Research Article

Effect of Amlodipine and l-Carnitin Separately and Collectively on Certain Body Parameters that Are Related to Bone Metabolism in Ovariectomized Albino Rats

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Abstract

Background: Amlodipine as calcium channel blocker has anti inflammatory through over augmentation of nitric oxide production, Anti oxidant effect by reduced superoxide radicals and increased the level of superoxide dismutase L-Carnitine has anti inflammatory through reacts with acyl group that accumulated in anoxic cells, Anti oxidant effect decreasing the superoxide anion production. **Objective:** Assessing the effect of Amlodipine and L-Carnitine Separately And Collectively in osteoporosis induced by ovariectomy in female albino rats. **Materials and Methods:** Fifty adult female albino rats of local strain were randomly divided into five groups of 10 animals each. **G1:** Negative control group that received no treatment **G2:** Postive control group Ovariectomized rats that will be received no treatment **G3**: Amlodipine treated group Ovariectomized rats that will be received Amlodipine (5mg/kg/day) dissolved in distilled water by intraperitoneal injection (after two months from ovariectomy operation) for three weeks. **G4**: L-Carnitine treated group Ovariectomized rats that will be received L-Carnitine (100 mg/kg/day) by intra-peritoneal injection (after two months from ovariectomy operation) for three weeks**. G5**: Amlodipine + L-Carnitine Treated Group Ovariectomized 10 rats that will be received both Amlodipine at dose (5mg/kg/day) and L-Carnitine at a dose of 100 mg/kg/ day by intraperitoneal injection (after two months from ovariectomy operation) for three weeks. All rats were sacrificed and blood was withdrawn for biochemical examinations of Estrogen, Osteocalcine, Tumor necrosis factor α (TNFα) and Alkaline phosphatase (ALP). Femurs were removed for histopathological examination. **Results:** The serum levels of Osteocalcin, TNFα and ALP were increased significantly in positive control group as compared to negative control group. Treatment with Amlodipine and L-Carnitine for three weeks after two months from ovareiectomy operation significantly decreased the serum levels of Osteocalcin, TNFα and ALP when compared to positive control group. These results suggest that amlodipine and L-Carnitine have protective role in osteoporosis induced by ovariectomy in female albino rats.

Key words: Amlodipine, L-Carnitine, Osteoporosis, Ovariectomized rats.

Introduction

Osteoporosis is a multifactorial skeletal disease, which is characterized by bone loss and is seen mostly in postmenopausal women at about a 30% incidence rate[(1)]. Deterioration of bone tissue increases the risk of fracture [(2)]**.** Estrogen deficiency is the most important factor for the development of osteoporosis in postmenopausal women and is related to heredity tendency, diet, physical activity, medication use, and coexisting diseases[(3)]. Due to the loss of ovarian function, postmenopausal women showed bone loss because of estrogen-deficiency.**[(4)]** However, estrogen influences exhibit antioxidative properties and many studies demonstrated that estrogen may block the inflammatory reaction and reduce the degree of inflammation and tissue damage[(5)]**.** Loss of estrogen in menopausal women causes an unregulated chronic inflammatory process by raising the local production of various cytokines, such as IL-1β, IL-6, and TNF-α and the regulation of bone resorption and bone turnover associated with inflammatory cytokines[(6)]**.** Postmenopausal bone loss is a

major public health concern[7]. Although drug therapies are available, women are interested in alternative/adjunct therapies to slow down the bone loss associated with ovarian hormone deficiency[8]. *Amlodipine*, is a type of calcium channel blocker commonly used to treat high blood pressure and coronary artery disease [9]. Amlodipine was patented in 1982 and approved for medical use in 1990. It is on the World Health Organization's List of Essential Medicines, which lists the most effective and safe medicines needed in a health system [10]. Previous studies demonstrated that amlodipine have anti-inflammatory and antioxidant effects. This anti-inflammatory effect is considered to be based on over augmentation of nitric oxide production and a reduction of oxidative stress[11]. Zhou, Jaimes and Raij, 2004 [12] reported that amlodipine in angiotensin-induced oxidative stress reduced superoxide radicals and increased the level of superoxide dismutase as an antioxidant effect. Calcium ion has an important role in intracellular regulation of osteoclasts: it contributes both in regulation and formation of mature osteoclasts [13]. The inositol trisphosphate receptors (IP3R) are located in the endoplasmic reticulum (ER) and play an important role in intracellular calcium release via extracellular signals [6]. Many extracellular signals cause an osteoclast response, and most of these signals cause IP3-dependent calcium release[14]. IP3 receptors have been shown in osteoclasts[15]. IP3-dependent intracellular calcium release causes apoptosis of osteoclasts . Another study showed that absence of IP3 inhibited osteoblasts formation[16]. It was shown that IP3R can be up-regulated via L-type calcium channels and this upregulation incr-eased the stimulation of osteoclast activation[17]. Amlodipine exert anti-osteoporotic effects by blockade of L-type calcium channels and both these are agents known to effect IP3R. These anti-osteoporotic effects of amlodipine may result from it blocking calcium channels, IP3R stimulation, intracellular calcium release and by stopping activation and maturation of osteoclasts.[18]. Carnitine (β-hydroxy-γ-*N*-trimethylaminobutyric acid, 3-hydroxy-4-*N*,*N*,*N*- trimethylaminobutyrate) is a quaternary ammonium compound involved in metabolism in most mammals, plants and some bacteria. Carnitine may exist in two isomers, labeled D-carnitine and L-carnitine.. Carnitine was discovered in 1905 as a result of its high concentration in muscle tissue[19]. Carnitine is involved in transporting fatty acids across the mitochondrial membrane, by forming a long chain acetylcarnitine ester and being transported by carnitine palmitoyltransferase I and carnitine palmitoyltransferase II[20]. As fatty acid oxidation provides an important part of energy for bone cells, carnitine may directly improve the metabolism of osteoblasts[21]. In addition, L-carnitine is capable of restoring the age-related changes in the functions of inflammatory cells[22]. Moreover, *L*-carnitine may play a protective role in the tissue destruction in inflammation by decreasing the superoxide anion production[23]. Carnitine is an essential substance for energy metabolism[24].

The aim of the present study is to evaluate the possible protective effect of Amlodipine and L-Carnitime in osteoporosis induced by ovary-ectomy in female albino rats.

Materials and Methods

Chemicals

Amlodipine was taken in the form of powder from EPICO Company dissolved in distilled water. L-Carnitine was taken in the form of ampoules from MEPACO Company (1g/5 ml).

Experimental animals

Fifty adult female albino rats (weighting 120-150 gm) of local strain will be chosen as an animal model for this study. All animals will be kept in suitable cages (20x32x20 cm for every 5 rats) in standard conditions with constant 12h light/12h dark cycle at temperature of 25±2 the animals will be supplied with commercial pellet food and water ad libitum. The animals will be acclimatized to the animal room condition for at least two week before the experiment.

Ovariectomy Operation

The animals were anesthetized with a combi-nation of ketamine (80 mg/kg) and xylazine (10 mg/kg), intraperitoneal. Sterilization of hair and skin of the lower half of the abdominal wall was carried using betadine solution. Skin, fascia and muscles of the lower abdominal wall were dissected by a sterile scalp.

Pelvic peritoneum was opened to expose the pelvic organs. The ovary, embedded in fat, was withdrawn out and tied with a silk ligature so as to enclose the whole ovary and avoid the inclusion of the adjacent fat as much as

possible[25]. Pelvic fatty tissues were picked up to determine the uterine rami and the ovaries were recognized at the end of each ramus surronded by fatty tissues.

A tie was done at the end of each ramus of the uterus using vicryl threads the ovaries were incised bilaterally one by one and preserved in formalin solution. Any ooze was controlled by pressure by gauze swaps. The muscles of the abdominal wall, fascia and skin were closed using silk.

After the ovariectomy, the rats were given 25 mg/kg Diclofinac sodium as an analgesic for 2 days and 1.75 mg/kg amoxicillin to prevent to infections. The rats will be randomly divided into three groups Each group contains ten rats, as follow: -

Group 1 (Negative Control Group):
Non-ovariectomized 10 rats that will be kept as negative control group.

Group 2 (Positive Control Group):
Ovariectomized 10 rats that will be received no treatment and kept as positive control group.

Group 3 (**Amlodipine Treated Group)**:
Ovariectomized 10 rats that will be received Amlodipine (5mg/kg/day) dissolved in distilled water by intraperitoneal injection (after two months from ovariectomy operation) for three weeks.

Group 4 (L-Carnitine treated group):
Ovariectomized 10 rats that will be received L-Carnitine (100 mg/kg/day) by intraperitoneal injection (after two months from ovariectomy operation) for three weeks.

Group 5 (Amlodipine + L-Carnitine Treated Group): Ovariectomized 10 rats that will be received both Amlodipine at dose (5mg/kg/day) and L-Carnitine at a dose of 100 mg/kg/ day by intraperitoneal injection (after two months from ovariectomy operation) for three weeks.

At the end of the study each rat was taken from it a blood sample < 1cm by capillary tubes retro-orbital; it was kept in a test tube in an upright position for 10 min till finishing all the rats.

Then it was centrifuged for about 20 min, where the serum was separated and taken by micro-pipette in an epindorph and put in the fridge for one day until transfer to the Lab. for assessment of serum estrogen Osteocacin, TNFα and ALP. Tissue was cleaned from the left and the right femurs. The left femurs were separated for histopathological examination.

Statistical analysis:
Results were expressed as mean ± standard error of mean (S.E.M). One-way analysis of variance (ANOVA) followed by the Tukey-Kramar post analysis test were used to analyze the results for statically significant difference. Graph pad prism was used for statistical calculations (version 3.02 for windows, Graphpad Software, San Diego California USA,www.graphpad.com).

Results

Body Weight

As Shown in figure[1] The final body weight was significantly increased in Positive, Amlodipine treated group, L-Carnitine treated group and Amlodipine + L-Carnitine treated group as compared to Negative control group. The final body weight was185.7 ± 2.542, 206.4±5.533, 212.9 ±6.801, 216.4± 5.200 and 212.1±3.247 in Negative, Positive ,Amlodipine group, L-Carnitine treated group and Amlodipine + L-Carnitine treated group respectively.

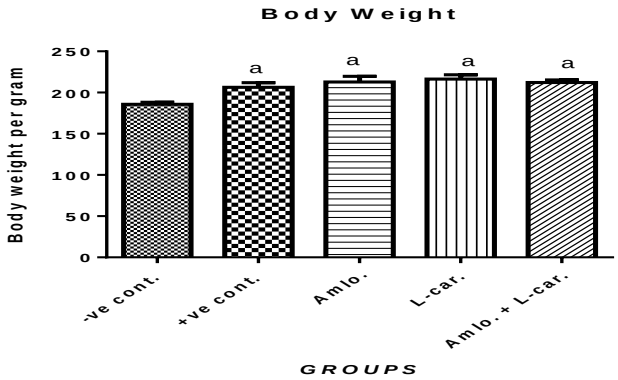


Figure 1: The final body weight in the different studied groups. **-ve cont**.: Negative control group, **+ve cont**.: Positive control group, **Amlo.**: Amlodipine treated group, **L-car.**: L-carnitine treated group, **Amlo. + L-car.**: Amlodipine + L-carnitine treated group. **a**: Significantly different from negative control group, $P < 0.05$. Data are expressed as mean ± S.E.M. of 10 rats in each group.

Serum Estrogen

As Shown in figure [(2)] the serum estrogen was significantly decreased in Positive, Amlodipine treated group, L-Carnitine treated group and Amlodipine + L-Carnitine treated group as compared to Negative control group. The serum estrogen was 29.14 ± 0.911, 17.57 ± 1.525, 15.29 ± 1.322, 16.14 ± 1.05, and 17.00 ± 1.055 in Negative, Positive, Amlodipine group, L-Carnitine treated group and Amlodipine + L-Carnitine treated group respectively.

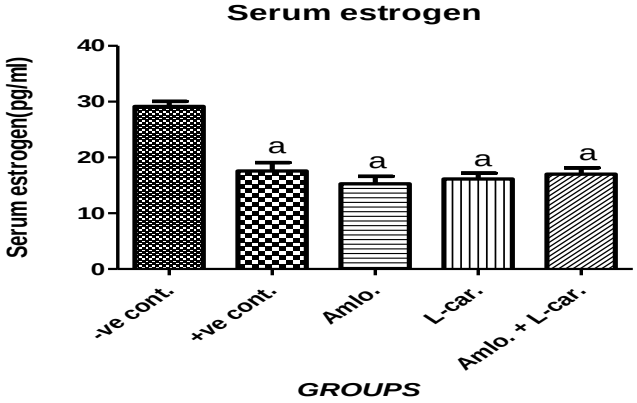


Figure 2: The serum estrogen in the different studied groups. **-ve cont**.: Negative control group, **+ve cont.**: Positive control group, **Amlo.**: Amlodipine treated group, **L-car**.: L-carnitine treated group, Amlo. + L-car.: Amlodipine + L-carnitine treated group. **a**: Significantly different from negative control group, $P < 0.05$. Data are expressed as mean ± S.E.M. of 10 rats in each group.

Serum Osteocalcin

As shown in figure (3) The serum Osteocalcin is significantly increased in positive control group as compared to negative control group on the other hand the serun Osteocalcin in Amlodipine treated group and L-Carnitine treated group is significantly decreased as compared to positive group also the serum osteoclacin in Amlodipine + L-Carnitine treated group is significantly decreased as compared to positive group, Amlodipine treated group and L-Carnitine treated group. The serum Osteocalcin was 20.07±2.154, 44.14 ±1.814, 33.01 ± 0.457, 33.97 ± 0.473 and 27.10 ± 0.604 in Negative, Positive, Amlodipine treated group, L-Carnitine treated group and Amlodipine + L-Carnitine treated group respectively.

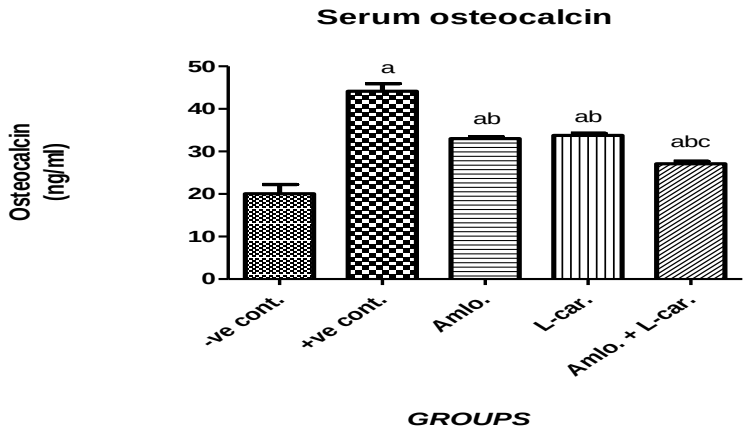


Figure 3: The serum osteocalcin in the different studied groups. **-ve cont**.: Negative control group, **+ve cont**.: Positive control group, **Amlo.**: Amlodipine treated group, **L-car.**: L-carnitine treated group, **Amlo.+ L-car.**: Amlodipine + L-carnitine treated group. **a:** Significantly different from negative control group, **b:** Significantly different from positive group, **c:** Significantly different from Amlodipine treated group and L-carnitine treated group, $P < 0.05$. Data are expressed as mean ± S.E.M. of 10 rats in each group.

Serum TNFα

As shown in figure[4] The serum TNFα is significantly increased in positive control group as compared to negative control group on the other hand the serum TNFα in Amlodipine treated group and L-Carnitine treated group is significantly decreased as compared to positive group also the serum TNFα in Amlodipine + L-Carnitine treated group is significantly decreased as compared to positive group, Amlodipine treated group and L-Carnitine treated group . The serum TNFα was 23.07 ± 0.713, 34.87 ±1.77, 30.56 ± 0.653, 30.39 ± 0.602 and 26.61 ± 0.578 in Negative, Positive, Amlodipine treated group, L-Carnitine treated group and Amlodipine + L-Carnitine treated group respectively.

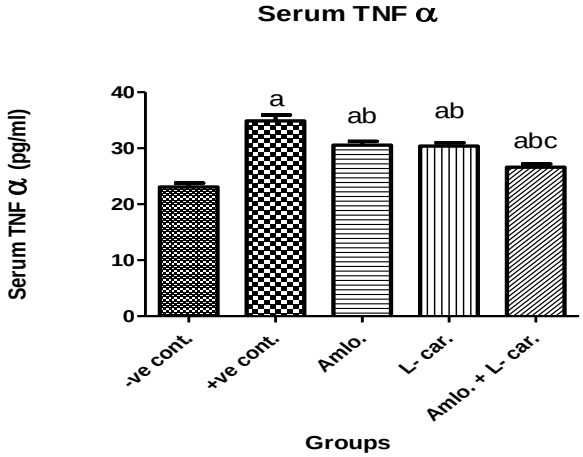


Figure 4: The serum TNF α in the different studied groups. **-ve cont**.: Negative control group, **+ve cont.:** Positive control group, **Amlo.**: Amlodipine treated group, **L-car**.: L-carnitine treated group, **Amlo. + L-car.**: Amlodipine + L-carnitine treated group. **a**: Significantly different from negative control group, **b**: Significantly different from positive group, **c**: Significantly different from Amlodipine treated group and L-carnitine treated group, $P < 0.05$. Data are expressed as mean ± S.E.M. of 10 rats in each group.

Serum ALP

As shown in figure [6] The serum ALP is significantly increased in positive control group as compared to negative control group on the other hand the serum ALP in Amlodipine treated group and L-Carnitine treated group is significantly decreased as compared to positive group also the serum ALP in Amlodipine + L-Carnitine treated group is significantly decreased as compared to positive group, Amlodipine treated group and L-Carnitine treated group . The serum ALP was 24.81 ± 0.514, 36.76 ± 0.342, 31.83 ± 0.560 , 31.37 ±0.576 and 28.76 ±0.215 in Negative, Positive, Amlodipine treated group, L-Carnitine treated group and Amlodipine + L-Carnitine treated group respectively.

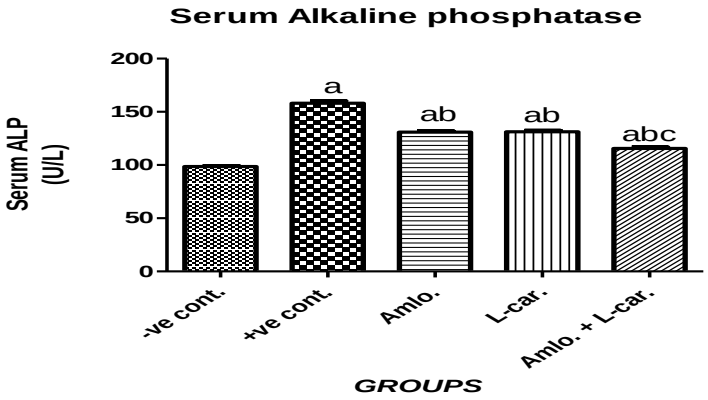
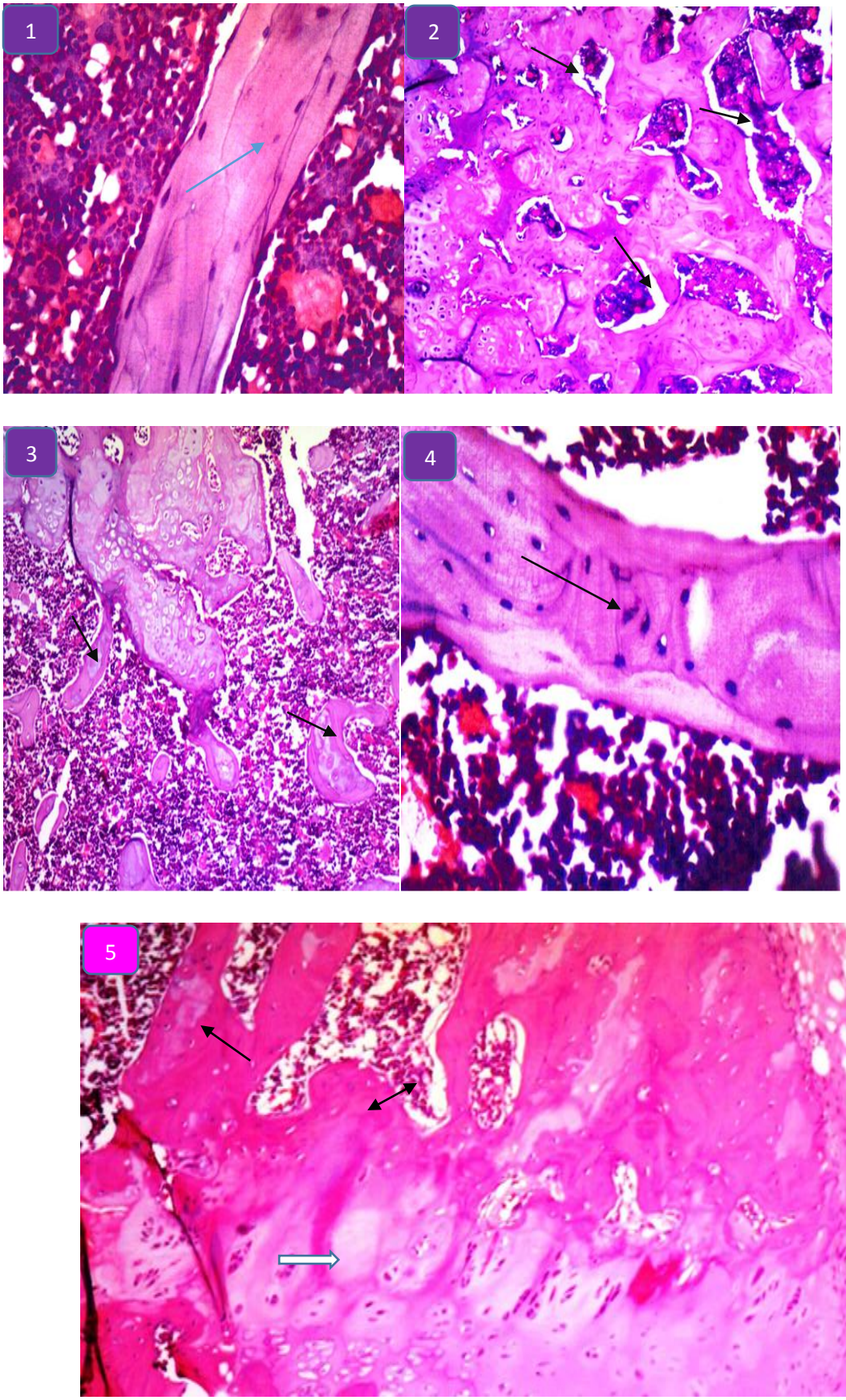
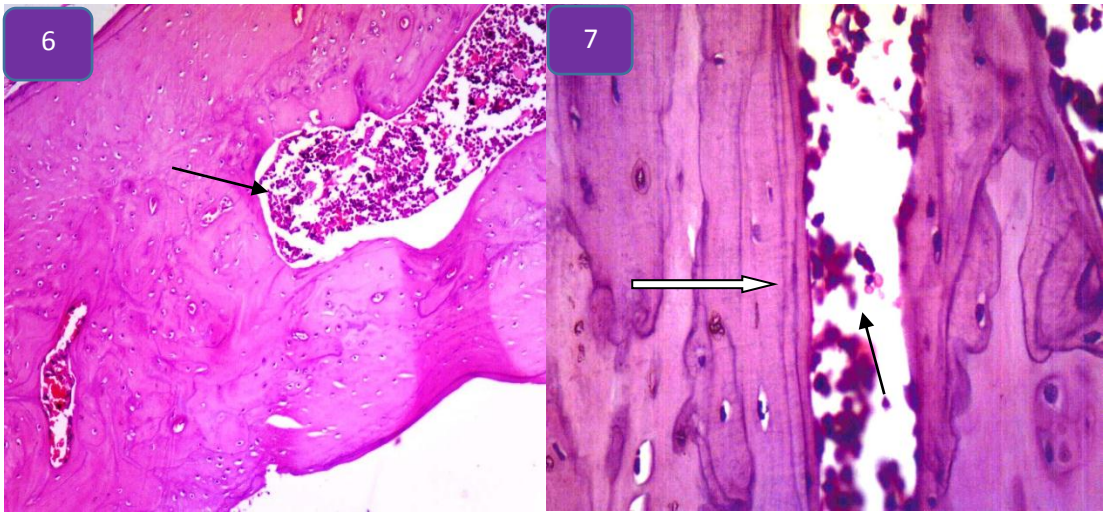


Figure 6: The serum Alkaline phosphtase in the different studied groups. **-ve cont**.: Negative control group, +ve cont.: Positive control group, Amlo.: Amlodipine treated group, L-car.: L-carnitine treated group, Amlo. + L-car.: Amlodipine + L-carnitine treated group. a: Significantly different from negative control group, b: Significantly different from positive group, c: Significantly different from Amlodipine treated group and L-carnitine treated group, $P < 0.05$. Data are expressed as mean ± S.E.M. of 10 rats in each group

Histological Results





Figures of Femurs in different rat groups stained with hematoxylin and eosin

1-Negative control group: shows intact and well-formed trabeculae (arrow) with normal trabecular spaces (Hx&E x400).

2-Positive control group: shows non homogenous matrix with multiple osteoporotic cavities (arrow) filled with a granulation tissue (H&E X100).

3,4-Amlodipine treated group: showing increased trabecular thickness (arrows) and narrowing of the widened spaces with newly formed osteoid tissue (arrow).

5- L-carnitine treated group showing increased trabecular thickness (arrows) and narrowing of the widened spaces with newly formed osteoid tissue (double headed arrow) with newly formed osteoid tissue (white arrow) (H&E X100).

6,7- Amlodipine + L-carnitine treated group showing nearly normal trabecular thickness (white arrows) and narrowing of the widened spaces with newly formed osteoid tissue (black arrow) (H&E X400).

Discussion

Osteoporosis is a multifactorial skeletal disease, which is characterized by bone loss and is seen mostly in postmenopausal women at about a 30 % incidence rate [(1)]. Deterioration of bone tissue increases the risk of fracture[(2)]**.** Estrogen deficiency is the most important factor for the development of osteoporosis in postmenopausal women and is related to heredity tendency, diet, physical activity, medication use, and coexisting diseases [(3)]. Due to the loss of ovarian function, postmenopausal women showed bone loss because of estrogen-deficiency [(4)]. However, estrogen influences exhibit antioxidative properties and many studies demonstrated that estrogen may block the inflammatory reaction and reduce the degree of inflammation and tissue damage[(5)]**.** Loss of estrogen in menopausal women causes an unregulated chronic inflammatory process by raising the local production of various cytokines, such as IL-1β, IL-6, and TNF-α and the regulation of bone resorption and bone turnover associated with inflammatory cytokines[(6)]**.** Postmenopausal bone loss is a major public health concern[(7)]**.** Although drug therapies are available, women are interested in alternative/adjunct therapies to slow down the bone loss associated with ovarian hormone deficiency[(8)]**.** Calcium channel blockers (CCBs) are widely used in various diseases and are most frequently used for hypertension and angina pectoris, myocardial infarction, hypertensive crisis, pulmonary hypertension, peripheral vascular diseases arrhy-thmia, left ventricular diastolic dysfunction, Raynaud's phenomenon, progressive systemic sclerosis,

chronic renal failure, Conn syndrome, migraine and esophageal spasm.[26].

Many anti-inflammatory and antioxidant substances have been experimentally studied, as in fractures occurring with osteoporosis, free oxygen radicals, inflammation and associated cytokines are all evident[27]. Carnitine plays a key role here. L-carnitine is a water-soluble molecule and has very important attributes related to mammalian metabolism, especially in the mitochondrial oxidation of normal fatty acid [28]. The ovariectomized rat bone loss model is considered a suitable model for studying human menopausal osteoporosis because of similar pathophysiological mechanisms in bone corruption. The imbalance between estrogen and bone turnover during menopause, in which absence of estrogen causes increased bone turnover, results in bone loss and susceptibility to osteoporosis[29].

The aim of the present study is to identify the effect of amlodipine as calcium channel blockers and L-Carnitine in treatment of osteoporosis induced by ovriectomy in female albino rats.

Our results revealed that the final body weight in the different studied groups was as following: in Negative Control group was 185.7 ± 2.54, in Positive Control group was 206.4 ± 5.53 and in L-Carnitine treated group 216.4 ± 5.20.

On contrast from our study, results in the study of [30] that assess effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats, in which body weight increased significantly in rats on the high fat diet compared with controls , while treatments with L-carnitine significantly reduced this gain during the treatment period. Treatments with L-carnitine significantly reduced the elevated body weight during the treatment period compared to high fat diet, Interest in the role of L-carnitine as a feed additive to improve whole body composition arose from the desire to partition nutrients away from lipid accretion, causing improvement of nitrogen balance; L-carnitine also attenuated visceral fat accumulation and accelerated the normalization of food intake[31].

The study on the hand, measured serum estrogen in the different groups and found that its level in Negative Control group was 29.14±0.91 ,in positive control group was 17.57±1.52 ,in Amlodipine treated group was 15.29±1.32 while in L-Carnitine treated group was 16.14± 1.05 and finally estrogen level in Amlodipine + L-Carnitine treated group was 17.00 ±1.05, and there was statistically significant difference between the four groups and the negative control group where p value < 0.05.

Bone loss is prevented by estrogen, which affects bone marrow and bone cells, reduces osteoclast formation, increases osteoclast apoptosis, and decreases the capacity of mature osteoclasts to resorb bone[32]. Estrogen regulates the production of pro- and antiosteoclastogenic cytokines by bone and bone marrow cells [33]. Estrogen deficiency related to aging causes inflammatory responses, which increase various osteoclastogenic cytokines in the cell in the microenvironment of bones [34] . Inflammation is a potential risk factor for osteoporosis [35]. Some proinflammatories, such as IL-1β, IL-6, TNF-α, all of which play a role in immune response, organize the activity and differentiation of osteoclast and osteoblasts [36].

Osteocalcin is a small g-carboxyglutamate protein secreted from osteoblasts and osteoclasts and is also expressed and secreted in bone marrow and many organs[37]. Osteocalcin acts as a regulator of bone mineralization, which is commonly used as a clinical marker of bone formation[38]. Ducy et al., 1996[39] reported that osteocalcin directly inhibits osteoblastic bone formation In osteoporosis, osteocalcin is secreted by osteoblasts and is responsible for bone mineralization and calcium ion homeostasis [40]. Liao et al., 2018[41] reported that serum osteocalcin levels increase in the context of osteoporosis. other studies on postmenopausal women showed that the serum level of osteocalcin is two times higher than premenopausal women[42].

The present study assessed the serum osteocalcin in the different studied groups and found that in the negative control group serum osteocalcin was 20.07± 2.15, in Positive Control group was 44.14 ± 1.81, in Amlodipine treated group was 33.01 ± 0.45 and in L-Carnitine treated group was 33.97±0.47 and finally in amlodipine+ L-Carnitine treated group was 27.10 ± 0.60.

In agreement with our results ,the study of Karakus et al., 2016 [43] that assess Effects of Administration of Amlodipine and Lacidipine

on Inflammation-Induced Bone Loss in the Ovariectomized Rat, in which osteoporosis induction significantly increased bone turnover markers osteocalcin and osteopontin (OC and OP) in the OVXinf (Ovariectomized + Inflammation) groups when compared with the sham group (P 0.05).

This data suggests that amlodipine effect osteoclast activation in inflammation-induced osteoporosis. The mechanism by which Amlodipine affect osteoclast activation as follow: Calcium ion has an important role in intracellular regulation of osteoclasts: it contributes both in regulation and formation of mature osteoclasts [13].

The inositol trisphosphate receptors (IP3R) are located in the endoplasmic reticulum (ER) and play an important role in intracellular calcium release via extracellular signals[6]. Many extracellular signals cause an osteoclast response, and most of these signals cause IP3-dependent calcium release[14]. IP3 receptors have been shown in osteoclasts[15]. IP3-dependent intracellular calcium release causes apoptosis of osteoclasts .

Another study showed that absence of IP3 inhibited osteoblasts formation[16]. It was shown that IP3R can be up-regulated via L-type calcium channels and this upregulation increased the stimulation of osteoclast activation[17]**.** Amlodipine exert anti-osteoporotic effects by blockade of L-type calcium channels and both these are agents known to effect IP3R**.** These anti-osteoporotic effects of amlodipine may result from it blocking calcium channels, IP3R stimulation, intracellular calcium release and by stopping activation and maturation of osteoclasts [18].

On the other hand, this finding suggests that L-carnitine has positive effects on osteoblast activation by improving its metabolism[21]. The positive effects of carnitine on osteoblasts have been shown in previous studies (Colucci et al., 2005 and Cibulka et al., 2007).

Inflammation is a potential risk factor for osteoporosis. Some proinflammatories, such as IL-1β, IL-6, and TNF-α, all of which play a role in immune response, organize the activity and differentiation of osteoclast and osteoblasts [46].

TNF-α, IL1β, and IL-6 have a relationship with osteoporosis as pro-inflammatory agents. These osteoclastogenic pro-inflammatory cytokines offer important characteristics for osteoporotic bone[47].

The present study assessed the serum tumor necrosis factor α (TNFα) in the different studied groups and found that in the negative control group was 23.07 ± 0.713, in Positive Control group was 34.87 ±1.77, in Amlodipine treated group was 30.56 ±0.653 and in L-Carnitine treated group was 30.39 ± 0.602 and finally in amlodipine and L-Carnitine treated group was 26.61 ± 0.578.

Pacifici et al., 1991[48] suggested that the level of blood cytokines increased and parallel with the condition of elevated bone resorption in ovariectomized women. Additionally, another study demonstrated that serum cytokines caused a differentiation of osteoclast and cytokines secreted free radicals and inhibited the differentiation of osteoblasts. From these cytokines, TNF-α and IL-1β stimulated osteoclast formation and bone resorption[49].

Another study showed that amlodipine decreased the level of cytokines, such as TNF-α and IL-6 [50].
Halici et al., 2008[51] indicate that amlodipine have anti-inflammatory properties. This anti-inflammatory effect is considered to be based on over augmentation of nitric oxide production and a reduction of oxidative stress.

The study on the hand assessed Serum alkaline phosphatase (ALP), and was found that its level in negative control group was 98.57 ± 0.84 , in positive control group was 158.0 ± 2.18, in Amlodipine treated group was 130.9 ± 1.29 ,and in L-Carnitine treated group was 131.3 ± 1.29 ,finally its level in Amlodipine + L-Carnitine treated group was 115.6 ± 1.36.

On contrast from our study, NISHIYA and SUGIMOTO, 2001[52] reported that blocking calcium channel stimulates ALP activity and mineral deposition. Here we showed that nifedipine and amlodipine weakly block calcium channel on osteoblast. Therefore, it is not surprising that nifedipine and amlodipine also

partially elevated ALP activity at higher concentrations.

The study of Liu et al., 2012[53] that assessed The adverse effects of long-term L-carnitine supplementation on liver and kidney function in rats, in which they founded that L-carnitine reduced body and fat weights, as well as serum, liver, and kidney lipid levels in rats. Simultaneously, hepatic fatty acid β-oxidation and lipid synthesis were disturbed in L-carnitine-fed rats. Moreover, L-carnitine accelerated reactive oxygen species production in serum and liver and alteration of serum alkaline phosphatase levels further confirmed liver dysfunction in L-carnitine-fed rats.

Additionally, L-carnitine may potentially disturb kidney function by altering renal protein levels of rat organic ion transporters. These observations may provide the caution information for the safety of long-term L-carnitine supplementation.

In conclusion, From the previous date we can say that both Amlodipine and L-Carnitine have anti osteoporotic, anti-inflammatory effects and both of them can use in treatment of osteoporosis but using them as a combination is more useful due to the difference in their mechanisms of action, Amlodipine act on osteoclast (By blocking calcium channel) and L-Carnitine act on osteoblast (By increasing its metabolism).

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