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The impact of carvedilol on level of interleukin-6, superoxide dismutase, elastin levels and epidermal thickness in experimentally aging induced mice model

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ABSTRACT: The study aimed to evaluate the antiaging activity of carvedilol, as it has antioxidant and anti-inflammatory activity, and also to investigate whether it's better to give carvedilol with or after aging. The study was conducted on six groups (I-VI) of male mice, each consisting of 10 animals who were administered, for 6 weeks, oral normal saline only, 500 mg/kg of D-galactose (induction model), d-galactose 500 mg/kg, and vitamin C 100 mg/kg concomitantly, D-galactose 500 mg/kg followed by vitamin C 100 mg/kg; D-galactose 500 mg/kg; and carvedilol 10 mg/kg concomitantly; and galactose 500 mg/kg followed by carvedilol 10 mg/kg; respectively. Animals were killed, and heart tissue was taken and homogenized for the measurement of interleukin-6 and superoxide dismutase by ELISA technique, while skin tissue was divided into two parts, one homogenized to determine elastin level and another for histopathological analysis to determine epidermal thickness. Findings showed a significant drop in the level of interleukin-6 with a substantial elevation in Superoxide dismutase (SOD) and elastin level, along with an improvement in the epidermal thickness in both carvedilol treated groups, which revealed that carvedilol may have an anti-aging effect by decreasing oxidative stress, restoring normal levels of inflammatory markers, and postponing the aging of the skin, which is obtained when the carvedilol is used with or after the aging induction, with superiority when used at the beginning of the process.

KEYWORDS: Carvedilol; elastin; epidermal thickness; interleukin-6; superoxide ismutase.

1. INTRODUCTION

Aging is a genetic physiological process that is characterised by morphological and functional changes in cellular and extracellular components. These changes are made worse by lifelong injury and eventually result in a progressive imbalance of the control regulatory systems of the organism, including hormonal, autocrine, neuroendocrine, and immune homeostatic mechanisms. Strength, stamina, response time, agility, basal metabolism, sexual activity, and hearing acuity are just few of the things that naturally decline as person aged. Important as it is, remember that becoming older is not a medical condition. [1]. Though several factors contribute to aging, the major reason is the gradual buildup of random unrepaired molecular damage, this causes tissue dysfunction and aging over time due to cellular abnormalities, as illustrated in Figure 1 [2].

Major biological theories are inflammageing and oxidative stress, which are concerned with explaining deleterious effects that lead to decreased function in an organism; age-related changes that occur gradually over time; and finally, intrinsic changes that can affect all members of a species because of chronologic age [3]. According to the inflammageing hypothesis, older organisms are at increased risk for cardiovascular disease and other age-related chronic diseases and adverse health outcomes because of their pro-inflammatory status, which is characterized by high levels of pro-inflammatory markers in cells and tissues [4–6]. These postulates make up what is known as the redox hypothesis of oxidative stress: Redox elements are found in all biological systems and play important roles in cell signaling and physiological regulation. The redox activity of these elements is organized and coordinated through components undergo a specific reaction, oxidative stress results [7-9].

Skin is one of the organs affected by getting older, aged skin experiences structural, biochemical, and neurosensory perception changes, as well as an increased prevalence of various skin conditions. Even though there are a constant number of cell layers, the skin gradually becomes thinner with age at an

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accelerating rate. In addition, the epidermis becomes thinner, especially in women and on the face, neck, upper part of the chest, and extensor surface of the hands and forearms [10, 11]. In addition, as skin ages, keratinocytes change shape, becoming shorter and fatter. Thickness declines by roughly 6.4% every decade on average [11]. Carvedilol is a nonselective third generation β-blocker/α1-blocker, it improves myocardial function and inhibits destructive remodeling in heart failure by lowering peripheral vascular resistance and causing vasodilation, carvedilol lowers blood pressure. It's antioxidant, anti-inflammatory, and anti-apoptotic qualities contribute significantly to carvedilol's positive effects. [12]. This work was aimed to evaluate the antiaging activity of Carvedilol on experimentally induced aging mice when administered one time concomitantly with initiation of aging induction and once again after aging model established.

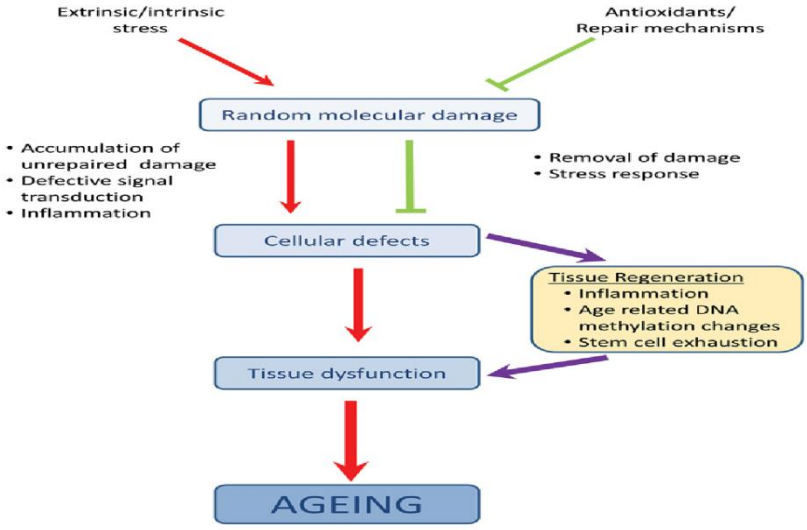

Figure 1. The underlying mechanisms of aging [2]

2. RESULTS

Regarding interleukin-6 (IL-6), the mean of inflammatory mediation was highly significantly elevated in the induction group (GII) compared to the control group (GI) [p-value <0.001]. There was no significant difference among groups GIII, GIV, and GV; meanwhile, all these groups were significantly lower than GII and significantly higher than GI. Furthermore, GVI was statistically significantly higher compared to all other groups except GII (it was lower), as illustrated by Table 1. Regarding superoxide dismutase (SOD), it shows a highly significant reduction in the induction group (GII) compared to the control group (GI) [p-value <0.001]. There was no significant difference among groups GIII, GIV, and GV; meanwhile, all these groups had significantly higher levels than GII and significantly lower levels than GI. Furthermore, GVI was statistically significantly lower compared to all other groups except GII (it was higher), as illustrated by Table 1. Regarding elastin level, the mean of elastin was highly significantly reduced in the induction group (GII) compared to the control group (GI) [p-value <0.001]. There was no significant difference between GIII and GIV. Of note, GV and GVI were significantly different from the other groups as well, as illustrated by Table 1. Regarding epidermal thickness, the mean was highly significantly reduced in the induction group (GII) compared to the control group (GI) [p-value <0.001]. There was no significant difference between GIII and GIV. Also, there was no significant difference between GV and GVI, but these groups were significantly different than the other groups, as illustrated by Table 1 and Figures 2–7.

Table 1. Evaluation of interleukin -6, superoxide dismutase, elastin levels and epidermal thickness according to study groups

Groups	IL-6 (Mean ± SD)	SOD (Mean ± SD)	ELS (Mean ± SD)	Epidermal thickness (µm) Mean ± SD
GI: Normal control	16.94±2.53[a]	1200.48±406.47[a]	1,455.96±76.69[a]	25.44±1.73[a]

GII: Induction	113.89±9.52[b]	148.29±26.91[b]	856.62±65.99[b]	12.50±2.42[b]
GIII: vitamin C 100mg/kg with induction	26.90±3.70[c]	833.87±189.17[c]	1,256.37±62.91[c]	21.96±1.98[c]
GIV: vitamin C 100mg/kg after end of induction	27.60±3.50 [c]	834.04±153.64[c]	1,245.48±66.74[c]	20.62±2.19[c]
GV: carvedilol10 mg/kg with induction	26.85±3.44 [c]	764.28±188.58[c]	1,075.86±69.68[d]	17.09±1.55[d]
GVI: carvedilol 10 mg/kg after end of induction	60.89±3.17[d]	456.32±66.98[d]	951.79±60.92[e]	16.87±2.06[d]
p-value	<0.001[***#]	<0.001[***#]	<0.001[***#]	<0.001[***#]

Column with similar letter indicate no significant difference (p-value ≥0.05), while different litters indicate significant difference (p-value<0.05)
[#] One Way ANOVA (Post *hoc* Tukey test)
[***] indicate highly significant difference
SD: standard deviation

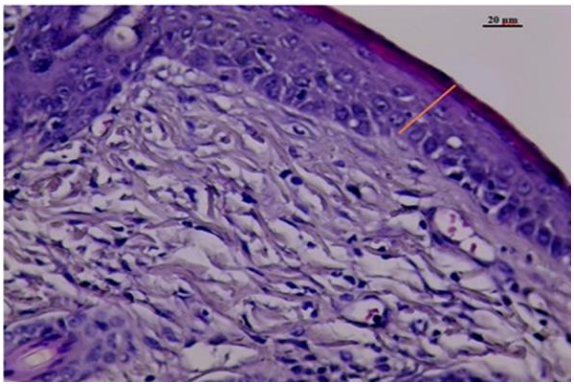

Figure 2. Cross-sections of hematoxylin- and eosin-stained under light microscopy of the dorsal skin in Group I. Thick organ line (—) represent the epidermal thickness (=28.44 µm). (Olympus BX51microscpose, X40).

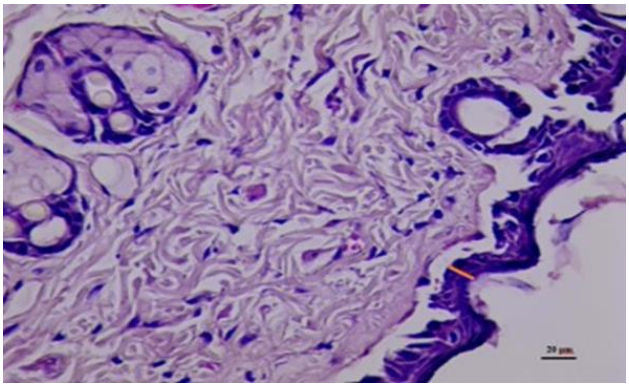

Figure 3. Cross-sections of hematoxylin- and eosin-stained under light microscopy of the dorsal skin in Group II. Thick organ line (—) represent the epidermal thickness (=13.6 µm). (Olympus BX51microscpose, X40).

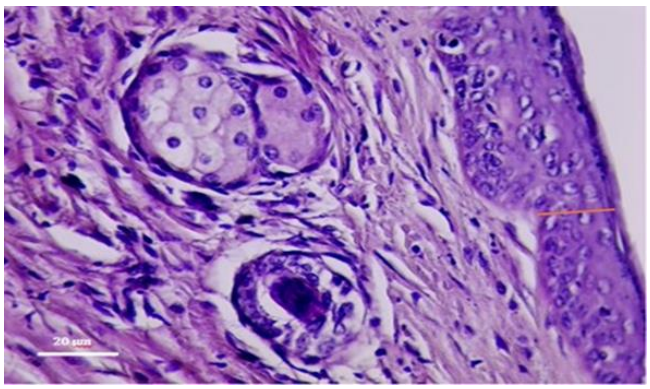

Figure 4. Cross-sections of hematoxylin- and eosin-stained under light microscopy of the dorsal skin in **Group III**. Thick organ line (—) represent the epidermal thickness (=19.2 µm). (Olympus BX51microscpose, *X40*).

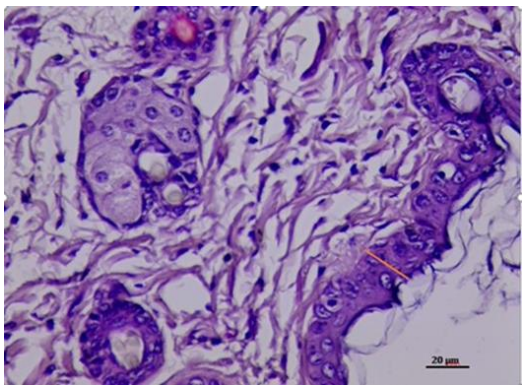

Figure 5. Cross-sections of hematoxylin- and eosin-stained under light microscopy of the dorsal skin in Group IV. Thick organ line (—) represent the epidermal thickness (=20.1 µm). Black line (—) and circle represent thin collagen fibers (Olympus BX51microscpose, X40).

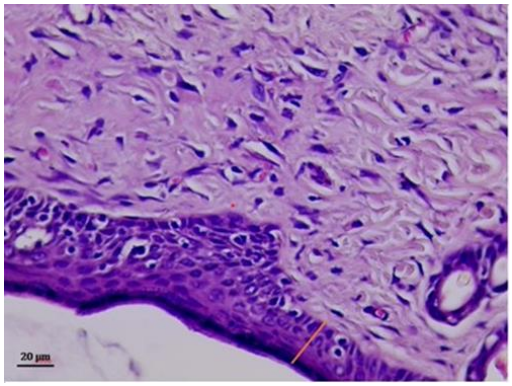

Figure 6. Cross-sections of hematoxylin- and eosin-stained under light microscopy of the dorsal skin in Group V. Thick organ line (—) represent the epidermal thickness (=18.69 µm). (Olympus BX51microscpose, X40).

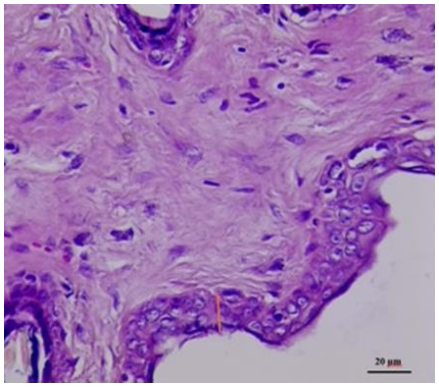

Figure 7. Cross-sections of hematoxylin- and eosin-stained under light microscopy of the dorsal skin in Group VI. Thick organ line (—) represent the epidermal thickness (=17.9 µm). (Olympus BX51microscpose, X40).

3. DISCUSSION

A deregulated immune response that leads to a long-lasting inflammatory state is one of the most important changes that occur with aging. Two examples of dysregulated proinflammatory mediators that are important in the development of chronic inflammation and immunosenescence are cytokines and chemokines. [13]. In the present study IL-6 obtained from heart homogenate content evaluated in different studied groups, as a IL-6 was significantly improved in mice received carvedilol which given with and after induction phase in comparison to induction group. Researchers observed that when given 24 hours after myocardial infarction, carvedilol decreased the protein expression of IL-6, while simultaneously increasing the protein expression of the anti-inflammatory cytokine IL-10 [14]. According to a research done by Zhang et al., which looked at the effects of doxorubicin on the hearts of mice, the IL-6 level in the carvedilol-treated group were significantly lower than those in the doxorubicin-treated group and the control group [15]. From the Mechanistic viewpoint, the action of carvedilol on the adrenergic receptors of cardiomyocytes modulates the generation of cytokines. In mice, -adrenergic stimulation increased cardiac fibroblasts' production of the IL-6 gene family while delaying the activation of cardiac STAT; carvedilol reversed this effect [16]. Furthermore, carvedilol increased levels of interleukin 10 (IL-10), a powerful anti-inflammatory molecule that has been demonstrated to prevent the activation of nuclear factor kappa B. The expression of adhesion molecules and chemokines is likewise suppressed by IL-10, and the generation of reactive oxygen intermediates is decreased in macrophages [17]. Also, a study carried by Alfieri *et al*, showed that in addition to substantial improvement in IL-10 level, there was a decrease in IL-18 (a proinflammatory cytokines). Since increase in IL-18 have potential to exacerbate the proinflammatory response within the myocardium by increasing the production of endothelial cell adhesion molecules and other proinflammatory mediators (such as IL-6), which are implicated in the modulation of myocardium [18].

Oxidative stress causes molecular damage by releasing reactive oxygen species (ROS) that are byproducts of normal cellular metabolism. Antioxidant enzymes that guard against ROS slow down the aging process, which has been linked to the build-up of endogenous oxygen radicals created inside cells and the subsequent oxidative alteration of biological components (lipids, proteins, and nucleic acid) [19]. A study carried by Kumar et al, showed that administration of D-galactose to mice significantly decreased SOD level which restored by carvedilol treatment [20] . the level of SOD in the heart tissue of untreated diabetic rats was decreased markedly in compared with control group, while treatment with carvedilol increase the level of Malondialdehyde (MDA) in compassion to both healthy groups and diabetic groups, even SOD and Glutathione peroxidase (GSH-Px) activities were increased significantly [21]. These studies were in accordance with the current findings. The innovative antioxidant property of carvedilol, found in the carbazole moiety of the molecule, makes it distinct from other beta-blockers drugs. This is just one of several mechanisms that explain why carvedilol has antioxidant activity [22]. Also, the ß$_1$-adrenergic receptor blocking effect of carvedilol is useful for inhibition of catecholamine-induced oxidative stress [23], since the

excessive amounts of circulating catecholamines are oxidized to aminochromes, which are highly reactive quinine compounds that cause subcellular alterations, intracellular Ca2+-overload and myocardial damage. In addition, oxyradicals, which are generate oxidative stress were formed during the oxidation process of catecholamines [24].

Elastin is considered a vital skin protein that give the skin its ability to stretch and contract [25]. Present study shows that given Carvedilol with and after induction showed significantly higher skin level elastin compared to induction group only. Also, mice given carvedilol with galactose showed higher levels when compared to mice given carvedilol after galactose induction for and it was statistically significant for all markers. Till the time of paper preparation, no single study examined the effect of carvedilol on elastin level on skin aging in both animals and humans. But there was a study examined the protective effects of carvedilol on Col in human joint cartilage, shown that pretreatment with carvedilol protects against IL-1β treatment reduction of elastin and reverse it in a dose dependent manner. The effect of carvedilol possibly related to a significant inhibition of both metalloproteinase 1 and -13 expressions in a dose dependent manner [26]. This favorable effect of carvedilol on elastin level can be directed toward their antioxidant properties. Accumulating reactive oxygen species (ROS) from free radicals is widely believed to be a significant cause of skin aging and damages of epidermis and penetrates deeper into the dermis, where it degrades elastin fibres via oxidative stress and activating MMPs [27]. The activation of MAPK is a common consequence of elevated ROS generation. elastin fibres production declines with aging because MAPK activation activates AP-1 (activated protein 1), which in turn raises MMP expression [28]. In the present study the level of SOD (antioxidant enzyme) were significantly increased in mice treated with carvedilol, which in part explain the antiaging effect of drugs on elastin.

Aged skin has thinner epidermis, a flatness of the dermal-epidermal junction, and less keratinocyte activity [29]. The current study is the pioneer in examining the effect of carvedilol on epidermal thickness in accelerated age mice model. One of postulated mechanism of ability of its to increase epidermal thickness, is that carvedilol showed antiapoptotic effect via inhibition of Fas receptor expression [30-32]. Reversing Fas-mediated apoptosis was shown to reverse changes in epidermal aging since it was connected with an increase in epidermal thickness and a decrease in keratinocyte Fas expression as well as apoptosis. [33-35].

4. CONCLUSION

The results of the present study suggested that carvedilol has a substantial anti-aging effect by decreasing oxidative stress, restoring normal levels of inflammatory markers, and postponing the aging of the skin. Although carvedilol's beneficial anti-aging effects are seen when it's administered both before and after the onset of aging, it's most effective when administered at the beginning of the process.

5. MATERIALS AND METHODS

5.1. Chemicals

D-Galactose was supplied as powder preparation by Sigma Aldrich®, USA. Carvedilol was supplied as powder preparation by Hangzhou Hyper Chemicals Limited®, China. Vitamin C was supplied as powder preparation by Hangzhou Hyper Chemicals Limited ®, China.

5.2. Animals

Male mice weighing 20–30 g aged 3-6 months was randomly divided into six groups each consisting of 10 animals. The mice were obtained from the national center for drug control and research, housed in a polypropylene cage under temperature-controlled environment (22±2 °C), with an inverted light-dark cycle (12/12 hours). The animals were maintained on standard pellet diet and free access to water ad libitum supplied by Al-Nahrain University – Biotechnology research center.

5.3. Experimental design of study

Experimental animals were grouped as following: Group I acted as (negative Control) given (normal saline) orally via gastric gavage once daily for six weeks (without D-galactose). Group II acted as (aging Induction model) by giving 500mg/kg of D-galactose orally by gastric gavage once daily for six weeks [36]. Group III (Vitamin C started with induction of aging) (positive control), given 500mg/kg of D-galactose orally by gastric gavage once daily and concomitantly given Vitamin C 100 mg/kg orally by gastric gavage once daily for six weeks duration [36, 37]. Group IV (Vitamin C started directly after ending of aging induction) (positive control), given 500mg/kg of D-galactose orally by gastric gavage once daily for six

weeks [36] and next day after aging induction achieved, Vitamin C 100 mg/kg was given orally by gastric gavage once daily and continued for another six weeks [37]. Group V (Carvedilol started with induction of aging) given 500mg/kg of D-galactose orally by gastric gavage once daily and concomitantly given Carvedilol 10 mg/kg orally by gastric gavage once daily for six weeks [36, 38]. Group VI (carvedilol directly after ending of aging induction) given 500mg/kg of D-galactose orally by gastric gavage once daily for six weeks [36] and next day after aging induction achieved, Carvedilol 10 mg/kg was given orally by gastric gavage once daily for another six weeks [38].

5.4. Induction and assessment of aging in mice

Galactose in a dose of (500 mg/ kg) was given orally by gastric gavage once daily for 6 weeks to 50 male mice to induce aging. Mice were evaluated for signs of aging, which included ruffled fur and a more rounded overall appearance, as well as signs of aging such as skin wrinkles and a lack of vigour and responsiveness. [39].

5.5. Outcome measures

Weight measurement for all mice was done at baseline and before euthanasia. Groups (I,II,III,V) mice were euthanized at the end of their substance administration period which is a total of 6 weeks. Groups (IV,VI) mice were euthanized at the end of their substance administration period which is a total of 12 weeks. The process of euthanasia for all mice was total anesthesia followed by exsanguination (cardiac puncture), a procedure suitable for tissue harvest and conservation [40, 41]. After the end of each group experimental period, dissection has been done for the euthanized mice, and the heart and a piece of 1mm of the dorsal skin area has been harvested. Skin tissue was divided into 2 pieces one piece was used for histopathological analysis, first washed with "Phosphate Buffered Saline (PBS), pH 7.4" Then processed by the traditional processing procedure which is paraffin-embedded method [42]. The other piece of skin tissue together with the heart tissue were isolated and rinsed with cold phosphate buffer saline "PBS, pH 7.4", then the tissue was dried with filter paper and used for ELISA analysis by (ELISA reader, Diagnostic Automation / Cortez Diagnostics®, California, USA) and weighed by sensitive balance. For ELISA each 50 mg of tissue was put in an Eppendorf tube (Eppendorf®, Hamburg, Germany) containing 0.45 ml of chilled PBS and then minced into small pieces. The tube containing the tissue was then put in an ice-containing beaker to keep it cold and then homogenized by the homogenizer machine (Electrical tissue homogenizer, Staruar®, England.), the homogenate was centrifuged for 20 minutes at 4°C and 2000 rpm in a cold centrifuge (Thermos scientific®, USA). The supernatant was isolated using a micropipette (Bioevopeak® , china), and stored at -20°C until the day of analysis [43].

5.6. Biochemical analysis

The resultant stored supernatant of the sampled mice homogenated heart and skin tissues was then thawed and sent for biochemical analysis by double-sandwich ELISA technique for interleukin 6 (il-6) by the kit (Mouse Interleukin 6 (IL-6) ELISA Kit,product ID SL0326Mo, Sunlong biotech®, China) , superoxide dismutase by kit (mouse super oxide dismutase(SOD) ELISA KIT ,ID SL0513Mo, Sunlong biotech®, China) and elastin level by (Mouse Elastin (ELN) ELISA Kit, SL0748Mo, Sunlong biotech®, China).

5.7. Light Microscopy

The thickness of the epidermis was evaluated. Hematoxylin and eosin (HE) were used to stain serial sections, which were then placed on slides coated with silane. A light microscope (Olympus BX51 Microscope, Olympus Corporation®, Japan) was then used to examine the stained sections in greater detail. The vertical epidermal thickness was measured using an ocular micrometre. [29]. One representative image of HE-stained dorsal skin section in each group was presented in part of results [44]

5.8. Ethical approval

The study was approved by the ethical committee of Al-Nahrain University/College of Medicine (Approval no. :20210914, on 9th of December 2021).

5.9. Statistical analysis

In the current study, Statistical Packages for Social Sciences (SPSS) software, version 25 was used to analyze data. The descriptive statistics were reported as mean ± standard deviation (SD). The analysis of variance (one-way ANOVA) test was applied to verify the significance of the difference between the studied

groups, then followed by the post hoc Tukey test. The differences between the groups were considered significant statistically when the P-value was less than 0.05 (P<0.05).

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