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Effects of Vitamin D and Simvastatin on Inflammatory and Oxidative Stress Markers of High-Fat Diet-Induced Obese Rats

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Abstract

Purpose: *This study was aimed to evaluate the antioxidant and anti-inflammatory effects of vitamin D and Simvastatin (SIM) on a high-fat diet (HFD) induced-obese rats.*

Methods*: 40 adult male rats were divided into four groups: control group, HFD, HFD + vitamin D, and HFD + SIM for 14 weeks. Vitamin D or SIM supplementation was done for the last 6 weeks. Vitamin D dosage was 500 IU/kg, while SIM dosage was 10 mg/kg. Interleukin-6 (IL-6) concentration and markers of oxidative stress including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and reduced glutathione(GSH) concentrations in serum were determined using ELISA kits and spectrophotometry methods, respectively.*

Results*: Treatment with vitamin D or SIM could significantly reduce IL-6 and MDA and increases SOD, GPx activities, and GSH levels. Oxidative stress can result not only from increased ROS production but also from dysfunctional antioxidant defenses.*

Conclusion*: From the experimental results, it was observed that SIM and vitamin D could attenuate oxidative stress and inflammation markers associated with obesity.*

1. Introduction

Obesity is a serious problem posing a considerable threat to human health (Ni *et al*., 2020). Obesity is a multifactorial disease caused by chronic positive energy surplus or secondary to genetic, hypothalamic, and endocrine diseases (Vallgårda *et al*., 2017). Obesity is characterized by excessive fat deposition into adipose tissue and non-adipose tissues (Kelishadi *et al*., 2017). Another characteristic feature coupled with obesity is the alteration of the redox state accompanied by metabolic risk factors (Warolin *et al*., 2014). Obesity and its related metabolic disorders are also strongly linked to oxidative stress. This oxidative status is closely associated with pro-inflammatory cytokine secretion, which can initiate oxidative stress in a continuous circle (Biswas, 2016). As a result, systemic oxidative stress and inflammation are critical factors in the pathogenesis of obesity-related diseases (Crujeiras *et al*., 2013).

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Several strategies have been developed to modulate oxidative stress and inflammation associated with obesity, which aims to decrease disease risk factors, including weight loss, physical activity, and dietary intervention (Bigornia *et al*., 2010). Previous studies have recommended that at least a 10% reduction of the total body weight could alleviate pro-inflammatory and oxidative stress parameters (Ford, 2002; Church *et al*., 2011). However, this weight reduction strategy may not be suitable for the general population (Bruun *et al*., 2006; Kelly *et al*., 2007).

Other studies have examined dietary intervention efficacy, including changes in macronutrient intakes and caloric restrictions in the management of oxidative stress and inflammation (Huang *et al*., 2015). However, one of the most effective dietary interventions is vitamin D. Vitamin D, a non-enzymatic antioxidant, has been defined as a potent preventive and therapeutic agent for the obese (Moukayed & Grant2019). Moreover, vitamin D is well known for its potential role in managing obesity-induced oxidative stress Reid & Li, (2001).due to its significant role in decreasing lipid hydroperoxide Garcion *et al*. (1999) and increasing total antioxidant status (TAS) and oxidative capacity in monocytes (Wu *et al*., 2011), as well as its anti-inflammatory effect (Gode *et al*., 2016).

Another strategy to attenuate inflammation and oxidative stress depend on using lipid-lowering agents such as statins (Sahid *et al*., 2017; Rodrigues *et al*., 2019). Simvastatin, a member of the statin group, inhibits rate-limiting enzymes in cholesterol biosynthesis. Recently, Simvastatin has attracted more attention due to its non-lipid properties. Hence, it could modulate inflammatory and oxidative stress cascades induced during obesity through its pleiotropic effects besides its lipid-lowering properties (Csonka *et al*., 2016).

This study aimed to evaluate the effect of HFD on inflammation and oxidative stress induction in serum of adult male Wistar rats. Moreover, to examine the impact of vitamin D and Simvastatin administration in ameliorating oxidative stress and inflammation in these animals.

2. Methodology and Procedures

Animals

Forty adult male Wistar rats (8 weeks of age), weighing about 145-165 g, supplied by the Animal House of the Research Institute of Ophthalmology, Giza, Egypt. The animals were individually housed in stainless-steel cages under conventional controlled conditions (12-h light/dark cycle with a temperature of 24 ± 1°C and relative humidity of 50 ± 10%), fed with a standard laboratory pellet diet, and had free access to distilled water *ad libitum*. At the end of the first week, the animals were randomly divided into four groups: (1) control group (Control n=10, fed with standard diet), (2) HFD group (HFD n=10 was fed with HFD), (3) vitamin D group (vitamin D n=10, fed with HFD and supplemented with vitamin D 500IU/kg/day at the last 6 weeks), (4) SIM group (SIM n=10 fed with HFD and treated with SIM 10 mg/kg/day). All four groups were given their respective treatments for 14 weeks (Fig.1). In addition, vitamin D and SIM were supplemented for the last 6 weeks by oral gavage. All experimental animal protocols

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were carried out according to the standards of the Guide for the Care and Use of Laboratory Animals and approved according to the Animal Experimentation Ethics Committee of Animal Research of the Research Institute of Ophthalmology, Giza, Egypt. The composition of the experimental diet (G/Kg diet) was according to the formula (Sobesky *et al*., 2014)

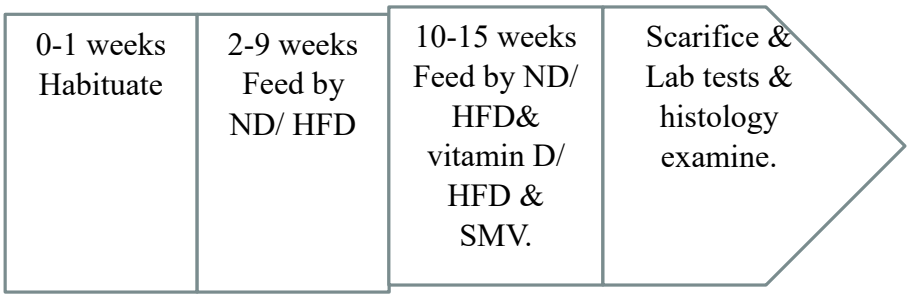

Figure 1: Overview of the design of the experiment

Diets and drugs

Standard diet and HFD were purchased from El-Gomhorya Company, Cairo, Egypt. HFD was preserved at 4°C until used. SIM (Zocor 10 mg) was purchased from Merck sharp & Dohme Ltd., UK. Vitamin D (Calcitriol 500 IU) was purchased from Sigma Aldrich, USA.

Sample collection

At the end of the experimental period (98 days), the animals were weighed and anesthetized by diethyl ether after overnight fasting. The blood samples were collected through the retro-orbital venous plexus. The blood samples used for serum assessment were collected without using an anticoagulant. First, the blood was allowed to clot for 30 min at 25° C. The samples were then centrifuged at 4,000 rpm for 15 min at 4° C. After that, the supernatants were stored at -80° C for further assessment, except IL-6 samples were stored at -20° C to avoid loss of bioactive rat IL-6.

Malondialdehyde assessment

Malondialdehyde levels were measured using the thiobarbituric acid reactive substances (TBARS) method (Ohkawa *et al*., 1979).

Glutathione peroxidase and superoxide dismutase assessment

SOD was assayed by a spectrophotometric method based on the inhibition of a superoxide-induced reduced nicotinamide adenine dinucleotide (NADH) oxidation according to Paoletti *et al*. method Boitard *et al*., (2014) by using Ransod, Randox kit (UK). GPx activity was measured according to Paglia and Valentine method Erion *et al*. (2014). using Ransel, Randox kit (UK).

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Reduced glutathione assessment

GSH was measured using the colorimetric method (Beutler *et al*., 1963) of Beutler *et al*. using BioAssay Systems (USA) kit.

Assessment of interleukin-6

IL-6 activity was measured according to the ELISA method of Engvall and Perlmann (Engvall & Perlmann, 1971). using a ThermoFisher kit (USA).

Statistical analysis

All statistical analyses were performed using SPSS software, version 20. Data were analyzed using one-way analysis of variance (ANOVA) followed by Post hoc-least significant difference (LSD). Data were expressed as the mean ± SD. $P < 0.05$ was considered statistically significant. All charts were performed using OriginPro for data analysis and graphing software (version 2018).

3. Results and Discussion

Change of MDA levels in the studied groups

According to our results (Fig.2), MDA levels were significantly increased in the HFD group. However, both vitamin D and SIM groups showed a marked depletion compared to the HFD group. Moreover, a significant decrease was detected in the SIM group compared to the vitamin D group.

Change of SOD and GPx activities in the studied groups

A notable decrease in SOD activities in the HFD group compared to the control group. Conversely, a significant rise was detected in vitamin D and SIM groups versus the HFD group; also, an increase was detected in the SIM group compared to the HFD group and (Fig.2). The current study revealed a significant upwards in GPx in the HFD group compared to the control group. In contrast, a marked decline was detected in the vitamin D and SIM groups compared to the HFD group. However, a non-significant decrease was detected between the two groups (Fig.2).

Change of GSH levels in the studied groups

Results from the current study showed a significant decrease in the HFD group compared to the control group in serum GSH levels. A further marked decline in GSH levels was detected in both vitamin D and SIM groups compared to the HFD group. However, there was a significant rise in the SIM group compared to the vitamin D group (Fig.2).

Change of IL-6 levels in the studied groups

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A significant rise was detected in IL-6 levels in the HFD group compared to the control group. A remarkable drop was detected in both vitamin D and SIM groups compared to the HFD group. However, the SIM group showed a significant decrease compared to the vitamin D group (Fig.2).

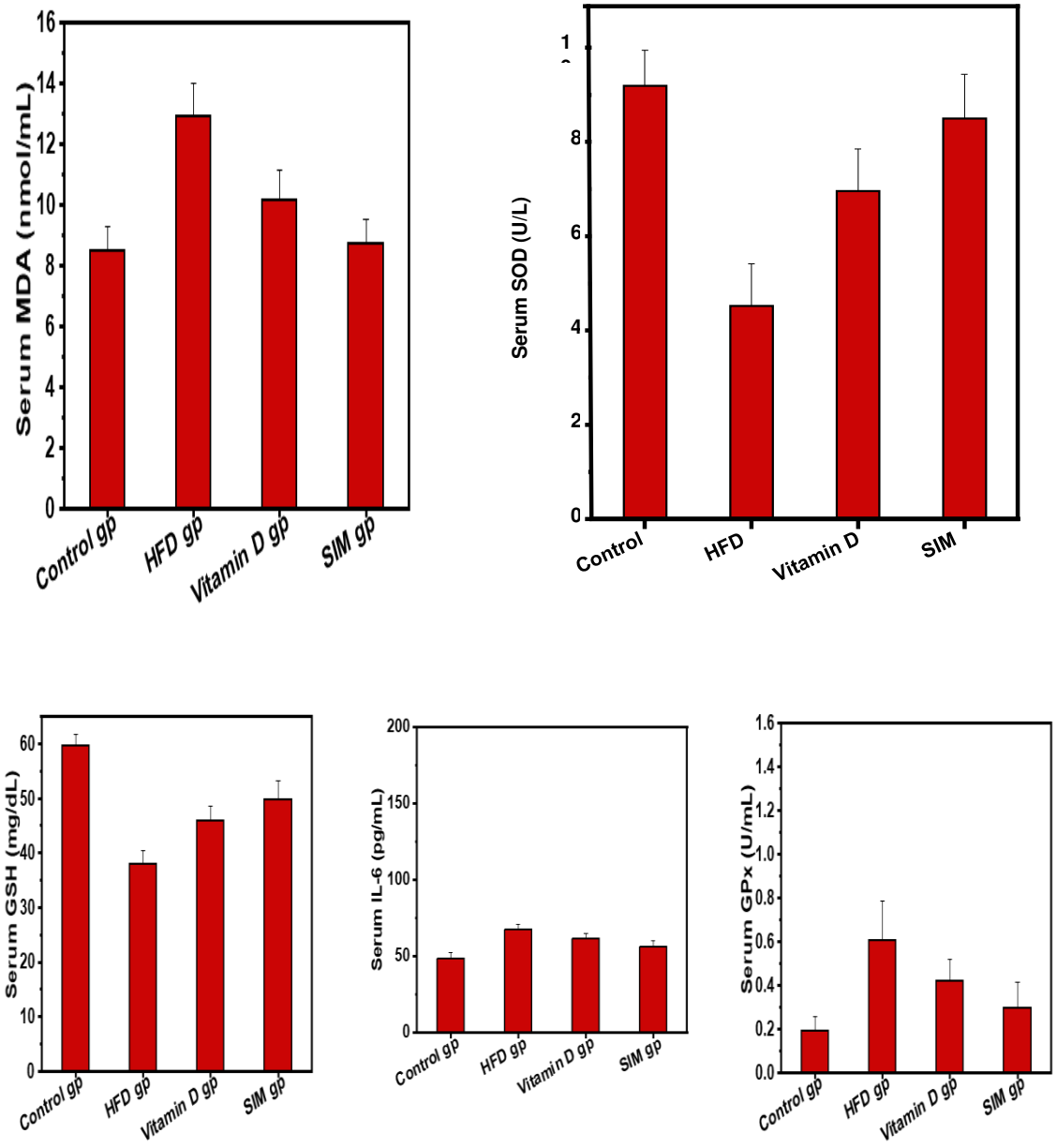

Figure 2: Effect of vitamin D and SIM on parameters of oxidative stress and IL-6 in obese male Wistar rats.

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Obesity is a real pathology as it represents a risk factor for various metabolic diseases (De Lorenzo *et al*., 2019). Regardless of whether obesity is a condition or a disease, it arises from multiple etiologic determinants, either acquired or inherited (d'Autume *et al*., 2012). These determinants favor positive energy balance and weight gain (Hall *et al*., 2009; Popkin & Hawkes, 2016). Feeding (HFD) to rats was proved to be a valuable model of the putative effects of dietary fat in humans(López *et al*., 2003). Therefore, rat models are valuable tools for inducing obesity as they will readily gain weight when fed high-fat diets (Von Diemen *et al*., 2006).

Oxidative stress is an imbalance between tissue free radicals, reactive oxygen species (ROS), and antioxidants (Fernández-Sánchez *et al*., 2011). Lipid peroxidation is the first step of cellular membrane damage (Repetto *et al*., 2012). It constitutes a complex chain reaction of free radicals that leads to degradation of polyunsaturated fatty acids (PUFAs) in the cell membrane (Halliwell & Gutteridge, (1984) and in turn, profound alternations of the cell membrane structure and function (van Ginkel & Sevanian, 1994). MDA is one of the end-products of the lipid peroxidation process. Thus, increased MDA level is a crucial indicator of lipid peroxidation and oxidative status (Ayala *et al*., 2014).

The results of the current study were supported by Mahmoudi *et al.,* which demonstrated that the elevation of serum MDA levels is an indicator of lipid damage due to obesity development (Mahmoudi *et al*., 2019). Also, reduction of MDA levels after vitamin D supplementation was matched by Mostafa *et al*., which elucidated vitamin D's significant role in ameliorating lipid peroxidation levels and, in turn, inhibiting HFD-induced oxidative stress in obese males Wistar rats (Mostafa *et al*., 2016). Furthermore, MDA levels were found to be markedly decreased, reaching almost the control level in the SIM group. These results agreed that Eweda *et al.* proved that SIM could prevent lipid peroxidation and ameliorate oxidative stress induced by the HFD (Eweda *et al*., 2018)

Furthermore, oxidative stress can result not only from increased ROS production but also from dysfunctional antioxidant defenses. SOD is the first defense against ROS toxicity (Fang *et al*., 2013) as it catalyzes superoxide radicals dismutation to the less toxic hydrogen peroxide (H_2O_2) (McCord, 1987). Also, GPx, a selenoenzyme, plays a significant role in H_2O_2 and hydroperoxide reduction to non-toxic products (Freeman, & Crapo, 1982). It also has a predominant role in the redox cycling of the reduced glutathione (GSH) to the oxidized glutathione (GSSG), necessary to maintain cells' thiol content to maintain the cell's redox state (Agrawal *et al*., 2014). Therefore, the decrease in SOD and GPx activities could be ascribed to the exhaustion of these enzymes in fighting free radicals generated during the development of obesity (Das *et al*., 2013; Noeman *et al*., 2011).

On the other hand, the antioxidant effect of vitamin D due to its ability to decrease superoxide anion formation; hence these enzymes will not be exhausted in encountering the radicals after vitamin D supplementation. Thus the concentration of SOD and GPx were up-regulated (Polidoro *et al*., 2013). Furthermore, Liang *et al*. and Pan *et al*. proved the enhanced expression of SOD and GPx activities after SIM treatment (Liang *et al*., 2020; Pan *et al*., 2016).

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Moreover, the thiol-based antioxidant system contributes a second line of cellular defense against ROS-mediated oxidative damage (Nazima *et al*., 2014). GSH is one of the significant intracellular non-enzymatic antioxidants and the most abundant component of an endogenous cellular "redox buffer" (Kurutas, 2015). The significant decrease could be owing to increased utilization of GSH by cells either as a scavenger of free radicals (Deng *et al*., 2019). Vitamin D increases GSH levels through the upstream regulation of glutathione reductase (GR) gene expression, a crucial enzyme involved in the synthesis of GSH (Kanikarla-Marie & Jain, 2016). Also, This upward trend of GSH levels after SIM administration was confirmed by (Feng *et al*., 2015).

Reactive oxygen species also activate NF-κB, a redox-sensitive transcription factor that acts as a master regulator of the inflammatory response (Uciechowski & Dempke, 2020). It is required to induce many inflammatory genes, including those encoding IL-6 (Wang *et al*., 2014) which could explain the increased levels of IL-6 during obesity. IL-6 is a member of the pro-inflammatory cytokine family, which induces various proteins expression responsible for acute inflammation (Uciechowski & Dempke, 2020). Decrease IL-6 levels in vitamin D groups was matched with Kim *et al*., which confirmed vitamin D impact in the downregulation of IL-6 secretion (Kim *et al*., 2020). Also, Nicholls *et al.* illustrated that statins could suppress the modification of proteins by myeloperoxidase-catalyzed reactive nitrogen species. Thus, these species have been demonstrated to promote multiple inflammatory pathways (Nicholls *et al*., 2006).

4. Conclusion and Suggestion

The previous biochemical investigations confirmed the therapeutic effect of vitamin D and Simvastatin against the deleterious effect of oxidative stress and inflammation induced by HFD, which could reduce the risk of obesity-related disease.

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