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Research Article

DUNALIELLA SALINA IMPROVED OBESITY-ASSOCIATED INFLAMMATION AND OXIDATIVE DAMAGE IN ANIMALS' RODENT

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ABSTRACT

Objective: The objective of this study is to investigate the efficacy of microalgae *Dunaliella salina* to improve apelin, oxidative damage, inflammatory, and apoptotic function implicated in high-fat diet (HFD)-induced obesity in rats.

Methods: Fifty male Westar albino rats weighing 150–160 g were fed on HFD for 12 weeks. Treatment of obese rats with *D. salina* was carried out in a dose 150 mg/kg body weight as compared to orlistat as anti-obesity standard drug. Blood nuclear factor kappa-B cells (NF-kB), apelin, apoptosis regulator (B-cell lymphoma 2 [BCl2]), monocytes chemoattractan protein-1, paraoxonase-1 (PON1) were determined in serum of different groups. Besides, lipid peroxidation (malondialdehyde [MDA]), glutathione (GSH) levels as well as histopathological examination were investigated in liver tissue of obese rats.

Results: Serum apelin, MDA, and NF-kB levels were significantly high, reached to 97.25, 158.18, 511.433, and 170.73%, respectively. While significant decrease in PON1 (47.82%), BCl2 (74.88%), and GSH (63.54 %), levels were detected in the obese rats compared to controls. Obvious improvement in all biomarkers under investigation upon treated obese rats with ethanol extract of *D. salina*. Histopathological examination of obese hepatic tissue showed dilatation in the central portal veins associated with inflammatory cells infiltration in the portal area and congestion. However, treatment of obese rats with *D. salina* confirmed biochemical analysis and declared less diffuse inflammatory cells infiltration as well as less focal infiltration in both hepatic parenchyma and portal area with higher improvement in *D. salina* than drug.

Conclusion: It could be concluded that *D. salina* has a great ability to improve inflammation associated with obesity as well as damaged hepatic architectures, hence it can be used as a promising anti-obesity nutraceuticals.

Keywords: Apelin, Adiponectin, Obesity, Nuclear factor kappa-B, Monocytes chemoattractant protein-1 – B-cell lymphoma 2, Paraoxonase-1, *Dunaliella salina.*

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INTRODUCTION

Obesity is considered to be major common health problem associated with low grade of inflammatory state [1,2]. Considerable evidence suggested the inflammatory response to dietary fat is mediated by toll-like receptor (TLR) signaling, which result in the activation of nuclear factor kappa-B (NF-kB) and production of inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNF-α) interferon, growth factors, cell adhesion molecules, immune receptors, monocytes chemoattractant protein-1 (MCP-1), and stress proteins [3].

It has been known that oxidative stress in obese subjects is a principle factor to the pathogenesis of obesity-linked with metabolic disorders. Adipocytes secreted apelin which is an adipocytokine. Apelin is well recognized for its anti-obesity and antidiabetic characteristic. Apelin reduced the generation of reactive oxygen species (ROS) in adipocytes, through apelin receptor (APJ) interaction. This is further documented by the knows that apelin elicits enhancement of antioxidant enzymes through pathways of MAPK kinase/extracellular signal-regulated kinase (ERK) and AMPK and decreases prooxidant enzyme production through AMPK pathway [4].

Obesity is connected with oxidative stress [5] which is a principle factor-associated obesity and other metabolic diseases [6]. Adipose tissue regulates homeostasis of metabolism through the release of different metabolic factors and adipocytokines. It was found that fat accumulation in adipocytes increases in parallel with the production of ROS [4]. In addition, ROS overproduction destroys functions of adipocyte. It, for examples, damages glucose uptake by insulin in adipocytes and elicits adipogenesis and lipolysis, resulting in free fatty acids overproduction [4]. Furthermore, overproduction of ROS in adipose tissue causes suppression of antioxidative enzymes, promotes NADPH oxidase expression, and abnormality in the regulation of adipocytokines expression (e.g., adiponectin and IL-6) [4].

It is well recognized that the activity of paraoxonase-1 (PON1) in serum is differ related to PON1 consumption to prevent oxidation [7]. Inhibition activity of PON1 in serum is considered as a risk factor for hypercholesterolemia [8], obesity, and Type 2 diabetes [9].

Apoptosis is considered as one of the most important features of obesity and non-alcoholic fatty liver (NAFLD) [10]. It has been demonstrated that Bax and caspase-3 were induced by cholesterol [11] which may be a principle apoptotic proteins; however, expression of p53 and B-cell lymphoma 2 (BCL2) in steatotic cells did not elevate by cholesterol, speculating critical effect for the mechanisms of cell death in hepatocytes [12].

On the other hand, microalgae are considered as a rich source of carotenoids [13]. In addition, up to 0.2% of carotenoids were constituted microalgae (Chlorophyceae family), with the highest content of β-carotene in *Dunaliella* (up to 1% dry weight). Hence, they are not only rich purified compounds source but also powerful functional foods that are currently being demonstrated as chemopreventive nutraceuticals against inflammatory and carcinogenic diseases [14].

β-carotene showed antioxidant and anticancer properties which are attributed to its retinol (Vitamin A) [13]. Retinoids have a wide range of biological activities and can improve several processes such as reproduction, embryogenesis, growth, differentiation, proliferation, apoptosis, vision, bone formation, metabolism, hematopoiesis, and immunological processes [13].

B-carotene as an anti-inflammatory activity has been demonstrated in many models *in vitro* and *in vivo* [15]. Although β-carotene could act preventively on intestinal inflammatory diseases. The efficacy was attributed to different molecular target attenuation, such as NF-kB, COX-2, and matrix metalloproteinase 9 (MMP-9). *Dunaliella bardawil* was used against bowel inflammation induced by acetic acid [13]. β-carotene rich in *D. salina* exhibited the growth inhibition activity and proapoptotic effects on human colon cancer cell lines, many types of cancer, and degenerative diseases *in vitro* and *in vivo* [13] probably due to their antioxidant and anti-inflammatory activities [16].

Hence, the present study is designed to evaluate the role of *D. salina* in improving oxidative damage, inflammatory, and antiapoptotic biomarkers associated with high-fat diet (HFD)-induced obesity in rats.

METHODS

Cultivation of *D. salina*

The organism was grown in conical flask 5 L containing BG11 nutrient media according to Stanier *et al*. as shown in Table 1 [17].

One ml of the micronutrient solution was added to the culture medium. After autoclaving and cooling, pH of medium is about 7. The culture was harvested by centrifugation, dried at 40°C, and then grounded into homogeneous fine powder.

Ethanolic extract preparation of *D. salina*

For the preparation of the ethanolic extract, 100 g of *D. salina* powder was soaked in ethanol (80%) and shacked on shaker (Heidolph UNIMAX 2010) for 48 h at 150 rpm. The extract was filtered using a Buchner funnel and Whatman No. 4 filter paper, and the algal residue was reextracted with the addition of fresh 80% ethanol for another 2 times. Combined filtrates were concentrated using rotary evaporator (Heidolph, Germany) at 40°C under vacuum. The resulting dry extract was evaporated on a rotary vacuum evaporator to dryness. The dry extract was stored at −20°C in a freeze and kept for further analysis [18].

Biological assay

Experimental animals

Male albino rats (n=50) weighted 150±20 g were obtained from the Animal House of the National Research Center (NRC). Animals were quarantined and allowed to acclimate for 10 days before beginning experimentation. They were housed 10/cage under temperature-controlled environment (26–29°C) with a fixed light/dark cycle with free access to water and food. All procedures of the present study were performed according to the Ethical Committee of the NRC, Egypt, provided that the animals will not suffer at any stage of the experiment.

Table 1: BG11 nutrient composition

Macronutrient	g/L
$NaNO_3$	1.5
K2HPO4#	0.04
MgSO4.7H2O	0.075
CaCl2.2H2O	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (disodium magnesium salt)	0.001
Na2CO3	0.02
Micronutrient	g/L
H3BO3	2.86
MnCl2.4H2O	1.81
ZnSO4.7H2O	0.222
Na2MoO4.2H2O	0.39
CuSO4.5H2O	0.079
Co (NO3) 2.6H2O	0.0494

Induction of obesity in rats

Obesity was induced in rats according to the method of Adaramoye *et al*. [19] by feeding rats' HFD (lard), cholesterol was orally administrated at a dose of 30 mg/0.3 ml olive oil/1 kg animal 5 times a week for 12 consecutive weeks, lard fat was mixed with normal diet (ND) (1 kg of animal lard was added to 5 kg of ND), and the occurrence of obesity was determined by measuring body weight gain percentages and visceral and fecal fat percentages.

Doses and routes of administration

Obese rats received an oral dose of 2 mg/kg body weight dissolved in distilled water of the anti-obesity reference drug, orlistat (12 mg\Kg body weight) for 6 weeks [20]. Obese rats treated with orally administered 150 mg/Kg body weight of *D. salina* ethanolic extract for 6 weeks [21].

Assay of paraoxonase activity

The rate of paraoxon hydrolysis was monitored the increase of absorbance at 405 nm and 25°C. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM CaCl2 in 0.05 M glycine buffer, pH 10.5. 1 unit (IU) of PON activity is defined as 1 μmol of p-nitrophenol formed per min, and activity was expressed as U/l of serum [22].

Determination of MCP-1 protein

MCP-1 protein levels in serum were measured using a solid-phase sandwich enzyme-linked immunosorbent assay (MCP-1 Quantikine enzyme-linked immunosorbent [ELISA] kit, R&D systems, Abingdon, UK). Briefly, 100 μl of duplicated samples or standards (recombinant human MCP-1) were incubated (2 h at room temperature) in the wells precoated with the primary antihuman MCP-1 antibody. After incubation, wells were washed 3 times and horseradish peroxidase-conjugated polyclonal antibodies against MCP-1 were added (for 2 h at room temperature). Finally, tetramethylbenzidine substrate solution was applied for 30min, and after stopping the reactions by 2 M sulfuric acid, the absorbance was measured at 450 nm (with correction at 540 nm). The data were evaluated with KIM-E software (USOL, Prague, Czech Republic); the detection limit of the MCP-1 assay was 5.0 Pg/ml.

Quantification of NF-kB

NF-kB was measured in serum using commercially available human NF-kB ELISA kit (Glory Science Co., Ltd., Del Rio, TX, USA) according to manufacturer instructions. The kit uses a double antibody sandwich ELISA assay to assay the level of NF-kB. The detection range of the kit is 100–2000 U/l.

Determination of lipid peroxide

Lipid peroxidation was assayed in liver tissue homogenate by measuring the thiobarbituric acid-reacting substances as previously described by Ruiz-Larrea *et al*. [23] and Prijant *et al.* [24] in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red-colored complex having peak absorbance at 532 nm (using ultraviolet-VI8 recording spectrophotometer, Shimadzu Corporation, Australia).

Determination of BCL2

The level of serum BCL2 was determined by double-antibody sandwich enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (Biosystems, Egypt,).

Apelin-12

Serum apelin-12 levels were determined by ELISA (apelin-12 ELISA kit, Phoenix Pharmaceuticals, Belmont, CA, USA) (sensitivity: [Minimum detectable concentration] = 0.15–0.25 ng/ml, intra-CV: 5%, and inter-CV: 14%).

Marker for antioxidant
Glutathione (GSH) was demonstrated using 5, 5′-dithiobis 2-nitrobenzoic acid acid in phosphate buffer and the formed color was measured at 412 nm as described by El-Baz *et al*. [25]

Experimental design
Fifty male Wistar albino rats (5–6 weeks-old) weighing at 155.00±5.00 g (mean ± standard deviation [SD]) (weight of rats on the day received from supplier) after adaptation period to the environment., the rats were randomly divided into five groups (n=10/group) as follows:

- Group (1): Control rats received normal diet (ND)
- Group (2): Control rats administered ND and treated with 150 mg/g body weight of ethanolic extract of *D. salina*
- Group(3): HFD treated rats for 12 weeks
- Group (4): Obese rats treated for 6 weeks with 150 mg/kg body weight of ethanolic extract of *D. salina* (HFD/DS)
- Group (5): Obese rats treated for 6 weeks with anti-obesity standard drug orlistat (12 mg/kg body weight) (HFD/odd ratio). Health conditions of all rats were monitored daily and no adverse events were observed throughout the study. At the beginning of the experiments, the weights of all rats were recorded at 155.00±5.00 g (mean ± SD) (weight of rats after 10 days of acclimatization). All experiments and biochemical analysis were conducted using 50 rats with triplicate measurements. The permission to conduct this study was according to the ethics of NRC, Egypt.

Blood sample
Blood samples were obtained following an overnight fasting state at the end of treatment (week 12) at 8 a.m. Samples were withdrawn from a cubital vein into blood tubes under diethyl ether anesthesia and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min, and they were stored until analyzing at −80°C [26]. After 12 and 18 weeks of treatment, all the rats were sacrificed and the liver was removed for biochemical analysis of antioxidant. Part of the liver was fixed in formalin (10%), for histopathological examination.

Homogenization of hepatic tissue
Homogenization of hepatic tissue was carried out using saline solution (1:10 w/v) for the determination of lipid peroxide (malondialdehyde [MDA]) and GSH.

Histological investigation
Liver tissue slices were fixed in 10% buffer formalin. After fixation, paraffin 4 pm hick sections were taken and stained by hematoxylin and eosin (H&E ×200) and examined microscopically [27].

Statistical analysis
We compared the data between the different groups using the SPSS computer program version 8 coupled with a costate computer program, where unshared letters are statistically significant at p≤0.05.

RESULTS

The present results demonstrated an insignificant change in control rats treated with *D. salina* as compared to control, while obese rats showed significant increase in NF-kB and MCP-1 levels upon treated obese rats with ethanolic extract of *D. salina* with percentages 511.433 and 170.73%, respectively, as compared to control. Whereas, a significant decrease in BCl2 reached to 74.88%. Marked amelioration in NF-kB, BCl2, and MCP-1 levels in obese rats treated with *D. salina* with percentages of amelioration 378.44, 36.16, and 112.19%, respectively, compared to reference drug (318.47, 38.65 and 63.41 respectively). (Table 2).

In addition, there was an insignificant change in apelin, PON, MDA, and GSH levels in control rats treated with *D. salina* as compared to untreated control one. However, obese rats demonstrated significant increase in apelin and MDA levels with percentages 97.25 and 158.18%, respectively, while there was a significant reduction in PON1 activity and GSH level with percentages 47.82 and 63.54%, respectively, as compared to control. Medication of obese rats with *D. salina* demonstrated marked improvement in apelin, PON1, MDA, and GSH levels with percentages 67.50, 52.17, 127.27, and 41.14%, respectively, compared to standard drug (58.78, 29.57, 114.55, and 16.83%, respectively) (Table 3).

Histopathological examination
Histopathological examination of control rats showed no histopathological alteration and normal histological structure of the central vein and surrounding hepatocytes in the parenchyma (Fig. 1). Furthermore, control rats treated with *D. salina* showed no histopathological alteration (Fig. 2). However, photomicrograph of obese rats fed HFD showed dilatation in the central portal veins associated with inflammatory cells infiltration in the portal area and congestion (Figs. 3a and 3b). Photomicrograph of obese rats treated with *D. salina* and orlistat standard drug showed less diffuse inflammatory cell infiltration as well as less focal infiltration in both hepatic parenchyma and portal area with higher improvement in *D. salina* than drug (Figs.4a and 4b).

DISCUSSION

It was suggested that the enhanced hypothalamic TLR4/NF-kB and the release of inflammatory cytokines were incorporated in the imperfect food intake after dietary fat exposure [28]. In the present study, we found that NF-kB NF-kB was significantly higher in obese rats induced by HFD compared with normal control rats. Similar results were found

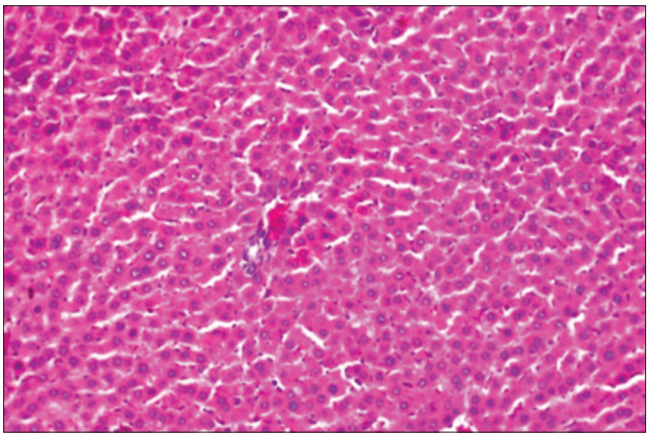

Fig. 1: Photomicrograph of control rats showed no histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes in the parenchyma (H and E ×200)

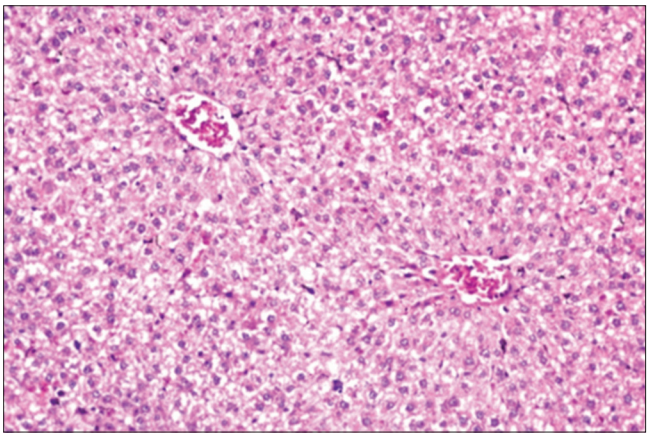

Fig. 2: Photomicrograph of control rats treated with *Dunaliella salina* and showed that there was no histopathological alterations (H and E ×200)

Table 2: Effect of *D. salina* on inflammatory and apoptotic markers in obese rats

Biomarkers	Groups				
	Control/ND	Control/ND/DS	HFD	HFD/DS	HFD/OR
NF-kB (U/l)	[a]52.50±5.00	[a]48.00±8.01	[b]321.00±21.9051	[c]122.32±2.90	[d]153.80±4.78
% change		−8.57	1.43	132.99	192.95
% improvement				378.44	318.47
BCl2 (µg/l)	[f]13.22±0.16	[f]13.00±2.00	[e]3.32±0.21	[a]8.10±0.34	[a]8.43±1.14
% change		1.66	74.88	38.73	36.23
% improvement				36.16	38.65
MCP-1 (Pg/ml)	[a]8.20±0.54	[a]8.00±2.00	[h]22.20±2.00	[c]13.00±0.99	[d]17.00±0.79
% change		2.40	170.73	58.54	107.31
% improvement				112.19	63.41

ND: Normal diet, ND/DS: Rats feed ND and treated orally with *D. salina* extract for 4 weeks. HFD/DS: Rats feed with HFD for 12 weeks and treated orally with *D. salina* for 4 weeks post-induction. FHD/OR: Rats feed HFD and treated orally for 4 weeks with standard drug orlistat. Statistical analysis is carried out using the SPSS computer program, combined with a costate computer program, where unshared letter is statistically significant at P≤0.05. NF-kB: Nuclear factor kappa-B, MCP-1: Monocytes chemoattractant protein-1, BCl2: B-cell lymphoma 2, HFD: High-fat diet, *D. salina: Dunaliella salina*, OR: Orlistat

Table 3: Effect of *D. salina* on apelin, antioxidant, oxidative, and apoptotic biomarkersin obese rats

Biomarkers	Groups				
	Control/ND	Control/ND/DS	HFD	HFD/DS	HFD/OR
Apelin(ng/l)	[k]400.00±23.90	[k]378.00±20.90	[l]89.00±21.89	[h]19.00±9.80	[d]553.86±11.00
% change		5.50	97.25	29.75	38.47
% improvement				67.5	58.78
PON-1 (kU/l)	[i]230.00±11.90	[i]227.00±10.00	[f]120.00±4.88	[j]200.00±3.80	[a]188.00±11.10
% change		1.30	47.82	34.78	18.26
% improvement				52.17	29.57
MDA (umol/mg protein)	[a]0.55±0.16	[a]0.45±0.06	[h]1.42±0.20	[g]0.72±0.08	[g]0.79±0.09
% change		18.18	158.18	30.90	43.64
% improvement				127.27	114.55
GSH (ug/mg protein)	[a]48.79±5.21	[a]55.70±5.21	[b]17.79±1.20	[c]37.88±0.90	[d]26.00±0.32
% change		14.16	63.54	22.36	46.71
% improvement				41.18	16.83

ND: Normal diet, ND/DS: Rats feed ND and treated orally with *D. salina* extract for 4 weeks. HFD/DS: Rats feed with HFD for 12 weeks and treated orally with *D. salina* for 4 weeks post-induction. FHD/OR: Rats feed HFD and treated orally for 4 weeks with standard drug orlistat. Statistical analysis is carried out using the SPSS computer program, combined with a costate computer program, where unshared letter is statistically significant at P≤0.05. PON-1: Paraoxonase-1, MDA: Malondialdehyde, GSH: Glutathione, HFD: High-fat diet, *D. salina: Dunaliella salina*, OR: Orlistat

by Zhang *et al*. [29]. It was found that inflammation of hypothalamus post-chronic consumption of HFD could not only make changes in feeding and body weight but also influence body weight-independent state to initiate systemic glucose intolerance, and the mechanism illustrated central leptin and insulin resistance induction [3].

Further, NF-kB is activated by oxidative stress, bacterial endotoxin, and cytokines. It induces liver damage related to different agents and pathogens [30], besides enhancement of NF-kB is consider a principle marker in the acute liver failure [30]. NF-kB and TNF-α roles are act as inflammatory response mediators in steatohepatitis nutritional model [31]. In obese condition, the pathways of pro-inflammatory response could initiate from stressed hepatocytes through NF-kB activation. Alternatively, NF-kB -activated Kupffer cells produced TNF-α, IL-1β, and other cytokines which could enhance NF-kB in neighboring hepatocytes [31].

In obese condition, excess fat lipotoxic effects may stimulate TNF-α production. Once produced, NF-kB/TNF-α cycle becomes self-maintaining [32]. It likely that TNF-α chronic exposure induces inflammatory cells accumulation in the liver, promoting hepatocytes damaging factors produced from activated monocytes [32]. This is indicated in the current results in the histopathological examination which revealed dilatation in the central portal veins associated with inflammatory cells infiltration in the portal area and congestion (Figs. 3a and 3b).

The present results revealed a significant decrease in BCL2 in obese rats which is run in parallel with the study of Briscini *et al*. [33], who demonstrated that obese rats' adipocytes recorded low ratios of BCL2/Bax mRNA and protein. BCL2 is proapoptotic and antiapoptotic factors. BCL2 and related proteins function are concerned with their efficacy to prevent apoptosis pathways of mitochondrial. Apoptotic process implies damage of outer membrane of mitochondria and the release of cytochrome C intermembrane and other proteins into the cytosol, which in turn stimulates death-driving caspase proteolytic enzymes that organize the disassembling of the cell, and this process is regulated by BCL2. Hence, by stopping proapoptotic protein Bax redistribution to the mitochondria, BCL2 prevents mitochondria cytochrome C from release and subsequent caspase proteins activation [34]. Further, the present investigation declared that HFD significantly increased serum MCP-1. These results are in concomitant with Chen *et al*. [35] who declared that the expression of MCP-1 and MCP-3 in rats was induced by HFD. The high expression level of MCP-1 in adipose tissue leads to the increase in plasma MCP-1 levels post 4 weeks HFD feeding. Monocytes slowly infiltrated and accumulated in the adipose tissue with an increase in the size of adipocytes, which subsequent stimulated expression of MCP-1. It was found that in response to increase in oxidized lipids, adipose MCP-1 and MCP-3 were induced. Furthermore, MCP-1 expression was stimulated by TNF-α in cultured adipocytes, and hence, may act to strengthen the expression of adipocyte MCP-1 [36]. Besides, hepatic MCP-1 has a principle effect in hepatic disorders through stimulation of Kupffer cells by HFD to release markedly high MCP-1 amounts [30].

Indeed, the specific overexpression of mice adipose tissue MCP-1, in turn, leads to insulin resistance development, inflammation, and hepatic

steatosis and fibrosis by eliciting hepatic stellate cells migration [37] (as indicated in the histological investigation, (Figs. 3a and 3b)).

Considering apelin which is a novel adipokine, the present results showed a significant increase in its level in obese rats induced by HFD. The apelinergic system has been demonstrated to be implicated in the high prevalence disorders such as obesity, glucose intolerance, and diabetes mellitus Type II [38]. Plasma levels of apelin are markedly elevated independently of diet composition in obese mice and humans with hyperinsulinemia [32]. It was found that apelin connected with APJ, which subsequent leads to protein kinase B (Akt) phosphorylation and ERK in different cell. Further, the system of APJ/apelin enhances the expression of adhesion molecule-1 (ICAM-1) through NF-kB/c-Jun N-terminal kinase (JNK) signal pathway [39]. In addition, Yasuzaki *et al*. [40] declared that apelin/APJ signaling may stimulate Fas-induced hepatic damage in mice injected with anti-Fas antibody (Jo2**)** through JNK phosphorylation.

On the other hand, treatment of obese rats with *D. salina* extract improved the inflammatory and antiapoptotic markers, MCP-1, and apelin levels. This biochemical observation is documented in histopathological examination of obese rats liver treated with *D. salina* (Figs. 4a and 4b), which may be attributed to *D. salina*-enriched carotenoids exhibited and anti-inflammatory activities [41]. In addition, *D. salina* treatment inferred a notable downregulation of NF-kB, MCP-1, apelin, and MDA levels, while the extract upregulated BCL2, GSH, and PON1levels. It could be due to the potential anti-inflammatory activity of carotenoids, especially carotenes [41]. Chiu *et al.* [41] added that the COX-2 protein expression was substantially downregulated by treatment with a carotenoid extract of *D. salina* in lipopolysaccharide-induced RAW 267.4 cell model. Likewise, Bai *et al.* [42] and Jang *et al*. [43] pointed out that β-carotene can inhibit the translocation of NF-p65 subunit from cytosol to the nucleus and thereby halt the inflammatory cascade. Hence, we speculate that *D.* extract rich in β-carotene can block the translocation of transcription factor (NF-p65/p50 subunit) and thus downregulate COX-2 protein expression and thereby exert anti-inflammatory activity as approved by the modulatory effect of *D. salina* on inflammatory reaction, resulting from obesity induced by HFD (Figs. 4a and 4b).

Jayappriyan *et al*. [44] and Palozza *et al*. [45] also indicated that β-carotene can interfere with cell growth (antiproliferative) by halting the cell cycle division through inhibiting cyclin A as well as induce apoptosis by downregulating antiapoptotic proteins such as BCL2 in human colon adenocarcinoma cell line. However, *D. salina* extract rich in carotenoids would not induce cell death in normal cells, rather only in cancer cells, and thus demonstrate its anticarcinogenic activity. In the present study, *D. salina* extract acts as an antiapoptotic agent, upregulated BCL2 anti-apoptotic marker. This statement clearly indicated that *D. salina* extract acted as an antiobesity-associated with anti- inflammatory agents not only by triggering anti-apoptosis rather it effectively reduced inflammatory cells reaction. Qiu *et al*. [46] proved that lycopene could regulate the expression of different protein involved in apoptosis, antiproliferation, antioxidant, andanti-inflammation, and thereby attenuate the progression of prostate cancer.

The current results showed inhibition of PON1 activity in serum of obese rats. The mechanism involved increased generation of ROS in the liver of obese rats as presented by the detected high levels of MDA. It was found that the inhibition activity of liver microsomal PON1 is considered as the starting point of biochemical alteration contributed to lipid peroxidation and hepatic damage noticed in rats injected with CCl_4-induced cirrhosis [47].

In another explanation, PON1 mRNA expression is downregulated by the pro-inflammatory cytokines as IL-1 and TNF-α in HepG2 cells. This cytokine promoted a decrease in the production of PON1 by the liver [48]. Hence, PON1 inhibition activity in sera of patients with chronic hepatic damage was suggested to be attributed to the degree of hepatic injury [22].

Obesity-induced pathology is thought to be occurred as a result of high level of lipid peroxidation. The data presented in the current study clarify that obesity elevated MDA level in hepatic tissue. This is in agreement with the results of Vincent *et al*. [49] and Amirkhizi *et al*. [50], who showed that obesity can produce a high level of lipid peroxidation by developing and growing cell damage as a consequence of the large body mass pressure. Cell damage stimulates cytokines production, particularly TNF-a, which initiates ROS from the tissues which lead to lipid peroxidation production [51]. Furthermore, the hypertriglyceridemia observed in obese rats may related to the change in the balance of oxidant-antioxidant status, suggesting that an elevation in the free fatty acids bioavailability can enhance lipid peroxidation [50]. It has been found that the body of animals had a powerful mechanism to stop tissue damage by free radical; this is achieved by a collection of proteins and endogenous antioxidant enzymes as glutathione S-transferase, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and GSH. Hence, when the production of ROS exceeds the defense of antioxidant, oxidative stress produce leading to different pathological conditions [51]. The decrease in the enzymes of antioxidant may be related to the fast consumption and exhaustion of enzyme storage in combating free radicals produced during obesity progress. The inhibition in PON-1 activity and reduced GSH level could be occurred as a result of changed synthesis and/or HDL secretion, secondary to destroyed activity of lecithin–cholesterol acyltransferase. PON-1 may be inhibited under oxidative stress, by S-glutathionylation, a redox-controlled mechanism, described by the combination between a protein thiol and oxidized GSH forming mixed disulfide [51].

The ameliorative effects of *D. salina* on MDA, GSH levels as well as PON1 activity may be due to the presence of several carotenoids such as all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-α-carotene, all-*trans*-β-carotene, and 9-*cis*-β-carotene. Nevertheless, all-*trans*-β-carotene and 9-*cis*-β-carotene are the major carotenoids present in *D. salina*

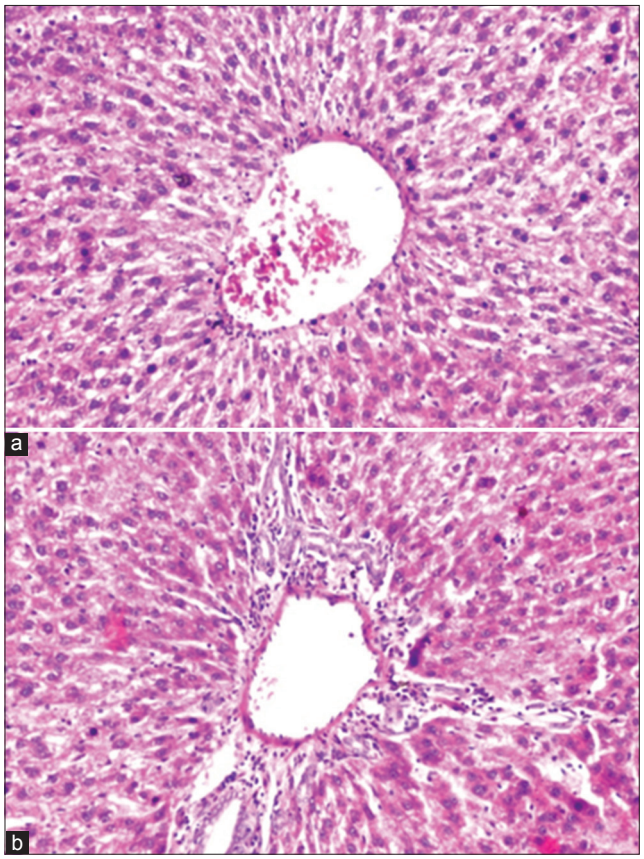

Fig. 3: (a and b) Photomicrograph of obese rats fed high-fat diet and showed dilatation in the central and portal veins associated with inflammatory cells infiltration in the portal area (H and E × 200)

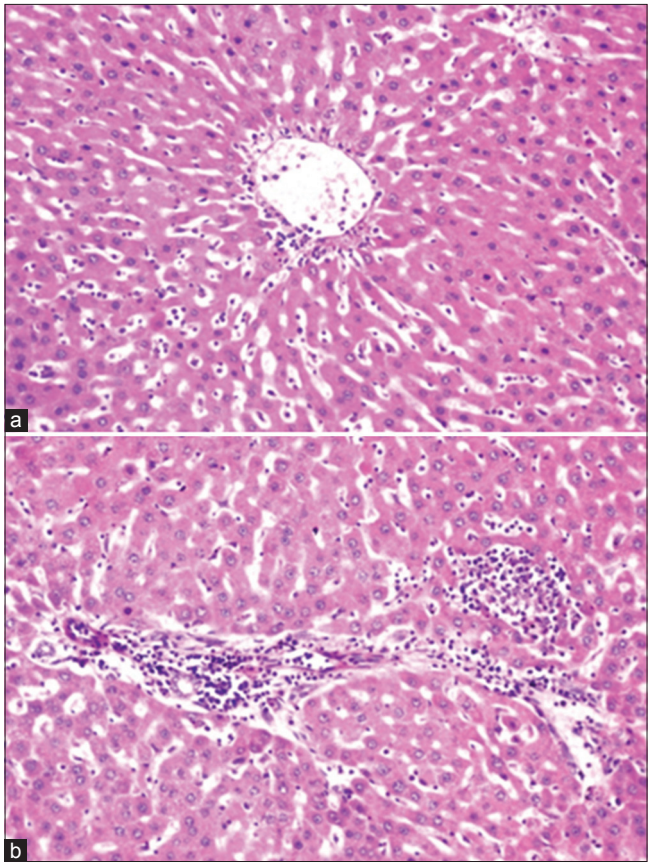

Fig. 4: (a and b) Photomicrograph of obese rats treated with (a) *Dunaliella salina* and orlistat standard drug (b) showed less diffuse inflammatory cells infiltration as well as less focal infiltration in both hepatic parenchyma and portal area with higher improvement in *D. salina* than drug (H and E ×200)

extract. Similarly, García-González *et al.* [52] and Yang *et al.* [53] also reported that all-*trans*-β-carotene and 9-*cis*-β-carotene were present in higher concentration than other carotenoids. Moreover, Ben-Amotz and Fishier [54] indicated that *Dunaliella* species (*D. bardawil*) contained about 50% of 9-*cis*-β-carotene of total carotenoid contents. The antioxidant analysis showed that the extract enriched with carotenoids would effectively scavenge free radical by improving the scavenging activity of DPPH and radicals as well as through elevated reducing and chelating efficiency in comparison with standard β-carotene. Existing evidence suggests that natural carotenoids can exist in the different isomeric forms (β-carotene-trans or cis, especially cis form is highly active) and hence effectively quench free radicals and thus act as a better antioxidant than other synthetic antioxidant [55,56].

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CONFLICT OF INTERESTS

Declared none.

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