EFFECT OF L- ARGININE AND L- CARNITINE SUPPLEMENTATION ON FERTILITY IN FEMALE RATS

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ABSTRACT

This study was carried out on forty female Spargue-Dawley rats. Animals were divided into four equal groups (ten rats each). The first group (CA) was considered a control adult female rat two months age and was feed on basal ration without feed additives. The other three groups were considered aged animals (one year old). The second group was considered a control aged group (CAG) feed on basal ration without feed additives. The third group (LA) was feed on basal ration supplemented with L-arginine (1.68%) and the fourth group (LC) was feed on basal ration supplemented with L-carnitine (0.5 mg%) for four weeks. After the end of the experiment, blood samples were collected from all the animals under experimental period (four weeks) for analysis of: estradiol, lipid profile and malondialdehyde, antioxidant enzymatic activities (SOD and catalase) and reduced glutathione (GSH). Tissue samples (ovary, kidney and liver) were also collected at the end of experiment for histopathological examination.

The present study revealed that, there were a significant decrease in SOD and catalase activities and reduced glutathione level in erythrocytes of aged rats. There was a significant decrease in serum HDL and estradiol. On other hand, there was a significant increase in the level of serum MDA, total lipid, triacyglycerol and LDL. More ever there was a non significant difference in serum total cholesterol level.

The oral administration of basal ration supplemented with L-arginine for aged rats decreased significantly the level of serum MDA, total cholesterol, triacylglycerol and LDL cholesterol. There was a significant increase in SOD catalase activities and glutathione level in blood as well as serum estradiol level. Meanwhile, there was a non significant change in total lipid and HDL cholesterol in comparison with control aged rats. The effect of basal ration supplemented with L-carnitine was manifested by increasing in SOD catalase activities and reduced glutathione level. There were also significant decreases in serum total lipid, total cholesterol, triacylglycerol, LDL cholesterol, and estradiol levels. A non significant difference in MDA & HDL cholesterol level in serum of this compared with aged group of rats. This study demonstrated that feeding of aged animals on basal ration supplemented with L-arginine and L-carnitine will change the undesirable effect of aging. Like reduction in free radicals, increase antioxi-

dants and enhancement of fertility. It was also evident from this study that, the use of L- arginine feed additive is more preferable than L- carnitine.

INTRODUCTION

Aging can be defined as a multi-factorial phenomenon characterized by a time-dependent decline in physiological functions. This physiological decline is believed to be associated with an accumulation of defects in the metabolic pathways. RNA, proteins and other cellular macromolecules are rapidly turned over and, consequently, are poor candidates for progressively accumulating damage over a lifetime. Therefore even early studies on mechanisms of aging focused on DNA. In mammalian cells, mitochondria and the nucleus are the only organelles that possess DNA. It appears obvious that the physiological integrity of the cell must critically depend upon the integrity of its genome, which is maintained by DNA repair machinery **(Mikhail et al., 2004)**.

Benign functions of reactive oxygen species (ROS) have been reported, including the activation of nuclear transcription factors, gene expression, and a defense mechanism to target tumor cells and microbial infections **(Simon et al., 2000)**. Superoxide anion may serve as a cell growth regulator **(Halliwell, 1997)**. Singlet oxygen can attack various pathogens and induce physiological inflammatory response **(Stief, 2003)**. On the other hand, it can cause oxidative damage to various biologic macromolecules such as proteins, DNA, lipids, and extracellular matrix **(Balazy, 2000)**.

GSH levels were significantly lowered in aged rats than young rats. Conversely, GSSG levels were significantly high in aged rats. GSH/GSSG molar ratio and redox index were found decreased in aged rats. The activities of GPx, GR, and G6PDH were found to be decreased in aged rats when compared with young rats **(Kumaran, et al., 2004)**. Aging is associated with elevated muscle triglyceride content **(Michelle and Lorraine, 2003)**. Lipoprotein analysis revealed that triacylglyeerol level in very-low density lipoprotein (VLDL), and cholesterol levels in low density lipoprotein (LDL), and in high density lipoprotein (HDL) were all significantly higher in aged rats than in young rat **(Yasukazu et al., 2004)**, there is non-significant decrease in catalase activity in aged rats **(El-Sayed et al., 2005)**. Aging affects oxidative metabolism in liver and other tissues **(Heidrun et al., 2002)**. L-Carnitine is a vitamin-like substance **(Paul and Andrea, 2000)**. L-Carnitine is a vitamin-like nutrient essential for energy production and lipid metabolism in many organs and tissues such as skeletal muscle and heart **(Jean et al., 2003)**.

L-arginine was shown to restore endothelial function in hypercholesterolemic rabbits by increasing NO production and protecting NO from inactivation by superoxide anions ($O_2^{\cdot-}$) **(Böger et al., 1995)**.

Reproductive aging in female mammals is characterized by alterations in the levels and release pattern of the sex steroid hormone, estrogen. In women, estrogen concentrations undergo a precipitous decline at menopause, and the risks and benefits of estrogen replacement therapy on the reproductive tract, bone, cardiovascular system, and brain are quite controversial **(Tandra et al., 2003)**. Testosterone level in serum of aged rats showed a significant decrease when compared with adult control level **(El-Sayed et al., 2005)**.

MATERIALS AND METHODS

Forty healthy female Spargue-Dawley rats were used in this study; ten young rats two months age and their average weights ranged between 120-140 gm and thirty aged rats at one year old, their average weights ranged between 290-300 gm, the animals were obtained from animal house at Tanta- Egypt. Rats were kept in metabolic cages (four rats per cage) in a controlled environment and maintained under a 12 hours light: dark cycle, air conditioned at 24 ± 2°c and 50-70% humidity. Throughout the study, rats were provided with basal diet and water ad-libitum and kept in the animal house at Faculty of Veterinary Medicine-Mansoura University. Animals were divided into four equal groups each of ten rats. The first group (CA) was considered a control adult female rat, two months age and kept on basal ration without feed additives. The other animals were considered aged rats (one year old) and divided into three equal groups ten aged rats each as follows: Control aged rats (CAG) group and fed on the same ration without feed supplements. L-arginine group (LA) was kept on the same ration supplemented with L-arginine (1.68%) and L-carnitine group (LC) was feed on the same ration supplemented with L-carnitine (0.5 mg %) for four weeks. At the end of the experimental period (four weeks), blood (serum & whole blood) samples were collected from rats under experiment for the biochemical analysis of: serum estradiol, lipid profile and malondialdehyde. Enzymatic (SOD and catalase) and non-enzymatic (GSH) antioxidant enzyme activities in the whole blood and tissue malondialdehyde content was determined. Tissue samples (ovary, kidney and liver) were collected for histopathological examination.

One blood sample from each animal was collected after decapitation of animals at the end of experiment and divided into two portions. The first one was collected in heparinized tube for determination of superoxide dismutase activity (SOD) **(Winterbourn et al., 1975)**, catalse activity **(Cohen et al., 1970)** and reduced glutathione level **(Ellmans's, 1959 and Beutler et al,. 1963)** The second blood portion was collected in sterile vial and fit at room temperature for 30 minutes then centrifuged at 3000 r.p.m. for collection of clear serum sample used for the biochemical analysis of serum malondialdehyde (MDA) **(Draper and Hadley 1990)**., serum triacylglycerol **(Buccolo and David, 1973)**, serum total cholesterol **(Meiattini et al., 1978)**, serum HDL-cholesterol **(Friedewald et al., 1972)**, serum LDL-cholesterol **(Friedewald et al., 1972)**, serum total lipid **(Kaplan, 1984)** and serum estradiol assay using Eliza technique **(Ratcliff, 1988)**.

Tissue specimens also were collected from liver, ovary and kidney and fixed in 10% neutral buffered formalin. Paraffin sections of 5µ thickness were prepared and stained with hematoxalin and eosin and examined microscopically according to **Wood and Ellis, 1994**. Statistical analysis is carried out by SPSS program **(Senedecor and Cochran 1989)**.

RESULTS & DISCUSSION

Aging is usually associated with increasing level of oxidation **(Rikans and Hornbrook, 1997 & Johnson et al., 1999)**. An imbalance between the formation and removal of reactive

oxygen species (ROS) and the development of OS plays an important role in aging and age-associated diseases **(Palomero et al., 2001)** ROS alters proteins, carbohydrates, and lipids, and inactivates enzymes and transporters, damages DNA and the transcriptional machinery, and initiates the chain reactions that peroxidize polyunsaturated fatty acids in membrane phospholipids **(Friedman, 2000)**. Determination of malondialdehyde by thiobarbituric acid is used as an index of the extent of lipid peroxidation **(Andallu & Varadacharyulu, 2003)**. MDA content in serum of normal control aged rats (1.019±0.108 µmol/L) is significantly higher (P≤0.05) than that of normal adult control animals (0.426±0.004 µmol/L) as shown in tables (1). **Traverso et al., (2003)** recorded that the plasma malondialdehyde (MDA), evaluated by means of the thiobarbituric acid test, and was significantly higher in the old age, confirming the presence of increased lipoperoxidation in old age. This result was supported by histopathological examination (Fig.1). This increase in MDA content might be due to an increase in oxygen free radicals that could be due to either increased production or decreased its destruction. Increased lipid peroxidation causes increased production of reactive oxygen species (ROS) due to autoxidation of monosaccharide which lead to the production of superoxide and hydroxyl radicals.

The mean values of MDA content in serum of LA treated rats are significantly lower than that of control aged rats. This result was in agreement with **Vanita et al., (2005) and Lubec et al., (1997)**. L-arginine has ability to ameliorate the oxidative stress and metabolic changes through reduction of malondialdehyde level in serum **(El-Missiry et al., 2004)**. This result was supported by histopathological examination (Fig. 7, 11 and15).

Serum of L-carnitine supplemented rats exhibited MDA level not significantly differed (P>0.05) from that of normal control aged animals. This result was supported by histopathological examination (Figures 9-16). The previous finding were disagree with the results of **Citil, et al., (2005)** who found that L-carnitine may improve tissues bioenergetics and lower the increased oxidative stress associated with aging. They added that in all brain regions except the hypothalamus, lipid peroxidation was higher for old rats than for young. Also they added that administration of L-carnitine reversed the age-associated changes in a duration-dependent manner.

Superoxide dismutase is the first line of defense against oxygen toxicity. It catalyzes the dismutation of superoxide anion producing hydrogen peroxide **(Norman and Kreinsky, 1992)**. The mean value of whole blood SOD activity in untreated aged rats of one year old (78.10±0.21µgm/ml) was significantly lowered (P≤0.05) than that of normal control adult rats of one month old (109.42±1.74µgm/ml). This result was in agreement with that reported by **El-Sayed et al., (2005)** in male rats and **El-Missiry et al., (2004)**.The decrease in SOD activity in aged rats could be attributed to the increased level of superoxide anion radicals and erythrocytes act as a sink for free radicals since both superoxide radicals and hydrogen peroxide have the ability to penetrate cell membranes. Consequently, erythrocytes are subjected to continuous flux of O2 and H2O2 arising from hemoglobin oxidation **(Aral et al., 1989)**. The mean value of whole blood SOD activity in the L-arginine group rats (94.705±0.356 µgm/ml) was significantly

increased (P>0.05) than that of normal aged control animals (78.103±0.207 µgm/ml) table (1). This result is supported by that of **Vanita et al., (2005) and Marra et al., (2007)** who found that superoxide dismutase activities increased under L-arginine treatment. SOD activity in the L-carnitine treated group of rats (90.784±1.404µgm/ml) was significantly increased (P>0.05) than that of normal aged control rats fed normal diet (78.103±0.207 µgm/ml) and significantly decreased (P≤0.05) than that of normal control adult animals (109.427±1.742 µgm/ml. This result was supported by the finding of **El-Sayed et al., (2005), and Mansour (2006)**. A decrease in the activities of the enzymes SOD and catalase can result in formation of O2. and H2O2, which in turn can form the hydroxyl radical (OH·) which can participate in a number of toxic reaetions **(Kumari and Menon, 1988)**.

Catalase activity in adult eontrol rats (0.24±0.0191 µmg/ml) was significantly increased (P>0.05) than that of normal eontrol aged rats fed the same diet (0.129±0.006 µmg/ml). This result was confirmed by that obtained by **El-Sayed et al., (2005) and Semsei et al., (1991)**. This may be due to increased free radical damage in the body **(Alper et al., 1998)**. Supplementing the aged rats group with L-arginine, significantly the activity of eatalase than that of normal aged control rats fed control ration (0.129±0.006 µmg/ml). This result was in agreement with that recorded by **Vanita et al., 2005 and Marra et al (2007)** who found that, catalase activity was increased under L-arginine treatment. L-arginine administration increases nitric oxide (NO) production.

Catalse activity in the L-carnitine supplemented group of rats (0.192±0.008 µmg/ml) was significantly increased (P>0.05) than that normal aged control rats (0.129±0.006 µmg/ml) and significantly decreased (P≤0.05) than that of normal adult control animals (0.24±0.0191 µgm/ml) table (1). These results are nearly similar to the results of **Rani & Panneerselvam, (2002)**.

GSH eontent in blood of aged eontrol rats (4.317±0.279mg/100ml) was significantly decreased (P≤0.05) than that of normal control adult animals (8±0.166mg/100ml) table (1). This decrement may be due to decrease its formation which requires NADPH+H+ and glutathione reductase **(Garg et al., 1996)**. The redueed availability of NADPH+H+ could be due to reduced synthesis in HMP shunt resulted in a decrease in the activity of glucose-6-phosphate dehydrogenase as this enzyme plays a very important role to maintain high ratio of NADPH+H+/NAPDP+ in the cell and plays a crucial role in regeneration of GSH from GSSG **(Jain, 1998)**.

The whole blood GSH content in L-arginine treated rats (6.631±0.230 mg/100ml) was significantly increased (P>0.05) than that of normal control aged animals (4.317±0.279mg/100ml) table (1). **Wan-teng et al., (2005)** found that L-Arginine supplementation may decrease free radicals and tubular membrane injury in nephrocalcinosis due to infiltrating leukocytes and decreased antioxidant enzyme activities in rats **(Ozturk et al., 2006)**.

GSH content in L-earnitine treated rats (5.333±0.184mg/100ml) was signifieantly increased (P 0.05) than that of normal control aged animals (4.317±0.279mg/100ml) but still signifieantly lower (P≤0.05) than that of normal control adult animals (8±0.166 mg/100ml) as shown in table (1). **Citil et al.,**

(2005), and Arsenian (1997), observed that a dministration of L-carnitine increased the level of GSH, where L-carnitine was found to produce complete proteetion against nephrotoxicity and pulmonary toxicity by increasing the antioxidant defense mechanism.

Serum total lipids in untreated aged rats (2159.996±238.67 mg/dl) was significantly increased (P>0.05) than that of normal adult control rats (1484.404±105.160 mg/dl). Serum triacylglycerol in untreated control aged rats was significantly different than that of normal control animals (298.675±4.8316 mg/dl) table (6) & graph (6). The mean value of serum total cholesterol in untreated aged rats (151.989±8.751 mg/dL) was none significantly increasel (P≤0.05) than that of normal control adult rats (144.988±6.378 mg/dL).

Regarding the mean value of serum lipoproteins. HDL cholestrol level in the control aged rats (14.224+0.738mg/dL) was significantly decreased (P≤0.05) than that of normal control adult rats (19.513±0.919 mg/dL). The serum LDL cholestrol in untreated aged rats (55.63±0.533mg/dL) was significantly increased (P>0.05) than that of normal adult control animals (45.395±0.377 mg/dL) table (9) & graph (9). These results were in agreement with that reported by **Kumaran et al., (2004) and Borum, (1991)** who stated that, the age-related changes in lipid eomposition are thought to account not only for the age-related accumulation of body fat, which is a risk factor for diabetes and atheroselerotic diseases, but also for age-related cellular hypofunction. Furthermore, **Michelle and Lorraine, (2002)** found that , aging was associated with higher triglycerides levels in muscles from old animals demonstrates a decreased ability to oxidize fatty acid that could in part explain the accumulation of muscle TG over time.

Coneerning L-arginine treated rats, the obtained result revealed that the mean value of serum total lipid in L-arginine treated rats (1852.859±162.84 mg /dl) was non significantly (P≤0.05) differed than that of normal adult control aged animals (2159.996±238.67 mg/dl). The mean value of serum triacylglycerol was 458.105±91.441mg/dL, and significantly decreased (P≤0.05) than that of normal control aged animals (710.309±112.97mg/dL). The mean value of serum total cholesterol in L-arginine supplemented rats (103.004±6.337 mg /dl) is significantly deereased (P≤0.05) than that of normal eontrol aged rats (151.989±8.751mg mg/dl).

The mean value of serum HDL cholesterol in L-arginine treated group of rats (15.669±1.864 mg/dL) was none significantly (P≤0.05) differed than that of normal control aged rats (14.224±0.738mg/dL). The mean value of serum LDL cholesterol in L-arginine treated rats (13.718±0.292mg/dL) was significantly decreased (P≤0.05) than that of normal untreated aged rats (55.63±0.533 mg/dl). **Yin et al., (2005)**, stated that oral L-arginine supplement improved endothelial function and redueed LDL level. On the opposite side, these results were disagreed with that of **Mendez and Balderas, (2001)** who observed that L-arginine tends to increase HDL.

Siani et al., (2000) reeorded that dietary L-arginine supplementation has been proposed to reverse endothelial dysfunetion in sueh diverse pathophysiologic eonditions as hypercholesterolemia, eoronary heart disease, and some forms of animal hypertension.

Hagen et al., (1998), concluded that, the dietary L-earnitine restored the function of liv-

er mitochondria in old rats. Treatment of aged rats with acetyl-L-carnitine (ALCAR) reversed age-associated increases in the levels of free and esterified cholesterol in plasma **(Ruggiero and Ruggiero., 1992)**, and restored age-associated decreases in cardiolipin levels in heart mitochondria **(Paradies et al., 1990)**. **Kumari and Menon, (1988)** found that The decreased levels of free fatty acids in serum in animals pretreated with carnitine may be due to decreased lipolysis, inercased uptake by mitochondria, or both.

The reproductive aging process is thought to be dictated by a gradual decrease in both the quantity and the quality of the oocytes held within the follicles present in the ovarian cortex **(Velde and Pearson 2002)**. In the current study, the mean value of serum estradiol level of normal control aged rats (12.511± 0.341 pg/ml) was significantly decreased ($P \leq 0.05$) than that of normal adult control animals (49.851±3.042 pg/ml). This result was supported by histopathological examination. This result was configared by Greenblatt et al., 1976 who stated that, estradiol levels showed no significant change in the aged male, but they were somewhat higher than in the aged female.

Serum estradiol level in L-arginine supplemented rats (37.942±1.853 pg/ml) was significantly higher ($P>0.05$) than that of normal aged control animals (12.511±0.341 pg/ml) table. This result was supported by histopathological examination (Fig. 7). **Battaglia et al., (1999)** studied the role of L-arginine in improving uterine and follicular doppler flow and in improving ovarian response to gonadotrophin in poor responder women and they concluded that oral L-arginine supplementation in poor responder patients may improve ovarian response, endometrial reeeptivity and pregnancy rate.

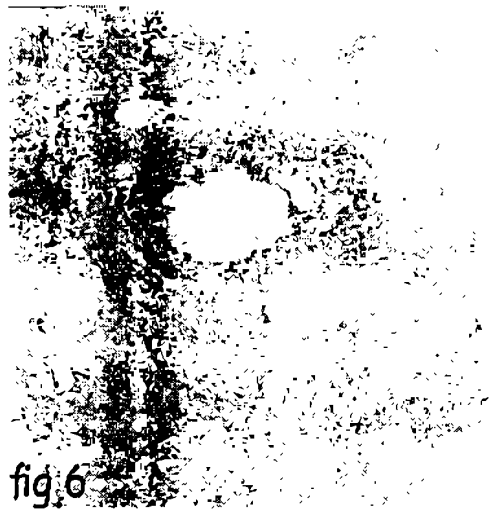
Serum estradiol level in L- carnitine supplemented rats (36.305±2.675 pg/ml) was significantly inereased ($P>0.05$) than that of normal aged control animals (12.51±0.34 pg/ml). This result was supported by histopathological examination (Fig. 8). Decreased follicle number increases FSH levels only in young rats, indicating aging-related alterations in the feedback regulation of FSH **(Anzalone et al., 2001)**. The progressive cessation of regular ovulatory function in aging female rats is preceded by a significant decrease in the magnitude of the proestrous LH surge during regular estrous cycles **(Lu et al., 1985)**.

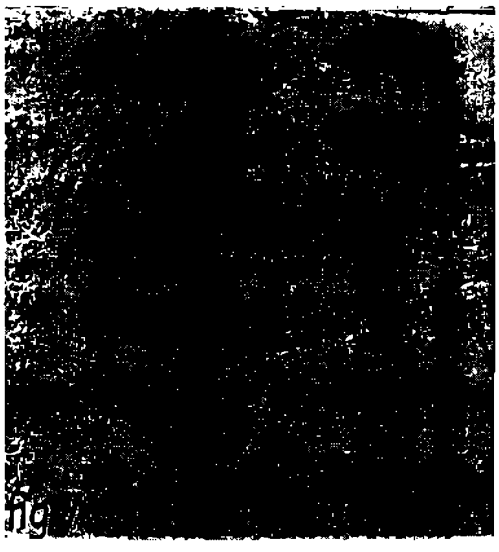
Aging is usually associated with increasing level of oxidation **(Rikans and Hornbrook, 1997 & Johnson et al., 1999)** An imbalance between the formation and removal of reactive oxygen species (ROS) and the development of OS plays an important role in aging and age-associated diseases **(Palomero et al., 2001)** ROS alters proteins, carbohydrates, and lipids, and inactivates enzymes and transporters, damages DNA and the transcriptional machinery, and initiates the chain reactions that peroxidize polyunsaturated fatty acids in membrane phospholipids **(Friedman, 2000)**.

The liver of aged rats showed an increase in number of cepoptotic cells and degeneration and dilated hepatic sinusoids (Fig. 14). The liver of rats received L-arginine showed an increase in the number of regenerated hepatic cell represented by diplocytes and increased mitotic activity (Fig. 15). The liver of rats received L-carnitine showed nearly the same results as that received L-arginine (Fig. 16). This result was confirmed by **Parola and Robino (2001) and Poli, (2000)** who ob-

served that the normal liver is a well equipped organ in terms of either enzymatic or non-enzymatic antioxidants. At molecular level, growth factors, cytokines and chemokines, changes in extracellular matrix (ECM) organization and composition as well as reactive molecules induced by OS, play a pathogenetic role. OS-related molecules may act as mediators to modulate tissue and cellular events responsible for the progression of liver.

L-arginine might decrease the oxidative stress in the liver and brain **(El-Missiry et al., 2004)**. These results were supported by the histopathological findings which revealed the absence of hepatic lesions in the liver of rats (LA) and (LC) groups. On the other hand, severe vacuolation and peripheral fibrosis were detected in aged rats (CAG).





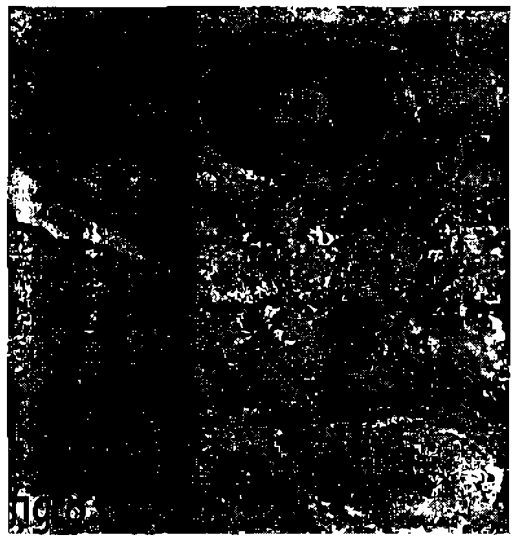


Fig. (5): The ovary shows mature graffian follicle at various stages of maturation.
Fig. (6): The ovary shows corpora lutea, besides degenerated cysts.
Fig.(7): The ovary of aged rat received L-arginine showed tendency toward retained its normal function represented by increase number of graffian follicles.
Fig. (8): The ovary of aged rat received L-carnitine showed tendency toward retained its normal function represented by increase number of graffian follicles.

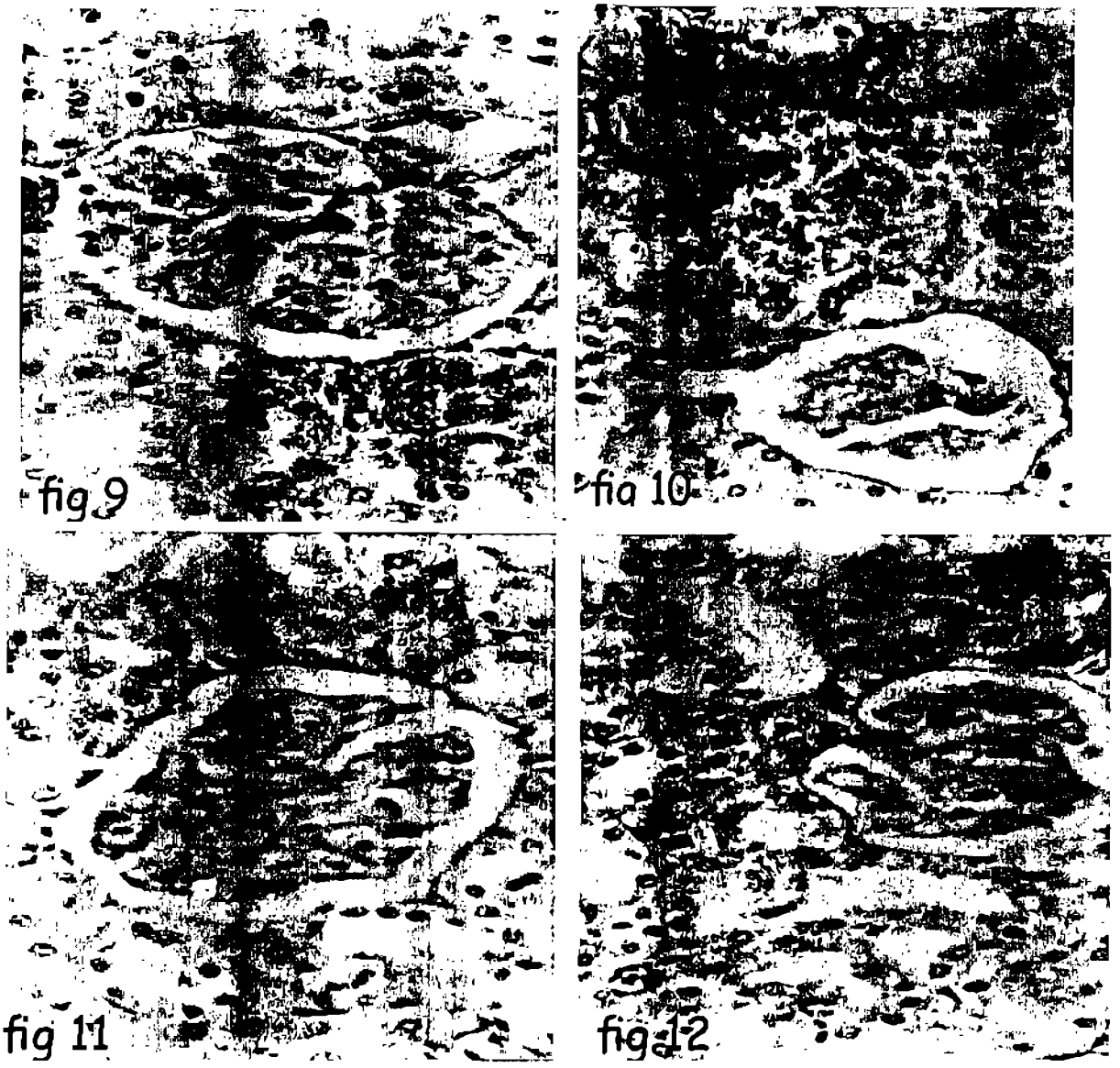


Fig. (9): Kidney of adult rat showed normal Microscopic picture.
Fig. (10): The kidney of aged rat shows increase number of lyalinize glomoruli beside congested capillaries and degenerated epithelial lining.
Fig. (11): Kidney of rat received L-arginine shows improvement of glomeruli tufts and renal epithelium structure.
Fig. (12): The kidney of rat received L-carnitine shows improvement of glomeruli tufts and renal epithelium structure.

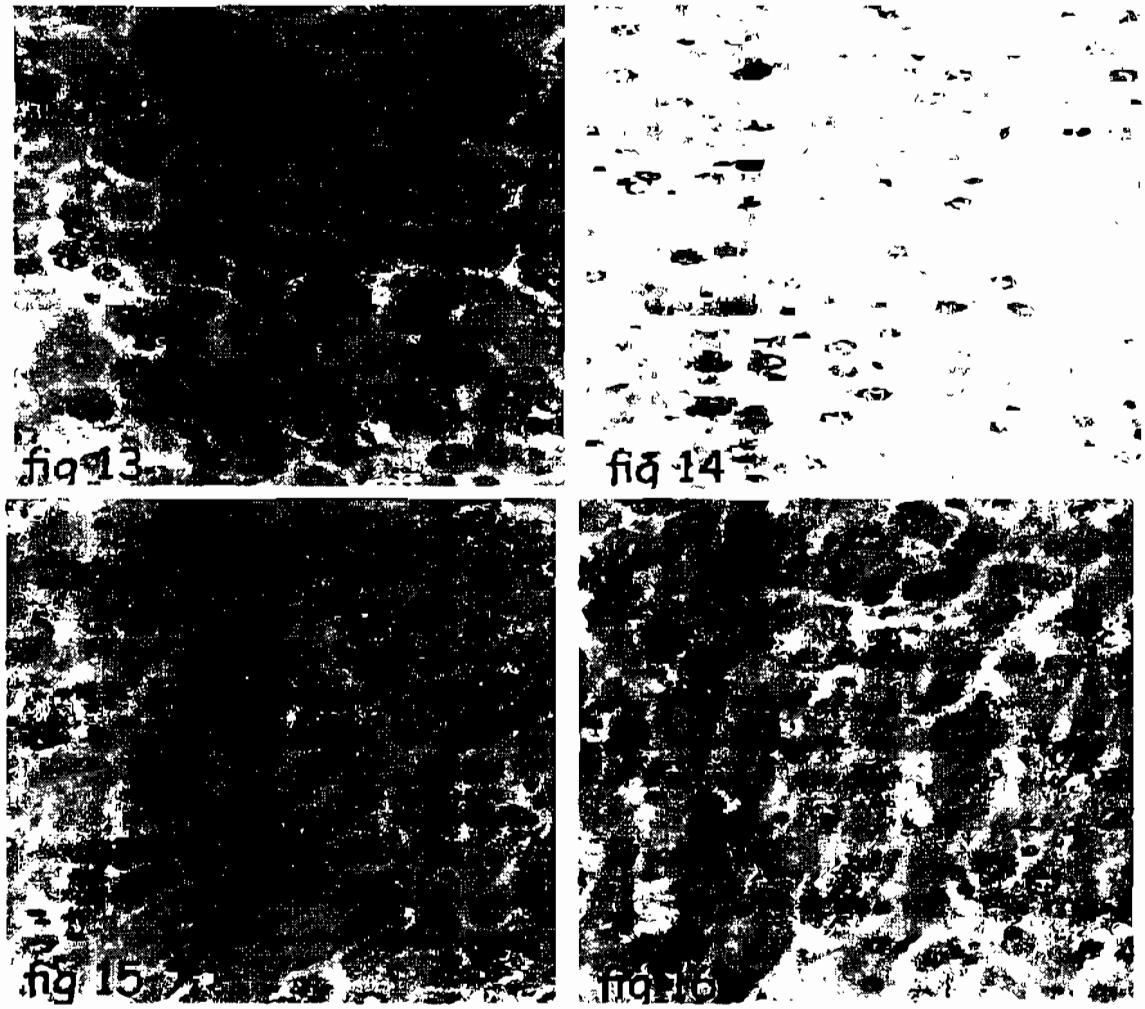


Fig. (13): The liver of adult rat shows microscopically picture normal.

Fig. (14): the liver of aged rats shows increase in number of cepoptotic cells and degeneration; moreover dilated hepatic sinusoids were seen.

Fig. (15): The liver of rats reeeived L-arginine showed increase number of regenerated hepatic cell represented by diplocytes, increases mitotic activity.

Fig. (16): The liver of rats received L-earnitine shows nearly same results of that rcccived l-arginine.

REFERENCES

Alper, G.; Sozmen, E.; Kanit, L.; Mentes, G.; Ersoz, B. and Kutay, F. (1998) : "Age-Related Alterations in Superoxide Dismutase and Catalase Activities in Rat Brain"Tr. J. of Medical Sciences. Vol. 28, 491-494.

Andallu, B. and Varadacharyulu, N. (2003) : "Antioxidant role of mulberry (Morus Indica L. cv. Anantha) leaves in streptozotocin-diabetic rats. Clin. Chim. Acta. Vol. 338, 3-10.

Anzalone, C.; Lu, J. and La Polt, P. (2001) : " Influences of age and reproductive status on ovarian ovulatory responsiveness to gonadotropin stimulation". Proc. Soe. Exp. Biol. Med. Vol. 217(4):455-60.

Arai, K.; Iizuka, S.; Tada, Y.; Oikawa, K. and Taniguchi, N. (1989) : "Increase in the glucosylated form of erythrocytes Cu–Zn superoxide dismutase in diabetes and close association of the non enzymatic glucosylation with the enzyme aetivity". Biochem. Biophys. Acta. Vol. 924, 292-296.

Arsenian, M. (1997):"Carnitine and it's derivatives in cardio-vascular disease. Progress in cardiovaseular diseases. Vol. 40, 265-286.

Balazy, M. (2000) : "Trans-arachidonic aelds: new mediators of inflammation". J. Physiol Pharmacol. Vol. 51, 597-607.

Battaglia, C.; Salvatori, M.; Maxia, N.; Petraglia, F.; Facchinetti, F. and Volpe, A. (1999): " Adjuvant L-arginine treatment for in-vitro fertilization in poor responder patients".Hum. Reprod. Vol. 14(7):1690-7.

Beutler, E.; Duran, O. and Kelly, B. (1963) : "Improved method for the determination of blood glutathione". J. Lab. Clinic. Med. Vol. 61, 882.

Böger, R.; Bode, S.; Mügge, A.; Kienke, S.; Brandes, R.; Dwenger, A. and Frölich, J. (1995) : "Supplementation of hypercholesterolaemic rabbits with L-arginine reduces the vascular release of superoxide anions and restores NO production". Atherosclerosis. Vol. 117, 273-284.

Borum, P. (1991) : "Carnitine and lipid metabolism". Bol. Asoc. Med. P. R. Vol. 83, 134-135.

Buccolo, G. and David, H. (1973) : "Quantitative determination of serum triglycerides by use of enzymes". Clin. Chem. Vol. 19, 476-482.

Citil, M.; Karapchilvan, M.; Marasli, S.; Atakisi, O. and Ogun, M. (2005): " Effect of oral L-carnitine supplementation on blood MDA and GSH levels in Tuj ewes". Indian Veterinary Journal. Vol. 82(2): 148-149.

Cohen, G.; Dembiec, D. and Marcus, J. (1970): "Measurement of catalase aetivity in tissue extracts". Anal. Bioch. Vol. 34, 30-38.

Draper, H. and Hadley, M. (1990): "Malondialdehyde determination as index of lipid peroxidation". Methods Enzymol. Vol. 186, 421-431.

Ellmans, G. (1959) : "Tissue sulfhydryl groups". Arch. Biochem. Biophys. Vol. 742, 214-226.

El-Missiry, M.; Othman, A. and Amer, M. (2004) : "L-Arginine ameliorates oxidative stress in alloxan-indueed experimental diabetes mellitus". J. Appl. Toxicol. Vol. 24 (2): 93-7.

El-Sayed, G; Laila, A. and El-shaeib, A. (2005): "Effect of L-carnitine and L-arginine supplementation on fertility by reduetion of oxidative stress in rats".4th International conference. Faculty of Vet.

Med. Mansoura University. Egypt. 923-942.

Friedewald, W.; Levy, R. and Frederickson, D. (1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the ultracentrifuge. Clin. Chem. Vol. 18, 499-502.

Friedman, S. (2000):"Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury". J. Biol. Chem. Vol. 275, 2247-2250.

Garg, M.; Ojha, S. and Bansal, D. (1996):"Antioxidant status of streptozotocin diabetic rats. Indian J. Exp. Biol. Vol. 34, 264- 266.

Greenblatt, R.; Oettinger, M. and Bobler, C. (1976):" Estrogen-androgen levels in aging men and women: therapeutic considerations". J. Am. Geriatr. Soc. Vol. 24 (4):173-8.

Hagen, T.; Moreau, R.; Suh, J. and Visioli F. (2002):"Delaying brain mitochondrial decay and aging with mitochondrial antioxidants and metabolites". Ann. N. Y. Acad. Sci. Vol. 959, 133-66.

Halliwell, B. (1997):"Antioxidants and human disease: A general introduction". Nutr. Rev. Vol. 55, 44-49.

Heidrun, K.; Sandra, L.; Thomas, K. and Alfred, L. (2002):" Dietary L-carnitine Stimulates Carnitine Acyltransferases in the Liver of Aged Rats". Journal of Histochemistry and Cytochemistry. Vol. 50, 205-212.

Jain, S. (1998):"Glutathione and glucose-6-phosphate dehydrogenase deficiency can increase protein glycation". Free Radic. Biol. Med. Vol. 24, 197-201.

Jean, D.; Béatrice G.; Caroline, R.; Marie-Charlotte, R.; Amélie, C.; Maud, S.; Serge L. and Françoise, L. (2003) : "Radioisotopic determination of L-carnitine content in foods commonly eaten in Western countries". Food Chemistry. Vol. 86, 137-142.

Johnson, F.; Sinclair, D. and Guarente, L. (1999) : " Molecular biology of aging". Cell. Vol. 96: 291-302.

Kaplan A. (1984) : Lipids. Clin. Chem. The C.V. Mosby Co. St Louis. Toronto. Princeton; 918-919.

Kumaran, S.; Savitha, S.; Anusuya, D. and Panneerselv, C. (2004) : " L-carnitine and DL-_-lipoic acid reverse the age-related deficit in glutathione redox state in skeletal muscle and heart tissues". Vol. 125(7): 507-512.

Kumari, S. and Menon, P. (1988): "Effect of carnitine administration on levels of lipid peroxides and activities of superoxide dismutase and catalase in isoproterenol-induced myocardial infarction in rats". J. Biosci, Vol. 13(3), 257-262.

Lubec, B.; Hayn, M.; Kitzmuller, E.; Vierhapper, H. and Lubec, G. (1997) : " L-Arginine reduces lipid peroxidation in patients with diabetes mellitus". Free Radic. Biol. Med. Vol. 22(1-2): 355-7.

Mansour, H. (2006) : " Protective role of carnitine ester against radiation-induced oxidative stress in rats". Pharmacol Res. Vol. 54(3): 165-71.

Marra, C.; Nella, J.; Manti, D. and de Alaniz, M. (2007):" Lipid metabolism in rats is modified by nitric oxide availability through a Ca++-dependent mechanism". Lipids. Vol. 42(3): 211-28.

Melattini, F.; Prencipe, L.; Bardelli, F.; Giannini, G. and Tarli, P. (1978): "The 4-hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic deter-

mination of serum cholesterol". Clin. Chem. Vol. 24. 2161- 2165.

Mendes, J. and Balderas, F. (2001):" Regulation of hyperglycemia and dyslipidemia by exogenous L- arginine in diabetic rats". Biochimie. Vol. 83(5):

Michelle, Z. and Lorraine, P. (2002): "Impaired fatty acid oxidation in muscle of aging rats perfused under basal conditions". AJP-Endo Articles In Press. Vol. 10, 1152.

Michelle, Z. and Lorraine, P. (2003):" Aging is associated with elevated muscle triglyceride content and increased insulin-stimulated fatty acid uptake". Am. J. Physiol Endocrinol Metab. Vol. 285, 827-835.

Mikhail, F.; Susan, P. and Glenn, L. (2004): " Mitochondrial DNA and aging". Clinical Science. Vol .107, (355-364).

Norman, I. and Kreinsky, N. (1992): "Mechanism of action of biological antioxidant". Proc. Soc. Expt. Biol.Med.Vol.200,248-253.

Ozturk, H.; Yagmur, Y. and Buyukbayram, H. (2006):" The effect of L-arginine methyl ester on indices of free radical involvement in a rat

Palomero, J.; Galán, A.; Muñoz, M.; Tunon, M.; Gonzalez-G. and Jimenez, R. (2001): "Effects of aging on the susceptibility to the toxic effects of cyclosporin A in rats. Changes in liver glutathione and antioxidant enzymes". Free Radic. Biol. Med. Vol. 30, 836-845.

Paradies, G. and Ruggiero, F. (1992): "Age related changes in the activity of the pyruvate carrier and in the lipid composition in rat-heart mitochondria". Biochim. Biophys. Acta. Vol. 1016, 207-212.

Paul, W. and Andrea, O. (2000) : " L-Carnitine, a 'Vitamin-Like Substance' for Functional Food. Proceedings of the Symposium on L-Carnitine". Annals of Nutrition & Metabolism. Vol. 44(2): 75-96.

Ratcliff, W. (1988) : "Estradiol assays: applications and guidelines for the provision of clinical biochemistry service". Ann. Clin. Biochem. Vol. 25, 466-483.

Rikans, L. and Hornbrook, K. (1997) : "Lipid peroxidation, antioxidant protection and aging". Biochim. Biophys. Acta. Vol. 1362, 116-127.

Ruggiero, F.; Cafagna, F.; Gadaleta, M. and E. Quagliariello. (1992) : "Effect of aging and acetyl-L-carnitine on the lipid composition of rat plasma and erythrocytes". Biochem. Biophys. Res. Vol. 170, 621–626.

Semsei, I.; Rao, G. and Richardson, A. (1991) : "Expression of superoxide dismutase and catalase in rat brain as a function of age". Mech. Ageing Dev. Vol. 58, 13-9.

Senedecor, G. and Cochran, P. (1989) : Statistical Methods, 8th ed., Iowa State University. Press, Ames, I.A.

Siani, A.; Pagano, E.; Iacone, R.; Iacoviello, L.; Scopacasa, F. and Strazzullo, P. (2000) "Blood pressure and metabolic changes during dietary L-arginine supplementation in humans". Am. J. Hypertens. Vol. 13, 547-51.

Simon, H.; Haj-yehia, A. and Levi-Schaffer, F. (2000) : "Role of reactive oxygen species (ROS) in the apoptosis induction". Apoptosis. Vol. 5, 415-18.

Stief, T. (2003) : "The physiology and pharmacology of singlet oxygen". Med. Hypotheses. Vol. 60(4): 567-72.

Tandra, R.; Chakraborty, L. and Andrea, C. (2003) : "Age-Related Changes in Estrogen Receptor β in Rat Hypothalamus: A Quantitative Analysis". Endocrin. Vol. 144(9): 4164-4171.

Traverso, N.; Patriarca, S.; Balbis E.; Furfaro, A.; Cottalasso, D.; Pronzato, M.; Carller, P ; Botta, F.; Marinari, U. and Fontana L. (2003) : " Anti malondialdehyde-adduct immunological response as a possible marker of successful aging". Exp. gerontol. vol. 38(10): 1129-1135.

Vanita, G.; Asheesh, G.; Shalini, S.; Harish, M.; D, Grover. and Ratan, K. (2005): "Anti-stress and Adaptogenic Activity of L-Arginine Supplementation". Evid Based Complement Alternat Med. Vol. 2(1): 93-97.

Velde. and Pearson, P. (2002) : "The variability of female reproductive ageing". Hum. Reprod. Vol. 8, 141-154.

Wan-teng, L.; Suh-ching, Y.; Kung-tung, C.; Chi-chang, H. and Ning-yuean, L. (2005): " Protective effects of L-arginine on pulmonary oxidative stress and anti- oxidant defenses during exhaustive exercise in rats". Acta Pharmacologica Sinica .Vol. 26, 992.

Winterbourn. C.; Hawking, R.; Brain, M. and Carrell, R. (1975) : "The estimation of red cell Superoxide dismutase activity". J. Lab. Clin. Med. Vol. 85(2): 337-341.

Woods, E. and Ellis, R. (1994) : Laboratory histopathology A complete References. 1st ed., Churchill Livingstone. Eden berg, Hong Kong, London, Madrid, New York and Tokyo.

Yasukazu, T.; Rorie, S.; Fumiko, F.; Hatsue, W.; Terue, K.; Mitsuyo, O.; Kyoko H. and Susumu. (2004) : " Acetyl-L-carnitine supplementation restores decreased tissue carnitine levels and impaired lipid metabolism in aged rats". Journal of Lipid Researeh. Vol. 45, 729-735.

Yin, W.; Chen, J.; Tsai, C.; Chiang, M.; Mason, S. and Lin, S. (2005) : "L-arginine improves endothelial function and reduces LDL oxidation in patients with stable coronary artery disease". J. Clin. Nutr. Vol. 24 (6): 988-997.

الملخص العربى

تأثير بعض الإضافات الغذائية على الخصوبة فى إناث الجرذان

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تعتبر الشيخوخة عملية ورائية معقدة تظهر فى الكائن الحى على مستوى الجينات والجزيئات والخلايا والأعضاء، بالرغم من أن آليتها الأساسية مازالت غير مفهومة، فإن تراكم الشقائق الحرة من أهم أسباب الشيخوخة.

تمت هذه الدراسة على عدد أربعون جرزاً وتم تقسيمها إلى أربعة مجموعات متساوية كل منها عشرة فئران، كانت المجموعة الأولى هى المجموعة الضابطة البالغة وعمرها شهرين فقط وتم تغذيتها على عليقة ضابطة بدون أى إضافات.

أما الفئران الباقية فكانت مسنة وعمرها سنة واحدة تم تقسيمها إلى ثلاث مجموعات متساوية من المجموعة الثانية حتى الرابعة تحتوى كل مجموعة منها على عشرة فئران.

المجموعة الثانية كانت مجموعة مسنة وكانت تتغذى على نفس العليقة بدون إضافات.

المجموعة الثالثة تغذت على نفس العليقة بإضافة إل-أرجنين، المجموعة الرابعة تم تغذيتها على نفس العليقة بإضافة إل-كارنتين.

فى نهاية التجربة تم تجميع عينات الدم من جميع الحيوانات لقياس الإستروجين، المالون داى ألدهيد، الدهون الكلية، الجليسريدات الثلاثية ، الكوليسترول الكلى، البروتينات الدهنية عالية الكثافة وقليلة الكثافة فى مصل الدم وأيضاً قياس نشاط إنزيم السوبر أكسيد ديسميوتيز والكاتاليز وكذلك نسبة الجلوتاثيون المختزل فى الدم بالإضافة إلى تجميع عينات الأنسجة (الكبد، المبيض، والكلى) من جميع الحيوانات لفحصها هستوباثولوجى.

أوضحت النتائج أن تأثير الشيخوخة على بعض القياسات الكيميائية مثل نقص معنوى فى نشاط إنزيم السوبر أكسيد ديسميتيز فى الدم، نشاط إنزيم الكاتاليز فى الدم وأيضاً نقص نسبة الجلوتاثيون المختزل فى الدم، بالإضافة إلى النقص المعنوى فى نسبة البروتينات الدهنية عالية الكثافة والاستراديول فى مصل الدم، على الجانب الآخر كانت هناك زيادة معنوية فى مستوى المالون داى ألدهيد مستوى الدهون الكلية، مستوى الجليسريدات الثلاثية ونسبة البروتينات الدهنية قليلة الكثافة فى السيرم، وعدم وجود فروق معنوية فى نسبة الكوليسترول الكلى فى المصل.

وقد لوحظ أن التغذية على إل-أوجنين لعليقة الفئران المسنة يؤدى إلى حدوث نقص معنوى فى مستوى المالون داى ألدهيد، مستوى الكوليسترول الكلى، مستوى الجليسريدات الثلاثية ونسبة البروتينات الدهنية قليلة الكثافة فى المصل، بالرغم من أن هناك زيادة معنوية فى مستوى نشاط إنزيم السوبر أكسيد ديميوتيز، نشاط إنزيم الكاتاليز فى الدم وفى نسبة الجلوتاثيون المختزل فى الدم، وأيضاً مستوى

الاستراديول فى المصل، ولم يكن هناك فرق معنوى فى مستوى الدهون الكلية ونسبة البروتينات الدهنية عالية الكثافة بمثيلاتها فى الفئران المسنة الضابطة.

نلاحظ أن تأثير ال-كارنتين على بعض القياسات الكيميائية توضح زيادة معنوية فى نشاط إنزيم السوبر أكسيد ديسميوتيز فى الدم، نشاط إنزيم الكاتاليز فى الدم وأيضاً نقص فى نسبة الجلوتاثيون المختزل فى الدم، كان هناك نقص معنوى فى مستوى الدهون الكلية ، مستوى الكوليسترول الكلى، مستوى الجليسريدات الثلاثية، نسبة البروتينات الدهنية قليلة الكثافة ومستوى الاستراديول فى المصل، ولايوجد فرق معنوى فى مستوى المالون داى ألدهيد ونسبة البروتينات الدهنية العالية الكثافة بالمقارنة مع الفئران المسنة.

ونستطيع أن نخلص إلى أن إضافة أل-أرجنين و أل-كارنتين سوف تقوم بتغير التأثير الغير مرغوب فيه للشيخوخة، والذى يتخلص نقص فى الشقائق الحرة، زيادة مضادات الأكسدة، وزيادة الخصوبة فى إناث الفئران، علاوة على ذلك فإن أل-أرجنين كان هو الأفضل من أل-كارنتين فى التأثير.