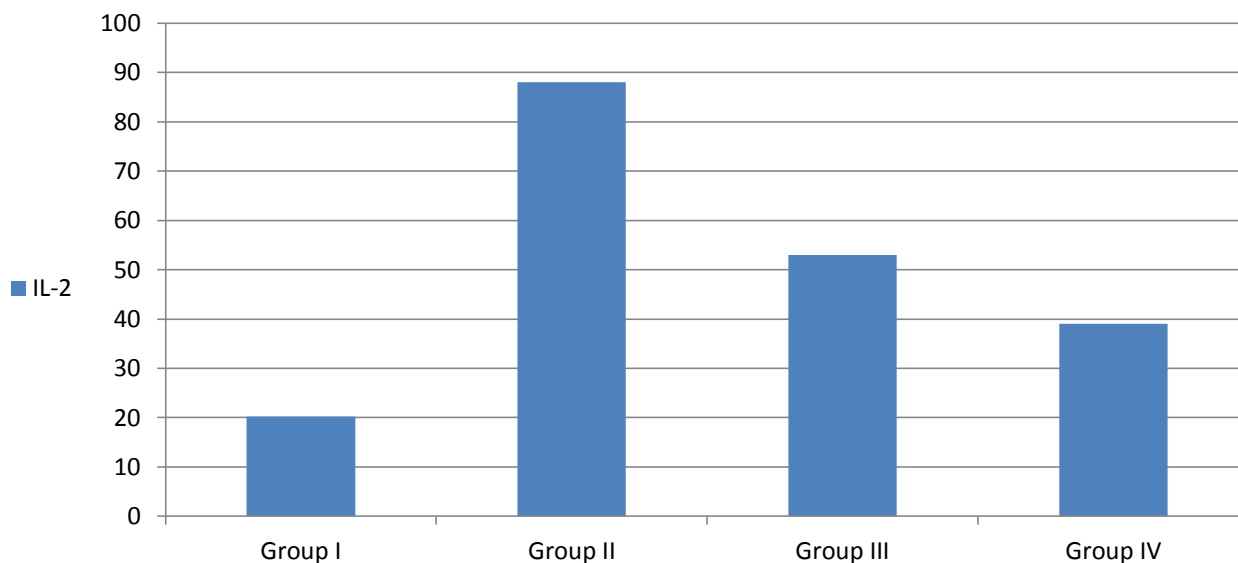


Figure 2:-Mean values of interleukin 2 in groups treated with L-carnitine at dose 200 mg/kg b.wt. , calf thymus extract at dose 0.5 mg/kg b.wt. and L-carnitine at dose 200 mg/kg b.wt. Plus calf thymus extract at dose 0.5 mg/kg b.wt. In male aged mice compared to control group. (n=5).

Histological investigation and immunohisto- anti-oxidative activity:-

Renal histological examination of the treated group showed sever dilatation in the renal collecting tubules, Interstitial lymphocytic infiltrations, and glomerular expansion , expansion of Bowman's capsule and thickening of the blood vessel wall is indicated in the (L- carnitine 200mg/kg.b.wt.) and milled in thymus group as well as in mixed group with thymus and carnitine than normal tissue, (*Figure3*).

Liver histological sections of treated mice showed a normal histological structure of hepatic tissue that involved arrangement of hepatocyte in radiating manner around central veins, with mild dilatation of hepatic sinusoid and central vein, slight shrinkage of hepatocyte, that was present in high degree in (L- carnitine 200mg/kg.b.wt.) and milled in thymus group as well as in mixed group with thymus and carnitine than normal tissue, (*Figure 4*).

Spleen histological sections of treated mice don't show marked or observable internal changes than normal except that of enlarged area of white pulp (lymphocyte producing cells) especially in thymus group, then in mixed group and in L-carntine group respectively, (*Figure 5*).

With regards to the immune-histochemistry of anti-oxidative activity of catalase enzyme, which seems as qualitative and semi quantitative immune activity and appears dark brownish color cells concentrated in perivascular area of liver tissue around central veins and portal vein of liver tissues of (L-carntine200 mg/kg.b.wt.) in high degree than control and other treated groups. Moreover, there are dark brown colors concentrated in renal collecting tubules of kidney tissues due to reaction of anti -oxidative investigations of treated extractions in aged mice was highly significant of L-carntine group, then in mixed group (combination between L-Carntine and thymus extract), then in thymus extract group respectively, (*Figure 6&7*).

Discussion:-

The investigation concerning the effect of L-carnitine on different tissues is rare, and further research is required for this purpose, (Boyacioglu *et al.*, 2014). The beneficial effects of L-carnitine have been documented in rat models of renal ischemia reperfusion injury and renal disease (Mister *et al.*, 2002 and Boyacioglu *et al.*, 2014), because, L-carnitine was known as an exogenous antioxidant agent in cells, due to its protective effects against lipid peroxidation and oxidative stress in the internal tissues, so can protect the tissues from damage by repairing oxidized membrane lipids, (Calò *et al.*, 2006 and Ye *et al.*, 2010). Therefore, it is reported to inhibit apoptosis. Additionally, supplementation with L-carnitine in aged rats improved the antioxidant status, (Boyacioglu *et al.*, 2014).

Our results in aged male mice show that L-carnitine administration stimulated CAT activities in renal and liver tissues is highly significant, similar to that observed in our results, L-carnitine may play a major role in stability of antioxidant status in kidney, liver tissue (Boyacioglu *et al.*, 2014).

The kidney plays the major role in carnitine biosynthesis, excretion, and acylation, kidney contains all enzymes needed to form carnitine from trimethyl lysine in activities exceeding that of the liver (Cibulka *et al.*, 2006), and providing a protective effect against lipid peroxidation and oxidative stress (Ye *et al.*, 2010), so our result in histology we observed that the presence of like, expansion of Bowman's capsule, dilatations in the tubule and interstitial infiltration. (Boyacioglu *et al.*, 2014) and Tousson *et al.* (2014) added that rat kidney section in post treated group with carnitine showed moderate organized tubular and glomerular enlargements with mild inflammatory infiltration, this may be because of the effect of dehydration as well as individual stresses of the rats. Our result matched with (Boyacioglu *et al.*, 2014) who elucidate that, it may be better to administer L-carnitine before an experiment. It may provide protection by reducing the concentration of oxidant products by scavenging free radicals and supporting the antioxidant system. Nevertheless, the therapeutic dose or application days of L-carnitine might have been insufficient in the present study.

The antioxidant and free radical scavenger activities of L-carnitine have been proposed to have several mechanisms by inhibiting free radical generation, preventing the impairment of fatty acid beta-oxidation in mitochondria and protecting tissues from damage by repairing oxidized membrane lipids (Calò *et al.*, 2006).

In the old age, the thymus gland is atrophic and difficult to detect macroscopically, that is manifested with multiple organ dysfunction and increasing of Reactive oxygen species (ROS) or the free radicals that causing deterioration of the immune system, and increased incidence of infection, impaired responses to vaccines, autoimmunity, and cancer, thus increasing the rates of morbidity and mortality in elder age, (Schulz *et al.*, 2015 and Haroun, 2018). The imbalance between oxidants like free oxygen radicals (reactive oxygen species, ROS) and antioxidants are considered as an important etiology of aging, (Desai *et al.*, 2010)

It seems that TFX may be a valuable therapeutic agent for the management of patients with chronic hepatitis B. (Skotnicki, 1989). It was concluded that the antibody-mediated chronic kidney graft rejection could be effectively inhibited or delayed with thymic hormones, (Gorski *et al.*, 1985).

The damage to the immune response either by the aged adult mice and by induction of thymectomy in newborn mice, can be prevented by repeated injections of calf thymus extract which is very effective, while the extracts derived from other calf organs as kidney or muscle, are ineffective (Trainin and Linker-Israeli, 1967) due to the existence of a diffusible product from thymus tissue in different strains and species, which, without being species specific, enables thymectomized animals to produce new lymphocytes and to develop immunologic competence (Law *et al.*, 1964). It has also been reported that injection of calf thymus extract into thymectomized mice protects from the fatal effect of a viral infection, prevents the appearance of a wasting syndrome, and increases the number of lymphocytes in peripheral blood (De Sommer *et al.*, 1963). Histologically, liver and spleen of these injected animals resembled those of neonatal mice (Czaplicki, & Błońska, 1989). Thymus calf extract treatment and neonatal thymus graft have similar corrective effects on age-related changes in mice (Basso *et al.*, 2005).

In this study, erythrogram, leucogram, phagocytic activity, phagocytic index, total protein, globulin, albumin, A/G ratio, ALT, AST, glucose level, interleukin 2 and relative spleen weight were demonstrated.

Our results showed an improvement in RBCs count, Hb concentration and PCV after intraperitoneal administration of 200 mg/kg b.wt. of L-carnitine in aged male mice. This agrees with the previous findings reporting that L-carnitine administration in male rats leads to an increase in RBCs count, Hb concentration and PCV (Qadir *et al.*, 2009). Jameel (2014) stated that RBCs count and Hb content were increased in chicks fed a diet supplemented by L-carnitine compared to those fed on a control diet. This result may be due to an effect of L-carnitine on erythrocyte stability and survival. Therefore, by this mechanism PCV can be increased. Bommer (1999) demonstrated that L-carnitine improved anemia and also, L-carnitine improved erythropoietin efficiency that reported by Hurot *et al.* (2002).

In this study, L-carnitine treated groups were shown to significantly decrease in neutrophil / lymphocyte ratio that may be attributed to an increase in lymphocyte production. This result is similar to results of Ohara *et al.* (2018) who reported that L-carnitine non-oral administration was decreased N/L ratio than oral route. In addition, lymphocyte cells % were significantly increased for duckling fed diets supplemented with L-carnitine than control group that demonstrated by Awadet *et al.*, (2016).

In regard to relative spleen weights were significantly increased than control, this referred to an increase in lymphocyte cell % (Boyacioglu *et al.*, 2014), and due to a decrease in the total number of thymocytes and splenocytes by the effect of stress which is also accompanied by a decreasing weight ratio of the spleen (Obmińska & Szczypka, 2005).

Our findings revealed an elevation in phagocytic activity and phagocytic index in group II (L-carnitine at dose 200 mg/kg b.wt.) in comparison to control group. These results agree with Thangasamy *et al.* (2008) who reported that L-carnitine at dose 300 mg/kg b.wt. for 7 successive days improved phagocytic activity and phagocytic index in aged rats. This result may be attributed to an increase in superoxide anion production in neutrophils of aged rats which leads to a decrease in phagocytosis in control group. Furthermore, superoxide anion production decreased by L-carnitine treatment. Therefore, by this mechanism phagocytic activity can be improved (Uysal *et al.*, 2003).

The present study revealed that L-carnitine had a hepatoprotective effect that determined by decreased levels of hepatic marker enzymes as AST and ALT. This result is compatible with previous studies, L-carnitine improved liver function enzymes may be attributed to its antioxidant effects leading to protection of membrane permeability, prevention of the leakage of intracellular enzymes (Mousah *et al.*, 2016).

Our results mentioned that L-carnitine reduced blood glucose level in aged male mice. These results may be due to its ability to stimulate fatty acid oxidation in mitochondria. This restores the intramitochondrial acyl-CoA/CoA ratio and stimulates pyruvate dehydrogenase activity and glucose oxidation. Malik *et al.* (2011) demonstrated that L-carnitine at dose 200 mg/kg b.wt. intraperitoneal for 6 days in rats was able to reduce blood glucose level. The studies by Balasaraswathi *et al.* (2008) had reported that L-carnitine at dose 300 mg/kg/day intraperitoneal was able to reduce blood glucose level. This is in contrast with few studies which stated that L-carnitine treatment did not lower the glucose level Patel *et al.* (2008). The difference between the results of our study and of their studies may be due to the difference in dose of L-carnitine or rat strain used and/ or route of administration.

In this study, L-carnitine significantly increased globulin and significantly decreased A/G ratio. This agrees with the previous findings reporting that L-carnitine significantly increased globulin, phagocytic activity, phagocytic index and significantly decreased A/G ratio (El-Kelawy and Elnaggar, 2017).

Our findings revealed that L-carnitine significantly increased interleukin 2 produced from T-helper cells. This agrees with the other studies demonstrating that L-carnitine in mice exhibiting a modification in production of a number of cytokines (interferon, tumor necrosis factor, IL2, IL-4, IL-6, vascular endothelial growth factor and insulin-like growth factor in serum (Vassiliadis and Athanassakis, 2011). This result may be attributed to a positive effect of L-carnitine on humoral immunity by increasing lymphocyte cells%, phagocytic activity, phagocytic index, globulins and interleukin 2 (Mast *et al.*, 2000 ; Thangasamy *et al.*, 2008 and El-Kelawy and Elnaggar, 2017).

Our results showed an improvement in RBCs count, Hb concentration and PCV after intraperitoneal administration of 0.5 mg/kg b.wt. of calf thymus extract in aged male mice. This agrees with the previous findings reporting that calf thymus preparation (TFX-Polfa) was significantly increased in the hemoglobin concentration and erythrocyte counts (Lewandowicz, 1990). There is a significant increase in the blood picture (RBCs, Hb and PCV) in irradiated Wistar rats treated with thymic extract (Jovanovic *et al.*, 2006).

In this study, thymus extract treated groups were shown significantly decreased in neutrophil / lymphocyte ratio that may be attributed to increase level of lymphocyte production. This results are similar to results of **Naik et al. (2005)** who reported that there was a very significant increase in number of lymphocytes in calf thymus extract treated groups, when compared to the control group, which is one of the probable reason for overall immunopotential and this could be due to the fact that thymic hormones activates T-cell and activates lymphocyte production. Relative spleen weights were significantly increased, this referred to increase lymphocyte cell % (**Abdel Fattah et al., 1999**).

In our study we documented a significant increased of phagocytic activity and phagocytic index. This agree with the previous findings reporting that thymic peptide mixtures (Thymosin fraction 5 thymulin) have been proved to stimulate the immune response and enhance phagocytosis (**Jablonska et al., 2005**). The increase in phagocytic activity of phagocytes in calf thymus extract treated group could be due to the ability of the thymus extract to act as an immunomodulator by exerting control on cytokine production by peripheral blood mononuclear cells (**Naik et al., 2005**).

The present study revealed that thymus extract had a hepatoprotective effect that determined by decreased levels of hepatic marker enzymes as AST and ALT. This results are compatible with previous studies, oral bovine thymus extracts in viral hepatitis induce broad-spectrum immune system enhancement presumably mediated by improved thymus gland activity and they induced accelerated decreases of liver enzymes (**Pizzorno, 2016**).

Our results mentioned that thymus extract reduced blood glucose level in aged male mice. This agree with the previous findings reporting that the efficacy of the use of thymalin in therapy of diabetes mellitus in children (**Kurbanovetal, 1989**). This is in contrast with few studies which stated that thymosin f5 (thymic hormone) produces hyperglycemic effect (**Sivas et al., 1982**). The difference between the results of our study and of their studies may be due to the difference in active principle, dose and/ or route of administration of thymus extract protects such animals from the fatal effect of a viral infection, prevents the appearance of a wasting syndrome, and increases the number of lymphocytes in peripheral blood.

In this study, thymus extract significantly increased globulin and significantly decreased A/G ratio. This agree with the previous findings reporting that thymus extract significantly increased globulin (**Naiket et al., 2005**).

In this study, thymus extract significantly increased interleukin 2 producing from T-helper cells. Our results are consistent with the hypothesis of (**Morozov and Khavinson, 1997**) who reported that thymalin was increased production of interferon and interleukin 2 by lymphocytes. In addition to report by (**colic et al.2008**) who stated that thymus extract was increased production of interleukin 2. This results may be attributed to a positive effect of intraperitoneal administration of calf thymus extract on humoral and cellular immunity by increasing lymphocyte cells% (**Naik et al., 2005**).

Conclusion:-

From this study, it can be concluded that: a) thymic involution with age negatively impacts the immune system, so there are disturbance of hematological parameters in aged male mice, which can be improved by exogenous supplementation of L-carnitine or calf thymus extract or combination of them. b) With aging, there are declined in immune functions and intraperitoneal administration of L- carnitine and calf thymus extract or combination of them had an as significant positive effect in enhancing immune response in aged male mice. C) The hepatoprotective effect of L-carnitine or calf thymus extract or combination between them in aged male mice by decreasing hepatic marker enzymes). Exogenous supplementation of L-carnitine or calf thymus extract or combination of them were decreased glucose level in aged male mice.D) The use of (thymus and L-carnitine) preparations improve the histological architectures in the internal tissues (liver, kidney and spleen) and a significant increase in CAT (catalase enzyme), in liver and kidney. These results clearly show the antioxidant and protective property of experimental preparations.

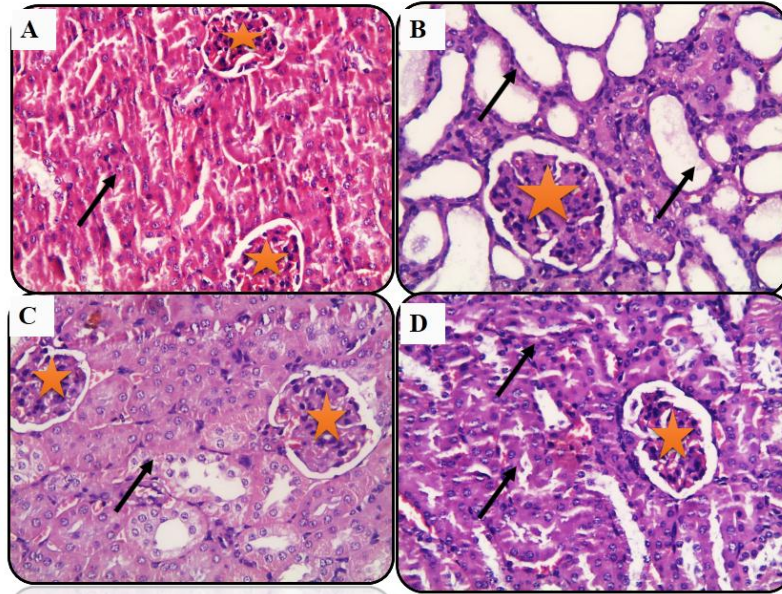


Figure 3:-Photomicrograph of kidney tissue of aged male mice, **A)** control group, **B)** treated group with L-carntine (200mg/kg b.wt.), **C)** treated mice with calf thymus extract (0.5 mg/ kg b.wt.), **D)** treated mice with both L-carntine (200mg/ kg b.wt.) and calf thymus extract (0.5 mg/ kg b.wt.) for successive 7days. Orange stars (renal glomeruli (Bowman's capsule, black arrows (convoluted-tubules). (**H&E stain.X200**)

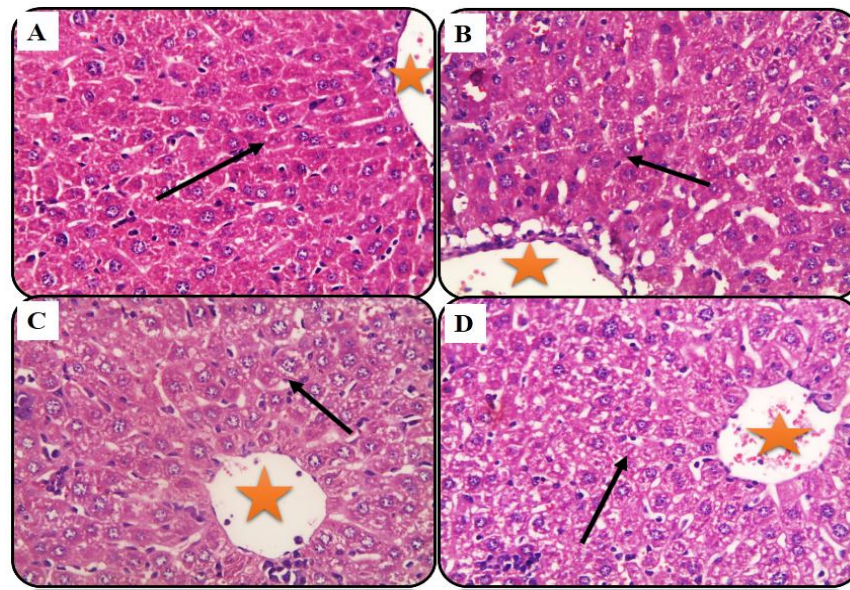


Figure 4:-Photomicrograph of liver tissue of aged male mice, **A)**control group, **B)**treated group with L-carntine (200mg/ kg b.wt.), **C)** treated mice with calf thymus extract (0.5 mg/ kg b.wt.), **D)** treated mice with both L-carntine(200mg/ kg b.wt.) and calf thymus extract(0.5 mg/ kg b.wt.), for successive 7days. Orange stars (central vein of liver), black arrows (radiating manner hepatocytes and hepatic sinusoids in between, (**H&E stain X200**).

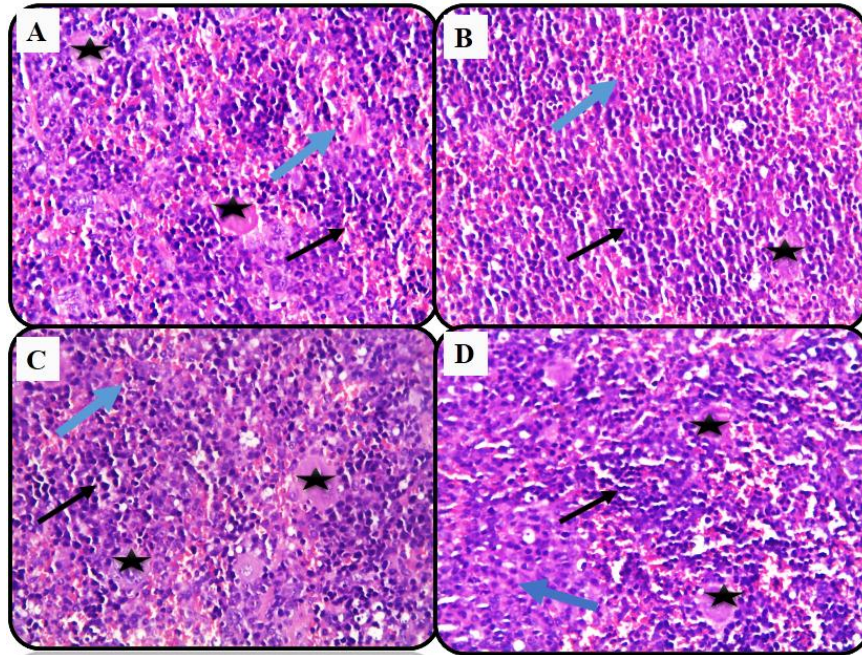


Figure 5:-Photomicrograph of spleen tissue of aged male mice, A)control group, B) treated group with L-carntine (200mg/ kg b.wt.), C) treated mice with calf thymus extract (0.5 mg/ kg b.wt.), D) treated mice with both L-carntine (200mg/ kg b.wt.), and calf thymus extract(0.5 mg/ kg b.wt.) for successive 7days. Black stars (central arteriole of spleen), black arrows (white pulp of spleen, that rich in lymphocyte) blue arrows (red pulp of spleen rich in macrophage, (H&E stain X200).

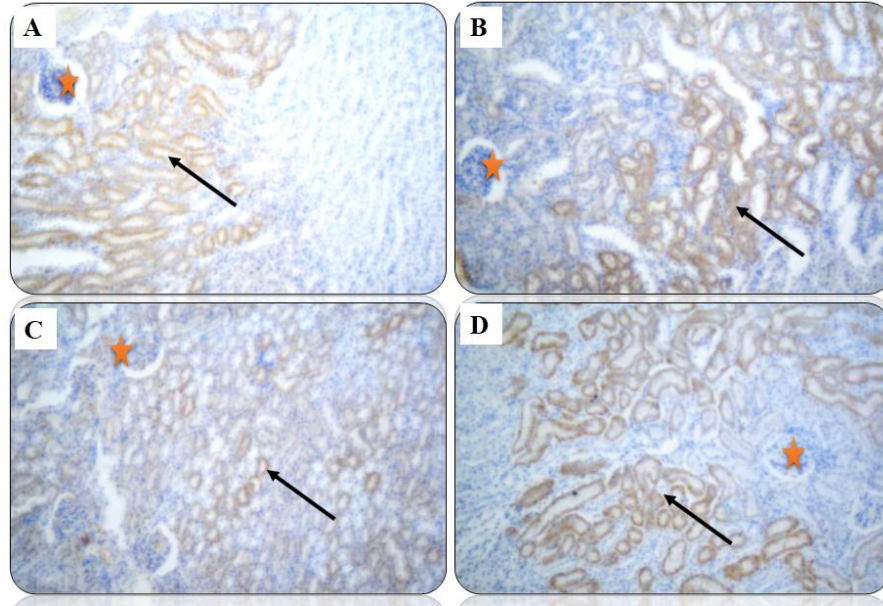


Figure 6:-Immune-histochemical localization of catalase enzyme (CAT) in kidney tissue of aged male mice in A)control group, B) treated group with L-carntine (200mg/ kg b.wt.), C) treated mice with calf thymus extract (0.5 mg/ kg b.wt.), D) treated mice with both L-carntine (200mg/ kg b.wt.) and calf thymus extract(0.5 mg/ kg b.wt.) for successive 7days, orang stars (renal glomeruli), black arrows (convoluted tubules that have dark brownish color due to catalase activity are concentrated at collecting renal tubules of kidney tissues).

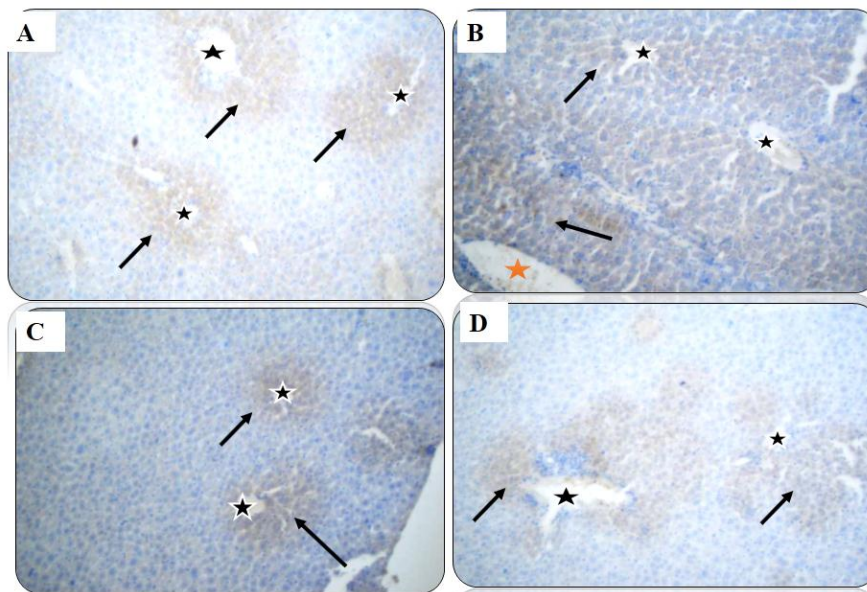


Figure7:-Immune-histochemical localization of catalase enzyme (CAT) in liver tissue of aged male mice, A) control group, B) treated group with L-carnitine (200mg/ kg b.wt.), C) treated mice with calf thymus extract (0.5 mg/ kg b.wt.), D) treated mice with both L-carnitine (200mg/ kg b.wt.) and calf thymus extract(0.5 mg/ kg b.wt.) for successive 7days, black stars (central veins of liver), orange star (portal vein) and black arrows hepatocyte have dark brownish color due to catalase activityare concentrated at perivascular area of liver tissue.

Reference:-

1. Abdel Fattah, A.M.; Mohamed, E.M.; Mohamed, E. and Ramadan, G. (1999): Effect of thymus extract on immunologic reactivity of chicken vaccinated with infectious bursal disease virus. *Journal of Veterinary Medical Science*, 61(7): 811-817.
2. Aniagu, S.O.; Nwinyi, F.C.; Akumka, D.D.; Ajoku, G.A.; Dzarma, S.; Izebe, K.S. *et al.* (2005): Toxicity studies in rats fed nature cure bitters. *Afr. J. Biotechnol.*;4(1):72-78.
3. Awad, A.L.; Fahim, H.N.; Beshara, M.M. (2016): Effect of dietary l-carnitine supplementation on productive performance and carcass quality of local duck breeds in summer season. *Egypt. Poult. Sci.*, 36 (1):11-27.
4. Balasaraswathi, K.; Rajasekar, P and Anuradha, C.V. (2008): Changes in redox ratio and protein glycation in precataractous lens from fructose- fed rats: Effects of exogenous L-carnitine. *Clin. Exp. Pharmacol. Physiol.* 35: 168-173.
5. Bancroft, J.D. and Stevens, A. (2008): *Theory and histological techniques*. 4th Edition, Churchill Livingstone, NewYork.
6. Barboza, G.; Rivera, S.; Parra, O.; Fernandez, G.; Ramirez, R. and Otero, C.(2000): Evaluation of immunological response in canines infected with demodectic mange after treatment with Amitraz or Thymostimulin Tp-1, *Revistacientifica-Fac de CienciasVeterinarias*, 10(2): 145-152.
7. Basso A, Rossolini G, Piantanelli A, *et al.* (2005): Aging reversibility: from thymus graft to vegetable extract treatment- application to cure an age-associated pathology. *Biogerontology*.,6(4):245–253.
8. Bommer, J. (1999): Saving erythropoietin by administering L-carnitine. *Nephrology Dialysis Transplantation*, 14(12): 2819-2821.
9. Boyacioglu, M.; Turgut, H.; Akgullu, C.; Eryilmaz, U.; Kum, C. and Onbasili, O.A. (2014): The Effect of L-Carnitine on oxidative stress responses of experimental contrast induced nephropathy in rats. *J. Vet. Med. Sci.*, 76(1): 1–8.
10. Calò L. A., Pagnin E., Davis P. A., Semplicini A., Nicolai R., Calvani M. and Pessina A. C. (2006): Antioxidant effect of l-carnitine and its short chain esters: Relevance for the protection from oxidative stress related cardiovascular damage. *Int. J. Cardiol.*, 107: 54–60. doi: 10.1016/j.ijcard.2005.02.053.

11. Cibulka, R., Široká, R., Rajdl, D. and Racek, J. (2006): Asymmetric dimethylarginine as a novel independent risk factor for cardiovascular disease in hemodialysis patients. XIV International Symposium on Atherosclerosis. 18: 22-26. Roma, Italy.
12. Colic, M.; Popovic, R.; Mrdakovic, D.; Radeta, M.; Novakovic, J.; Pavlovic, B. and Vucevic, D. (2008): Evaluation of the immunomodulatory properties of a calf lipid thymus extract and standardization of a method for quantification of its biological activity. American Journal of immunology, 3(2): 35-44.
13. Colucci, D. G.; Puig, N.R. and Hernandez-Pando, R. (2013): Influence of anaesthetic drugs on immune response from inflammation to immunosuppression. OA Anaesthetics, 1(3):21.
14. Czaplicki, J. and Błońska, B. (1989): Do active substances of the thymus influence the processes of aging? I. Effects of treatment with embryonal and early fetal thymic calf extracts (ETCE) on some organs of grown-up young mice. Acta Med Pol., 30(1-2):59-75.
15. Desai, K.M., Chang, T., Wang, H., Banigesh, A., Dhar, A., Liu, J., Untereiner, A. And Wu, L. (2010): Oxidative stress and aging: is methylglyoxal the hidden enemy? Can. J. Physiol. Pharmacol., 88(3):273-84. Doi: 10.1139/Y10-001.
16. De Sommer, P., Denys, P., Jr., and Leyten, R. (1963): Activity of a Noncellular Calf Thymus Extract in Normal and Thymectomized Mice. Life Sci., 2: 810-19.
17. Duncan, D.B. (1955): Multiple range and multiple F-tests. Biomtrics. 11:1-42.
18. Edres, H.A.; Taha, N.M.; Mandour, A.A. and Lebda, M.A. (2018): Impact of L-carnitine on bisphenol A-induced kidney damage in rats. Alexandria Journal of Veterinary Sciences, 56(1): 11-17.
19. El-kelawy, M.I. and El naggar, A.S. (2017): Effect of L-carnitine on production performance, blood parameters, lipid metabolism and antioxidative properties of broiler chicks. Egypt. Poult. Sci. J., 37(III): 873-892.
20. Fallah, R.; Nazari, P. and Mirzaei, E. (2016): Effect of different levels of terbutaline and L-carnitine on the growth performance, carcass traits, blood parameters and immune system of broiler chickens. Mal. J. Anim. Sci. 19(2): 57-69.
21. Franceschi, C.; Cossarizza, A.; Troiano, L.; Salati, R and Monti, D. (1990): Immunological parameters in aging: studies on natural immunomodulating and immunoprotective substances. International Journal of Clinical Pharmacology Research, 10: 53-57.
22. Ganie, S.A.; Dar, T.A.; Zargar, B.; Hamid, R.; Zargar, O.; Dar, P.A.; Abeer, S.U.; Masood, A.; Amin, S. and Zargar, M.A. (2014): Antioxidant and hepatoprotective effects of Crataegussongarica methanol extract. J. Environ. Pathol. Toxicol. Oncol. , 33(2):131-143
23. Gorski, A., Orłowski, T., Korczak-Kowalska, G., Nowaczyk, M., Wasik, M. and Podobinska, I. (1985): Suppression of immune functions *in vivo* with cyclophosphamide: enhancement of suppression by thymosin (TFX). *Transplant Proc* 17, 1339.
24. Hajinezhad, M.R.; Hajian, S.H.; Saghayei, S.; Samzadeh-Kermani, A.R. and Nabavi, R. (2016): comparison the protective effects of L-carnitine and acetyl L-carnitine on blood glucose and lipid peroxidation level in diabetic rats. Horizon Med Sci., 22 (3): 229-235.
25. Haroun, H.S.W. (2018): Aging of thymus gland and immune system, MOJ Anat& Physiol. 2018; 5(3):178-181.
Hurot, J.; Cucherat, M.; Haugh, M. and Fouque, D. (2002): Effect of L-carnitine supplementation in maintenance hemodialysis patients. A systematic Review. J. Am. Soc. Nephrol, 13: 708-714.
26. Jablonowska, E.; Tchorzewski, H.; Lewkowicz, P. and Kuydowicz, J. (2005): Reactive oxygen intermediates and serum antioxidative system in patients with chronic C hepatitis treated with IFN- α and thymus factor X. Arch. Immunol. Ther. Exp., 53: 529-533.
27. Jameel, Y.J. (2014): Effect of the content of fish oil, L-carnitine and their combination in diet on immune response and some blood parameters of broilers. Int. J. Sci. Nat., 5: 501-504.
28. Janeway C, Travers P, Walport M, Shlomchik M. Immunobiology. The immune system in health and disease, 5th edn. NY: Garland Publishing, 2001
29. Jovanovic, M.; Lazarevic, M.I.; Popovic, N. and Durdevic, D. (2006): The influence of thymic extract on hematological parameters values in irradiated Wistar rats. ActaVeterinaria . 56 (2-3):137-148.
30. Kabuto, H.; Amakawa, M. and Shishibori, T.(2004):Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice.Life Sci., 74(24):2931-2940.

31. Kabuto, H.; Hasuike, S.; Minagawa, N. and Shishibori, T. (2003): Effects of bisphenol A on the metabolisms of active oxygen species in mouse tissues. *Environ. Res.*, 93(1): 31-35.
32. Karlic, H. and Lohninger, A. (2004): Supplementation of L-carnitine in athletes: does it make sense. *Nutrition*, 20: 709-715.
33. Khavinson, V.K.H. and Morozov, V.G. (2003): Peptides of pineal gland and thymus prolong human's life. *Neuroendocrinology Letters*, 24(314): 233-240.
34. Kolodziejczyk, J.; Saluk-Juszczak, J. and Wachowicz, B. (2011): L-Carnitine protects plasma components against oxidative alterations. *Nutrition*. 27(6):693-699.
35. Kurbanov, T.G.; Kalbieva, B.M. and Zakieva, G.G. (1989): The efficacy of using thymalin in diabetes mellitus in children. *Problem Endokrinologii*. 35(6): 7-9.
36. Law, L. W., Trainin, N., Levey, R. H., and Barth, W. F. (1964): Humoral Thymic Factor in Mice: Further Evidence. *Science*, 143: 1049-1051.
37. Lewandowicz, J. (1990): Effect of thymus extract TFX (polfa) on hemoglobin level and peripheral blood erythrocyte count. *Pol. Tyg. Lek.* 45(23-24): 471-472.
38. Malik, M.O.; Hussain, M.M. and Bin-Aleem, S. (2011): Levo-carnitine normalizes blood glucose and lipid profile in insulin resistant type II diabetic rats. *Park Armed Forces Med. J.*, 61(1): 105-110.
39. Mast, J.; Buyse, J. and Goddeeris, B.M. (2000): Dietary L-carnitine supplementation increases antigen-specific immunoglobulin production in broiler chickens. *British Journal of Nutrition*, 83: 161-166.
40. Mister, M.; Noris, M.; Szymczuk, J.; Azzollini, N.; Aiello, S.; Abbate, M.; Trochimowicz, L.; Gagliardini, E.; Arduini, A.; Perico, N. and Remuzzi, G. (2002): Propionyl-L-carnitine prevents renal function deterioration due to ischemia/reperfusion. *Kidney Int.*, 61: 1064-1078.
41. Morozov, V.G. and Khavinson, V.K.H. (1997): Natural and synthetic thymic peptides as therapeutics for immune dysfunction. *International Journal of Immunopharmacology*, 19(9-10): 501-505.
42. Mousah, H.A.; Sahib, H.B. and Kadhum, H.H. (2016): Protective effect of L-carnitine, Atrovastin, and vitamin A on acetaminophen induced hepatotoxicity in rats. *Int. J. Pharm. Sci. Rev.Res.*, 36(2); 21-27.
43. Naik, B.M.C.; Babu, H. and Mamatha, G.S. (2005): The immunomodulatory role of calf thymus extract on humoral and cell mediated immune response in chicken vaccinated against New Castle disease virus. *Int. J. Poult.Sci.*, 4(8): 580-583.
44. Obmińska-Mrukowicz, B. and Szczyńska, M. (2005): Effect of calf thymus extract and zinc supplementation on the cellular response of mice exposed to restraint stress. *Pol. J. Vet. Sci.*, 8(1): 1-9.
45. Ohara, M.; Ogawa, K.; Suda, G.; Kimura, M.; Maehara, O.; Shimazaki, T.; Susuki, K; Nakamura, A.; Umemura, M.; Izumi, T. *etal.*, (2018): L-carnitine suppresses loss of skeletal muscle mass patients with liver cirrhosis. *J. Recommend. Serv.*, 2(8): 910-922.
46. Patel, J.K.; Goyal, R. and Bhatt, P. (2008): beneficial effects of levo-carnitine on lipid metabolism and cardiac function in neonatal streptozotocin rat model of diabetes. *Int. J. Diabetes and metabolism*, 16: 29-34.
47. Pizzorno, J.E. (2016): in the *Clinician's Handbook of Natural Medicine (Third Edition)*.
48. Qadir, A.B.; Maulood, I.M. and Majeed, Z.R. (2009): Effects of omega-3 and L-carnitine on some hematological parameters in sucrose treated male albino rats. *J. Duhok Univ.* , 12(1): 125-128.
49. Schulz, A.R., Mälzer, J.N., and Domingo, C. *et al.* (2015): Low thymic activity and dendritic cell numbers are associated with the immune response to primary viral infection in elderly humans. *J Immunol.* , 195(10):4699-4711.
50. Severa, M.; Zhang, J.; Garaci, E.;Giacomini, E.; Rizzo, F.; Enta, M.P.; Cruciani, M.;Garaci, E.; Chopp, M. and Coccia, E.M. (2019): Thymosins in multiple sclerosis and its experimental models: moving from basic to clinical application. *Multiple Sclerosis and Related Disorders*, 27:52-60.
51. Sivas, A.; Uysal, M. and Oz, H. (1982): The hyperglycemic effect of thymosin f5, a thymic hormone. *Horm. Metab. Res.*, 14(6): 330-331.
52. Skotnicki, A.B. (1989): Therapeutic application of calf thymus extract (TFX). *Med. Oncol. & Tumor Pharmacother.* 6(1): 31-43.
53. Thangasamy, T.; Subathra, M.; Sittadjody, S.; Jeyakumar, P.; Joyee, A.G.; Mendoza, E. and Chinnakkanu, P. (2008): Role of L- carnitine in the modulation of immune response in aged rats. *Clinica Chimica Acta*, 389: 19-24.

54. Tousson, E., Zaki, Z.T., Abu-Shaeir, W.A. and Hassan, H. (2014): Methotrexate-induced Hepatic and Renal Toxicity: Role of L-carnitine in Treatment. *Biomedicine and Biotechnology*. 2 (4):85-92.
55. Trainin, N. and Linker-Israeli, M. (1967): Restoration of Immunologic Reactivity of Thymectomized Mice by Calf Thymus Extracts. *Cancer Research* 27 Part 1, 309-313, February.
56. Uysal, N.I.; Arzu, A.; Karadogan, I. and Derin, N. (2003): Effect of L-carnitine on neutrophil functions in aged rats. *Mechanisms of Aging and development*, 124(3): 341-347.
57. Vassiliadis, S. and Athanassakis, I. (2011): A conditionally essential nutrient, L- carnitine, as a primary suspect in endometriosis. *American Society for Reproductive Medicine*. 95(8): 2579-2760.
58. Wilkinson, P.C. (1981): *Techniques in clinical immunology*. 2nded, Black well scientific publications, London. 287-288.
59. Wilson, J.L. (1999): Thymus extracts: An international literature review of clinical studies. *Physicians Research and Information*, 1-25.
60. Yapar, K.; Kart, A.; Karapehlivan, M.; Atakisi, O.; Tunca, R.; Erginsoy, S. and Citil, M. (2007): Hepatoprotective effect of L-carnitine against acute acetaminophen toxicity in mice. *Experimental and toxicologic Pathology*, 59(2): 121-128.
61. Ye, J., Li, J., Yu, Y., Wei, Q., Deng, W., Yu, L. (2010): L-carnitine attenuates oxidative injury in HK-2 cell via ROS-mitochondrial pathway. *Regulatory Peptides*. 161(1-3): 58-66.