Resolvin D1 Prevents the Impairment in the Retention Memory and Hippocampal Damage in Rats Fed a Corn Oil-Based High Fat Diet by Upregulation of Nrf2 and Downregulation and Inactivation of p66Shc

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ORIGINAL PAPER

Resolvin D1 Prevents the Impairment in the Retention Memory and Hippocampal Damage in Rats Fed a Corn Oil-Based High Fat Diet by Upregulation of Nrf2 and Downregulation and Inactivation of p^{66}Shc

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Abstract

This study investigated the effect of a high-fat diet rich in corn oil (CO-HFD) on the memory retention and hippocampal oxidative stress, inflammation, and apoptosis in rats, and examined if the underlying mechanisms involve modulating Resolvin D1 (RvD1) levels and activation of p^{66}Shc. Also, we tested if co-administration of RvD1 could prevent these neural adverse effects induced by CO-HFD. Adult male Wistar rats were divided into 4 groups (n = 18/each) as control fed standard diet (STD) (3.82 kcal/g), STD + RvD1 (0.2 µg/Kg, i.p/twice/week), CO-HFD (5.4 kcal/g), and CO-HFD + RvD1. All treatments were conducted for 8 weeks. With normal fasting glucose levels, CO-HFD induced hyperlipidemia, hyperinsulinemia, increased HOMA-IRI and reduced the rats' memory retention. In parallel, CO-HFD increased levels of reactive oxygen species (ROS), malondialdehyde (MDA), cytoplasmic cytochrome-c, and cleaved caspase-3 and significantly decreased levels of glutathione (GSH), Bcl-2, and manganese superoxide dismutase (MnSOD) in rats' hippocampi. Besides, CO-HFD significantly reduced hippocampal levels of docosahexaenoic acid (DHA) and RvD1, as well as total protein levels of Nrf2 and significantly increased nuclear protein levels of p-NF-κB. Concomitantly, CO-HFD increased hippocampal protein levels of p-JNK, p53, p^{66}Shc, p-p^{66}Shc, and NADPH oxidase. However, without altering plasma and serum levels of glucose, insulin, and lipids, co-administration of RvD1 to CO-HFD completely reversed all these events. It also resulted in similar effects in the STD fed-rats. In conclusion, CO-HFD impairs memory function and induces hippocampal damage by reducing levels of RvD1 and activation of JNK/p53/p^{66}Shc/NADPH oxidase, effects that are prevented by co-administration of RvD1.

Keywords Corn oil · Hippocampus · Oxidative stress · Resolvin D1 · p^{66}shc · Apoptosis

Introduction

Chronic consumption of high-fat diet (HFD) and obesity are associated with neurodegeneration and cognitive deficits in both humans and animals and are considered to be major risk factors for the development of dementia and Alzheimer's disease (AD) [1–6]. Covering data from epidemiological, clinical, and experimental studies have shown that oxidative stress and neuroinflammation are the major mechanisms responsible for the brain and neural adverse effect of HFD [1, 3, 5–7]. Yet, oxidative stress has been identified to be the earlier event to appear in the brain of animal models of HFD and is the central key initiator for all the other neural events including inflammation and apoptosis [1, 4, 6, 8].

However, the precise mechanism by which HFD and obesity induce brain and hippocampal ROS and inflammation

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remained not completely understood and are believed to be as a consequence of HFD-induced peripheral and central (brain) insulin resistance (IR) [3–5, 7]. Indeed, peripheral and brain IR are strongly correlated with neurodegeneration, dementia, depression, and impairment in cognitive function [9, 10]. Mechanisms behind this are well explained in excellent reviews [3, 4, 11]. However, it is still unknown if dietary fats may act also centrally and independent of their metabolic effects, an observation that needs further investigation.

Recently, the emerging role of p^{66}Shc in mediating oxidative stress and cellular apoptosis has been placed under extensive research in a variety of disease conditions. Currently, the activation of p^{66}Shc is a major mechanism for oxidative stress-induced and neurodegeneration, depression and cognitive decline [12–17]. Indeed, it was shown that p^{66}Shc is a potent pro-oxidant and apoptotic protein that can increase the intracellular levels of ROS and induces intrinsic cell death by numerous pathways including impairing mitochondria electron transport chain (ETC), activation of NADPH oxidase, the release of cytochrome-c, and suppressing endogenous antioxidant synthesis by inhibition of FOXO-3a [12, 18, 19]. However, activation of p^{66}Shc requires phosphorylation at Ser^{36} mediated by JNK, p53 or PKCβII [20, 21]. Despite these findings, the possible involvement of p^{66}Shc in HFD-induced neurodegeneration was never shown before.

On the other hand, the brain is one of the richest organs with polyunsaturated fatty acids (PUFAs) of both omega-3 and omega-6 (n-3/n-6 PUFAs). Currently, it has been reported that a balanced ratio of n-3/n-6 PUFA (1:1–4) is essential to maintain our mental health through the life cycle [22]. Generally, n-3 PUFAs are neuroprotective [23–26]. However, higher brain levels of n-6 PUFA are associated with a decline in the cognitive function with poor-described and almost unknown mechanisms [27, 28].

Nonetheless, Resolvin D1 (RvD1) is a specialized pro-resolving mediator (SPM) derived from the catabolism of docosahexaenoic acid (DHA), a major n-3 PUFA in the brain of mammals [29]. RvD1 has potent antioxidant, anti-inflammatory, and anti-apoptotic effects in various tissues including the liver, lung, kidney, and brain and was shown to act mainly by activating the nuclear factor erythroid 2-related factor-2 (Nrf2) and inhibiting the activation of the nuclear factor kappa B (NF-κB) transcription factors [29–35]. Interestingly, lower levels of RvD1 are associated with higher expression levels of NADPH oxidase, ROS generation, and lipid peroxidation in an animal model of atherosclerosis [36]. Besides, RvD1 has potent antidepressant effects [37, 38]. Of note, HFD reduced brain levels of DHA and concomitantly reduced neural plasticity and altered the behavior of the animals [39], thus implicating that HFD may negatively affect the brain and neural levels of RvD1.

However, it was shown that different fatty acids have a different impact on our brain [22]. Our ancestors have evolved on a ratio of n-3/n-6 PUFAs of 1:1–4 [22]. During the last decades, our modern diet was shifted toward a diet that is rich in n-6/n-3 PUFAs (20:1) [22]. Linoleic acid (LA, 18 : 2n-6) is the major n-6 PUFA that is found mainly in many dietary vegetable oils such as sunflower and corn oils [40]. Unfortunately, there are not enough studies that have examined the direct effect of n-6 PUFAs on the brain/hippocampus health or cognitive function. Therefore, in this study, it was worthy to evaluate the effect of HFD rich in corn oil (CO-HFD) on memory retention function and hippocampal oxidative stress, inflammation, and apoptosis in male rats from the perspective of its effect on the levels of RvD1 and activation of p^{66}Shc. Also, we investigated the neuroprotective effect of exogenous administration of RvD1 in this animal model.

Materials and Methods

Animals

Adult Wistar male rats (Charles River, Strain code: 003) (120 ± 10 g) were supplied from the animal house at King Khalid University, Abha, Kingdom of Saudi Arabia (KSA). During the whole period of the experimental procedure, all rats were kept in an automated controlled room (Temperature of 23 ± 1 °C, humidity of 60%, and 12/12 h light/ dark cycle). During the adaptation period of 1 week, all rats were fed a standard diet and had free access to drinking water, ad libitum. All procedures used in this study were approved by the animal ethics and use committee at King Khalid University where their regulations follow the guidelines established by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Experimental Design

Rats were divided into four groups (n = 18/each) as (1) STD rats: were rats that were fed a standard control diet (3.82 kcal/g; the energy of 76.6%, 9.4%, and 14% from carbohydrates, fats, and proteins, respectively) and received 1% ethanol solution diluted in phosphate buffer saline PBS (pH 7.4), as a vehicle; (2) STD + RvD1: were control rats as in group 1 and co-treated with RvD1 (0.2 µg/kg, i.p/twice/week), prepared in 1% ethanol; (3) CO-HFD rats: rats that were fed CO-HFD (5.4 kcal/g; the energy of 46%, 40%, and 14%, from carbohydrates, fats, and proteins, respectively), and received 1% ethanol solution, as a vehicle; (4) CO-HFD + RvD1: rats that were fed CO-HFD as in group 3 and co-treated with RvD1 (0.2 µg/Kg, i.p/twice/week).

All treatments were conducted for 8 weeks. RvD1 (Cat. No., Cay10012554-50) was purchased from Cayman Chemical (Ann Arbor, MI, USA) and was always prepared in absolute ethanol and diluted to the desired concentration in PBS (pH 7.4) where the final ethanol concentration was less than 1%. Preliminary data revealed no significant variation in the memory tests and hippocampal oxidative stress between control + normal saline and control + 1% ethanol. For this reason, the control group presented in this study was the one that was administered with ethanol. The dose of RvD1 and route of administration of RvD1 was selected based on the study of Krashia et al. [41] who have shown it to blunt neuroinflammation and improves motor function in a rat's model of Parkinson's disease. All diets ingredients were purchased form Dyets (Bethlehem, PA, USA) and are shown in Table 1. Soya bean oil was added to both diets in equal quantities to provide the rats with essential fatty acids.

Measurements of the Retention of Rats' Memory

Retention of rats' memory of all experimental groups was measured using the Morris Water Maze (MWM) [42] and Passive Avoidance Learning Test (PALT) [43]. the MWM is composed of a circular swimming pool (diameter of 1.7 m/depth of 60 cm) that is filled with milky water (temperature of 22 ± 1 °C). The pool was divided into 4 equal hypothetical quadrants (N, S, E, and W). A hidden escape platform (12 cm in diameter) was submerged 2 cm below the water level in the middle of the SW quadrant (target quadrant). At the end of the treatment regimen, each rat was placed at one of 3 starting positions (N, E, NE) and released to find the hidden platform. This procedure was repeated for 5 days with 3 trails/day (separated by 5 min interval) each conducted by releasing the rat from a different position (N, E, or SE) (for 90 s/each) keeping the platform fixed in the same quadrant (SW). The selection of these starting points minimizes the variation in path length from the goal and creates equidistance paths. If the rat was unable to find the platform, it was directed by the investigator and left on the platform for 15 s. In addition, an extra probe trial was conducted on each rat 1 h after the last trial where the platforms were removed and the total number of times crossed the place where the hidden platforms were initially placed were recorded. The training and probe sessions were carried out by a blind observer who was unaware of all experimental groups. All sessions were performed for n = 18 rats/group. On the other hand, the PALT measures the ability of the rat to remember a previous electrical shock in a dark room and avoid re-entering it. The apparatus is made of wood (50 × 50 × 35 cm) and contains 2 rooms, one large lighted room and another small dark room that is supplied with an electrical stimulator on the grid floor. Both rooms are separated by a door. The test consisted of three phases, exploration, training and testing. During the exploration day which was conducted the next day after completing the MWM, all rats were placed in the large lighted room with an open door and were allowed to freely explore the whole apparatus (3 trails/each of 5 min). Next day, the rats were placed in the lighted area with an open door and once they stepped into the dark area, the door was closed and they were exposed to an electrical foot shock of 60 Hz/1.5 mA for 1 s and were kept in dark for an additional 15 s (training). The next day, the procedure was repeated and the time (in sec.) spent by each rat in the lighted area was recorded (testing). Normally, rats with poor retention memory enter the darkroom faster.

Table 1 The composition of the standard (STD) and high-fat diet (HFD) rich in corn oil

Ingredients	STD (g/kg)	HFD (g/kg)
Casein (≥ 85% protein)	134	189
Starch	582	521
Sucrose	100	100
Soy oil	40	40
Corn oil	00	200
Fish oil	0	0
Wheat bran/cellulose	146	50
Salt mix[a]	35	35
Vitamin mix[b]	10	10
Vitamin E acetate (500 IU/g)	0.008	0.008
DL-Methionine/L-cystine	1.8	1.8
Choline bitartrate	2.5	2.5
Caloric value (kcal/g)	3.82	5.4

[a]Cat. no. 200301

[b]Cat. no. 310025 (Dyets, Bethlehem, PA USA)

Collection of Serum and Hippocampi

At the end of the memory testing, all rats were fasted overnight and then were anesthetized with an i.p. bolus of sodium pentobarbital (60–70 mg/kg). The chest of each rat was opened and blood samples (2 ml/each) were collected from each rat into plain or EDTA-tubes, centrifuge at 1500×*g* to collect serum and plasma, respectively. All samples were stored at – 20 ℃ until used. Then, the brains were collected on ice and all hippocampi were isolated under a dissecting microscope and snap-frozen in liquid nitrogen and stored at − 80 °C for further use.

Analysis of the Biochemical Parameters in the Brain and Hippocampi

Fasting plasma levels of glucose and insulin, as well as serum levels of triglycerides, cholesterol, low-density lipoprotein cholesterol (LDL-c), were measured using an

automatic analyzer (cobas® 8000 modular analyzer series. Roche Diagnostics). For the measurement of the other biochemical parameters in rat's hippocampi, parts of hippocampi from all groups (25 mg) were homogenized individually in 250 µl ice-cold PBS (pH 7.4) containing 5 µl protease inhibitor (Cat. No. P8340, Sigma-Aldrich, St. Louis, MO, USA), centrifuged at 1000×*g* for 10 min to collect the supernatants which were stored at -20 until the time of the use. Hippocampal levels of (MDA) were determined using a colorimetric kit (Cat. No. ab118970, Abcam, UK, respectively). Total levels of ROS were measured using a Green Fluorescence kit (Cat. No. STA-347, OxiSelect, Cell Biolabs, Inc. CA, USA). Levels of manganese superoxide dismutase (MnSOD) were measured using a rat's ELISA kit (Cat. No. MBS2881838, MyBioSource, CA, USA). Levels of reduced glutathione (GSH) were measured using a colorimetric kit (Cat. No. 7511-100-K, Trevigen, Gaithersburg, USA). Hippocampal levels of DHA and RvD1 were measured using special rats' ELISA kits (Cat. No. MBS2025500 and Cat No. MBS047677, CA, USA, respectively). Brain levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels were measured using rats' ELISA kits (Cat. No. CSB-E11987r and CSB-E04640r, and Cat. No. CUSABIO technology LLC, TX, USA, respectively). All procedures and analyses were done per each kit instructions.

Hippocampal Histology Study

The hippocampi from all groups of rats were fixed in 10% neutral buffered formalin for 24–48 h. Then, they were dehydrated in ascending graded alcohol series, cleared in xylene, and embedded in paraffin wax. The blocks were sectioned at 5 µm and stained with hematoxylin and eosin (HE). A pathologist who is unaware of the experimental groups examined and evaluated all tissues under an Olympus DG 03506 light microscope.

Preparation of the Nuclear and Cytoplasmic Fractions

The nuclear and cytoplasmic fractions from 6 hippocampi were prepared using a special kit (Cat No. 78835 and ThermoFisher Scientific) per the manufacturer's instructions. In brief, the frozen hippocampus of each rat (20 mg) was placed in a microcentrifuge tube and washed twice with ice-cold PBS (pH 7.4), centrifuged at 500×*g* (4℃, 5 min) to collect the pellet. The pellet of each sample was homogenized in 200 µl of the CERI lysis buffer that is provided with the kit. The mixture was then mixed and incubated in ice for an extra 10 min. Then, 11 µl of an ice-cold CER II solution (provided with the kit) was added to each tube, mixed and incubated for 1 min in ice. Then, the tube of each sample was centrifuged at 4℃ for 5 min at 16,000×*g* and the supernatant containing the cytoplasmic extract was removed and stored at -80℃ until use. After that, the insoluble remaining pellet in each sample (nuclei) was suspended in 100 µl NER solution, mixed, and then kept on ice with continuous vortexing every 10 min for a total of 40 min. Then, all samples were centrifuged at 16,000×*g* for 10 min at 4℃ to collect the supernatants which contain the nuclear extract fractions and stored at − 80 °C until use.

Western Blotting

For western blotting analysis, hippocampus tissue from each group (20 mg) was homogenized in 0.5 ml RIPA buffer (50 mM Tris–HCl (pH 8.0), 0.5% sodium deoxycholate, 150 mM sodium chloride, 1.0% NP-40, and 0.1% SDS) containing 5 µl the protease inhibitor cocktail). All samples were centrifuged at 10,000×*g* at 4 ℃ to collect the supernatants which were stored at − 80 °C until the time of use. Protein levels in the nuclear, cytoplasmic and total protein homogenates were determined using a Bradford assay-based kit (Cat. No 23300, ThemoFisher Scientific, MA, USA). Proteins (40 µg) of all samples were separated on SDS–polyacrylamide gel (8–12%) and then transferred onto nitrocellulose membranes and blocked with skimmed milk (prepared in TBST buffer). Then, membranes were incubated with primary antibodies against p-p^{66}Sch (Cat. No. ab54518, 67 kDa, 1:500, Abcam, Cambridge, UK), antibodies against p^{66}Sch (Cat. No. sc-967, 46/52/66 kDa, 1:500), JNK (Cat. No. sc-7345, 46/54 kDa, 1:1000), p-JNK (Cat. No. sc-6254, 46/54 kDa, 1:1000), Nrf2 (Cat. No. sc-365949, 60 KDA, 1:1000), Bcl-2 (Cat. No. sc-7382, 1:000, 26 kDa, 1:1000) and β-tubulin (Cat. No sc-390996. 35 kDa, 1:1000) (Santa Cruz Biotechnology, USA) and antibodies against p53 (Cat. No. 9282, 1:000, 53 kDa), cytochrome-c (Cat. No. 11,940, 1:500, 14 kDa), cleaved caspase-3 (Cat. No. 9661, 1:500, 17/19 kDa), NADPH oxidase (p47 phox) (Cat. No. 4312, 1:500, 47 kDa), MnSOD (Cat. No. 13,194, 1:1000, 22 kDa), p-NF-κB (Ser^{536}) (Cat. No. 3031, 65 kDa, 1:500), lamin B (Cat. No. sc-374015, 67 kDa 1:500), β-actin (Cat. No. 3700, 45 kDa, 1:2000) (Cell Signalling Technology, USA). Membranes were then incubated with an appropriate secondary antibody (prepared in TBST buffer). Antigen–antibody reactions were detected, photographed and analyzed using a Pierce ECL kit (ThermoFisher, USA, Piscataway, NJ) and C-Di Git blot scanner (LI-COR, USA). Membranes were stripped up to 5 times and phosphorylated forms were detected first. An internal known standard protein was run between gels and used for standardization.

Statistical Analysis

All analyses were done on GraphPad Prism statistical software package (version 8). The comparison in the MWM

test was done by 2-way ANOVA with repeated measures. The analysis of all others between the various groups was performed using two-way ANOVA followed by Tukey's Post hock test. Data are presented as means with standard deviation (mean ± SD). Significance was considered at $P < 0.05$.

Results

Changes in Body Weights, Serum Glucose, and Insulin Levels, and Lipid Profile

As compared to control rats fed STD, CO-HFD-fed rats showed a significant increase in their final body weights ($F = 48.06/P = 0.0001$), fasting serum levels of TGs ($F = 151.2/P < 0.0001$), CHOL ($F = 143.1/P < 0.0001$), and LDL-c ($F = 81.85/P < 0.0001$), as well as in the levels of their plasma insulin ($F = 39.24/P = 0.0002$) and HOMA-IRI (Table 2). However, there was no difference in fasting plasma glucose between STD and CO-HFD-fed rats (Table 2). On the other hand, there was no significant difference in rats' final body weights nor in the levels of any of these serum or plasma-related biochemical parameters when a comparison was made between STD + R_VD1 vs. STD-fed rats or between CO-HFD- RvD1 vs. CO-HFD (Table 2). The F and P values after RvD1 treatment for the changes in rats' final body weights, serum levels of TGs, CHOL, LDL-c, and plasma levels of insulin were $F = 0.1103/P = 0.7484$, $F = 0.1271/P = 0.9130$, $F = 1.129/P = 0.319$, $F = 0.5521/P = 0.4787$, and $F = 1.125/P = 0.3199$, respectively. The levels of interaction between HFD and RvD1 treatment for these markers were $F = 0.9115/P = 0.3677$, $F = 0.3341/P = 0.5791$, $F = 1.782/P = 2.186$, $F0.06135/P = 0.8106$, and $F = 0.09354/P = 0.7675$, respectively. These data suggest that all the other neural effects afforded by RvD1 in STD or COHFD-fed rats are not related to the changes in body weights or modulating plasma glucose or insulin levels.

Alterations in the Retention of Rat's Memory

Retention memory of all was tested using MWM and PALT. The MWM was tested over 5 days (3 trials/day) after the end of the experimental procedure and then followed by a probe test to count the total number of times by which the rat crosses the place where the hidden platforms was initially placed but this time was removed. CO-HFD-fed rats spent longer latencies to find the platform over days 2–5 as compared to STD-fed rats (Fig. 1 A, B). Besides, they had less number of times to cross the place of the hidden platform (Fig. 1C) and had a shorter time to enter the dark area after being trained to be exposed to a foot electrical shock in that area in PALT (Fig. 1D). On the contrary, all these memory-related parameters were significantly improved in both STD + RvD1 and CO-HFD + RvD1 as compared to STD or CO-HFD-fed rats which were administered the vehicle, respectively. These data indicate the ability of RvD1 to improve the retention of the memory in both control and CO-HFD-fed rats (Fig. 1A–D). The F and P values for HFD, RvD1 treatment and the interaction between them for the number of crossing were $F = 11.57/P = 0.0037$, F 8.798/P = 0.0091, and $F = 9.27/P = 0.0077$, respectively and were $F = 26.04/P < 0.0001$, $F = 12.52/P < 0.0001$, and $F = 3/P = 0.0018$, respectively, for the time to enter the dark area.

Table 2 Changes in final body weights, serum lipid profile, and plasma glucose and insulin levels in all groups of rats

Parameter	STD	STD + RvD1	CO-HFD	CO-HFD + RvD1
Final body weight (kg)	398 ± 15.4	389.2 ± 11.8	547 ± 24.3[ab]	533 ± 34.7[ab]
CHOL (mg/dl)	68.5 ± 6.5	64.8 ± 5.1	123.5 ± 9.7[ab]	128.3 ± 6.5[ab]
TGs (mg/dl)	45.5 ± 7.6	48.7 ± 4.3	134.4 ± 14.3[ab]	129 ± 18.7[ab]
LDL-c	34.5 ± 5.4	31.7 ± 4.1	67.8 ± 8.4[ab]	65.8 ± 7.5[ab]
Plasma glucose (mg/dl)	107 ± 6.2	103 ± 7.8	105.2 ± 7.1	107.3 ± 5.4
Plasma insulin (ng/ml)	4.3 ± 0.35	4.8 ± 0.83	9.4 ± 0.8[ab]	8.7 ± 1.5[ab]
HOMA-IRI	1.16 ± 0.07	1.22 ± 0.13	2.6 ± 0.3[ab]	2.3 ± 0.45[ab]

Data were considered significantly different at $P < 0.05$. Data are presented as mean ± SD of n = 18 rats/group

STD standard diet, *CO-HFD* a high-fat diet rich in corn oil, *RvD1* Resolvin D1, *HOMA-IRI* Homeostasis Model Assessment of Insulin Resistant Index

[a]Significantly different when compared with STD-fed rats

[b]Significantly different as compared to STD + RvD1

[c]Significantly different when compared with CO-HFD-fed rats

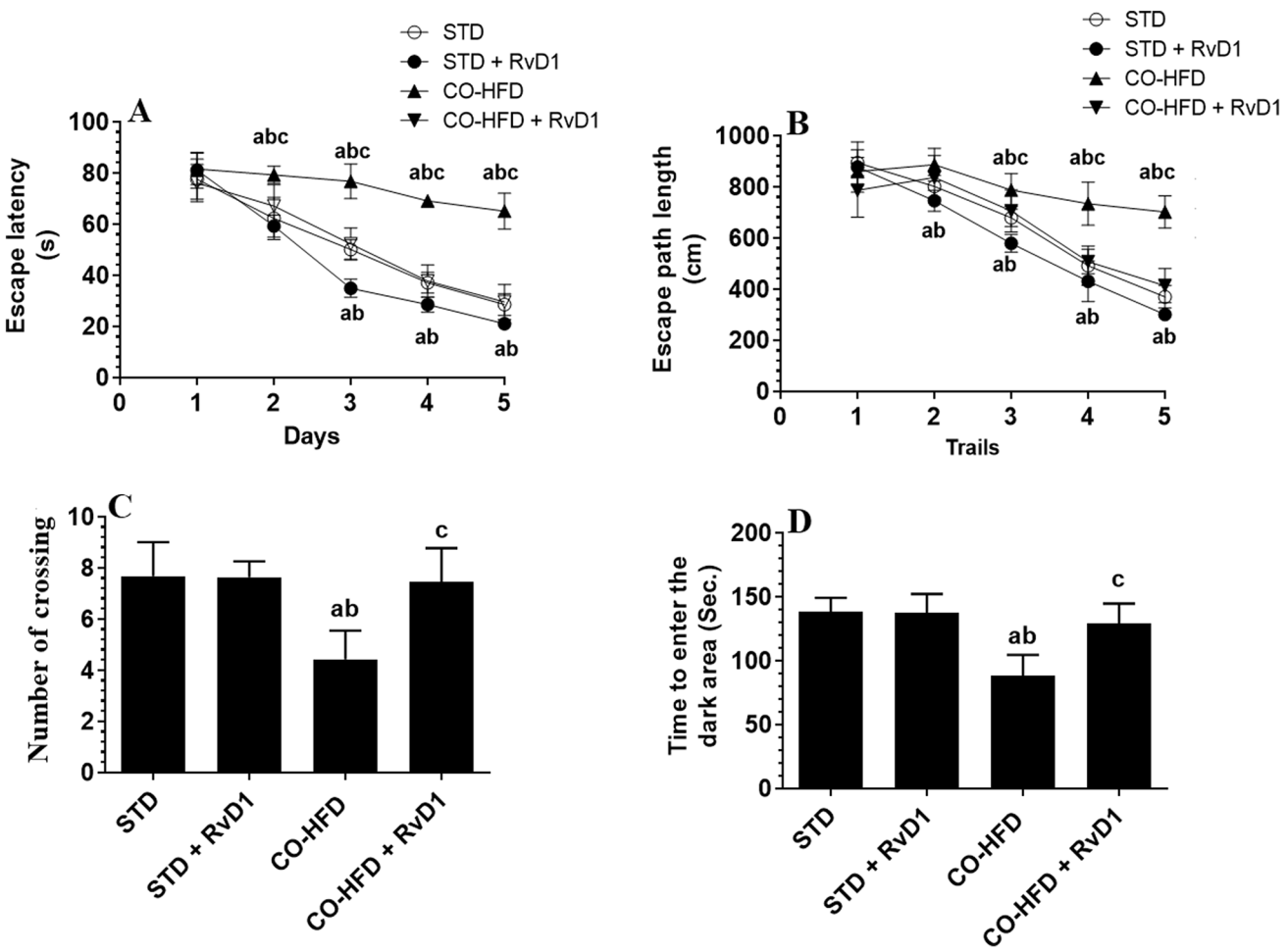


Fig. 1 The escape latency (**A**), length of the path (**B**), and the number of crossing (**C**) recorded in Morris Water Maze (MWM) test, as well as time spent by the rats in the illuminated (lighted) area before entering the dark area during the retention memory test (**D**). Data were considered significantly different at $P < 0.05$. Data are presented as mean ± SD of n = 18 rats/group. In **A**, **B**: [a]significantly different when compared to STD-fed rats [b]significantly different as compared to CO-HFD + RvD1. [c]significantly different when compared with STD + RvD1. In **C**, **D**: [a]significantly different when compared with STD-fed rats. [b]Significantly different as compared to STD + RvD1. [c]Significantly different when compared with CO-HFD-fed rats. *STD* a standard diet, *RvD1* Resolvin D1, *CO-HFD* a high-fat diet rich in corn oil

Alterations in the Hippocampal Biochemical Parameters

CO-HFD-fed rats had a significant increase in the intracellular levels of ROS ($F = 22.91/P = 0.0014$) and levels of MDA ($F = 30.04/P = 0.0006$), TNF-α ($F = 46.03/P < 0.0001$), IL-6 ($F = 138.3/P < 0.0001$), and a significant decrease in the levels of MnSOD ($F = 33.59/P = 0.004$), GSH ($F = 75.05/P < 0.0001$), DHA ($F = 311.1/P < 0.001$), and RvD1 ($F = 122.8/P < 0.0001$) in their hippocampi compared to STD-fed rats (Table 3). However, levels of MnSOD, GSH, DHA, and RvD1 were significantly increased whereas levels of ROS, MDA, TNF-α, IL-6 were significantly decreased in the hippocampi of both the STD + RvD1 and CO-HFD + RvD1 as compared to STD or CO-HFD-fed rats which were administered the vehicle, respectively (Table 3). The F and P values after RvD1 treatment for ROS, MDA, TNF-α, IL-6, MnSOD, GSH, DHA, and RvD1 were $F = 10.76/P = 0.0112$, $F = 8.824/P = 0.0206$, $F = 15.09/P = 0.0046$, $F = 66.36/P < 0.0001$, $F = 26.1/P = 0.0009$, $F = 42.58/P = 0.002$, $F = 113.8/P < 0.0001$, $F = 372.8/P < 0.0001$, respectively. However, the F and P values for the interaction between the diet and RvD1 treatment for ROS, MDA, TNF-α, IL-6, MnSOD, GSH, DHA, and RvD1 were $F = 0.3499/P = 0.5705$, $F = 2.331/P = 0.1654$, $F = 3.662/P = 0.0920$, $F = 15.56/P = 0.0034$, $F = 0.0025/P = 0.9614$, $F = 7.911/P = 0.227$, $F = 17.29/P = 0.0029$, $F = 19.5/P = 0.0020$, respectively. These data suggest that CO-HFD lowers levels of DHA and

Table 3 Biochemical analysis in the hippocampi of all experimental groups of rats

Parameter	STD	STD + RvD1	CO-HFD	CO-HFD + RvD1
ROS/RNS (DCF/mg)	27 ± 4.6	19.5 ± 3.8[a]	41.5 ± 6.7[ab]	31 ± 6.5[bc]
MDA (nmol/g)	0.5 ± 0.087	0.32 ± 0.04[a]	1.45 ± 0.45[ab]	0.85 ± 0.16[bc]
MnSOD (pg/g)	5.2 ± 0.87	7.9 ± 1.3[a]	2.1 ± 0.47[ab]	4.8 ± 0.97[bc]
GSH (nmol/g)	45.6 ± 7.8	76.7 ± 8.3[a]	31 ± 4.4[ab]	42.2 ± 6.6[bc]
TNF-α (pg/ml)	1.1 ± 0.15	0.76 ± 0.12[a]	2.6 ± 0.39[ab]	1.6 ± 0.36[abc]
IL-6 (pg/ml)	4.3 ± 0.58	2.4 ± 0.43[a]	11.4 ± 1.1[ab]	5.9 ± 0.85[abc]
RvD1 (pg/ml)	78 ± 8.7	282 ± 23.3[a]	19.8 ± 5.2[ab]	156.2 ± 19.5[abc]
DHA (pg/ml)	313 ± 28.9	535.2 ± 32.3[a]	116 ± 13.2[ab]	209 ± 24.9[ab]

Data were considered significantly different at $P < 0.05$. Data are presented as mean ± SD of n = 6 rats/group

STD a standard diet, *RvD1* Resolvin D1, *CO-HFD* a high-fat diet rich in corn oil

[a]Significantly different when compared with STD-fed rats

[b]When compared with STD-fed rats. Significantly different as compared to STD + RvD1

[c]Significantly different when compared with CO-HFD-fed rats

RvD1 in the hippocampi of rats, an effect that is associated with the production of ROS, oxidative stress, and inflammation and is reversed by the co-treatment with RvD1.

Alterations in the Structure of Hippocampi of Rats

Normal architectures with intact three layers, pyramidal layer, molecular layer (M), and a polymorphic layer were observed in the hippocampi of STD-fed rats which were administered the vehicle and STD + RvD1 treated rats (Fig. 2 A, B). In both groups, the pyramidal layer contained 4–6 layers of intact pyramidal cells most of which have normal vesicular nuclei. However, the hippocampi of CO-HFD-fed rats showed a decrease in the number of layers in the pyramidal layer where most of the pyramidal cells were shrunk and dark. In addition, apoptotic nuclei were abundant (Fig. 2C). On the other hand, almost normal architectures of the hippocampus were observed in CO-HFD + RvD1-treated rats (Fig. 2D).

Alterations in Cell Signaling

Hippocampal protein levels of total JNK were not significantly altered with any treatment (Fig. 3). However, protein levels of total P^{66}Shc ($F = 534.2/P < 0.0001$), p-p^{66}Shc (Ser^{36}) ($F = 119.3/P < 0.001$), p-JNK (The^{183}/Tyr^{185}) ($F = 80.51/P < 0.0001$), p53 ($F = 83.87/P < 0.0001$), NADPH oxidase ($F = 407.8/P < 0.0001$), and nuclear levels p-NF-κB p65 (Ser^{536}) ($F = 159.5/P < 0.0001$) were significantly increased but total protein levels of Nrf2 ($F = 10.53/P < 0.0118$) were significantly decreased in the hippocampi of CO-HFD-fed rats as compared to STD -fed rats (Figs. 3A–D, 4A, B). On the other hand, total protein levels of P^{66}Shc in the hippocampi of STD + RvD1 were not significantly changed as compared to their corresponding levels measured in STD rats administered the vehicles whereas total protein levels of P^{66}Shc were significantly decreased in the hippocampi of CO-HFD + RvD1 as compared to CO-HFD fed rats (Fig. 3C). In addition, RvD1 significantly reduced the protein levels of p-p^{66}Shc (Ser^{36}), p-JNK (The^{183}/Tyr^{185}), p53, NADPH oxidase and nuclear levels p-NF-κB and significantly increased protein levels of Nrf2 in the hippocampi of STD + RvD1 and CO-HFD + RvD1 as compared to their levels in the hippocampi of STD or CO-HFD which were administered the vehicle, respectively (Figs. 3A–D, 4A, B). The F and P values for RvD1 treatment for p^{66}Shc, p^{66}Shc(Ser^{36}), JNK, p53, NADPH oxidase, p-NF-κB p65 (Ser^{536}), and Nrf-2 were $F = 384.4/P < 0.0001$, $F = 409.4/P < 0.0001$, $F = 82.55/P < 0.001$, $F = 34.18/P = 0.0004$, $F = 130/P < 0.0001$, $F = 71.66/P < 0.0001$, and $F = 184.4/P < 0.0001$, respectively. The F and P values for the interaction between diet and RvD1 for p^{66}Shc, p-p^{66}Shc (Ser^{36}), JNK, p53, NADPH oxidase, NF-κB p65(Ser^{536}), and NrF-2 were $F = 125.3/P < 0.0001$, $F = 409.4/P < 0.0001$, $F = 11.09/P = 0.0140$, $F = 10.9/P = 0.0180$, $88.36/P < 0.0001$, $F = 20.88/P = 0.0021$, and $F = 8.318/P = 0.0204$, respectively. These data suggest that CO-HFD activates JNK/p66Shc/NADPH oxidase and NF-κB and inhibits Nrf2 in the hippocampi of rats, all of which are reversed by the treatment with RvD1.

Alterations in Protein Levels of MnSOD and the Apoptotic Markers

Total protein levels of MnSOD ($F = 219.1/P < 0.0001$) and Bcl-2 ($F = 17.76/P = 0.0029$) were significantly decreased but total protein levels of cleaved caspase-3 ($F = 83.92/P < 0.0001$) and cytoplasmic levels of cytochrome-c ($F = 165.4/P < 0.0001$) were significantly

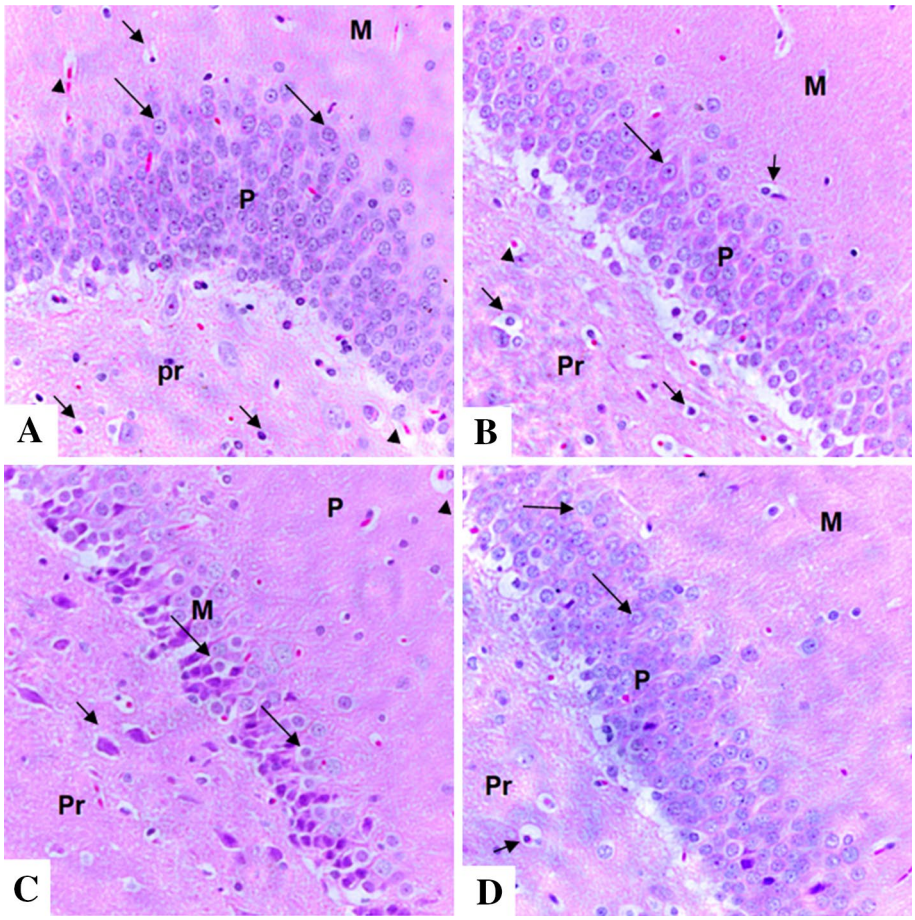


Fig. 2 Photomicrographs of histological features of rats' hippocampi of all groups of rats. All samples were photographed from the CA1 region of the hippocampus. **A**, **B** were taken from STD and STD + RvD1-treated rats, respectively and showing normal three layers including a pyramidal layer (P) that contain normal 4–6 layers of the pyramidal cells most of which contain normal vesicular nuclei (long arrow), a molecular layer (M) and a polymorphic layer (Pr) both of which contain abundant glial cells (small arrow) and blood capillaries (arrowhead). **C** was taken form a CO-HFD-fed rat and showing all three layers of the hippocampus with a reduced number of layers in the (P) layer, presence of apoptotic cells (long arrow). Also, most of the pyramidal cells were shrunk and have dark nuclei and cytoplasm indicating cell death. **D** was taken from a CO-HFD + RvD1-treated rat and shows an obvious increase in the number of layers and size of the (P) layer and the number of dark cells. Most of the cells were looks like those observed in the STD fed groups with vesicular nuclei

increased in the hippocampi of CO-HFD-fed rats as compared to STD-fed rats administered the vehicle (Figs. 4C, D, 5 A, B). On the contrary, total protein levels of MnSOD and Bcl-2 were significantly increased but the total protein levels of cleaved caspase-3 and cytoplasmic levels of cytochrome-c were significantly decreased in the hippocampi of both STD + RvD1 and CO-HFD + RvD1 when compared to their control groups (STD or CO-HFD, respectively) (Figs. 4C, D, 5 A, B). The F and P values after RvD1 treatment for MnSOD, Bcl-2, cytochrome-c, and cleaved caspase-3 were $F = 49.61/p = 0.0001$, $F = 103.1/P < 0.0001$, $F = 201.9/P < 0.0001$, and $F = 48.96/P < 0.0001$, respectively. The F and P values for the interaction between the drug and the treatment for MnSOD, Bcl-2, cytochrome-c, and cleaved caspase-3 were $F = 1.494/P = 0.2563$, $F = 4.481/P = 0.0590$, $F = 32.86/P = 0.0004$, and $F = 57.03/P < 0.0.0001$, respectively.

Discussion

The salient findings of this study show that chronic administration of HFD rich in corn oil (CO-HFD) for 8 weeks, like any other HFD, induced oxidative stress, inflammation, and apoptosis in rats' hippocampi and dampened their memory retention. In particular, CO-HFD reduces hippocampal levels of DHA and RvD1, the expression of Nrf2 and MnSOD. Besides, it increased the production of ROS, nuclear accumulation of p-NF-κB (Ser^{536}), and the production of TNF-α and IL-6. It also decreased hippocampal

levels of MnSOD and GSH and activated the mitochondria-mediated cell apoptosis. These effects were associated with activation of JNK, upregulation of NADPH oxidase, p^{66}Shc, and p53, and increase phosphorylation of p^{66}Shc at Ser^{36} in rats' hippocampi. Of interest, the administration of RvD1 to CO-HFD-fed rats reversed all the events and improved rats' memory retention. Also, similar effects were observed in control rats administered RvD1. Hence, these data suggest that higher hippocampi levels of RvD1 protect the rats from CO-HFD-induced hippocampal damage and memory deficits, at least by, (1) activation of Nrf2 and upregulation of endogenous antioxidants, (2) inhibition of NF-κB and production of inflammatory cytokines, and (3) decreasing the production of ROS and inhibition of cell apoptosis by downregulation/inhibition of JNK, p53, p^{66}Shc, and NADPH oxidase axis (Fig. 6).

The negative impact of obesity and HFD on brain health and memory function is well reported in humans and experimental animal models and mechanisms behind this are believed to be due to the development of peripheral and central IR [3, 4]. The brain is one of the riches organs in polyunsaturated fatty acids (PUFAs) and a balanced ratio of n-3/n-6 PUFA (1:1–4) was shown to be essential to preserve our brain function and mental health [22]. In this study, we particularly were interested to observe the effect of CO-HFD (an n-6 PUFA) on rat's memory retention and hippocampal damage in rats. This was based on the big shift in our diet toward a diet that is rich in n-6 PUFA and poor in n-3 PUFA that is coincided with an increase in idiopathic chronic disorders such as cardiovascular disorders, diabetes mellitus, depression, psychological disorders, and dementia [22, 40].

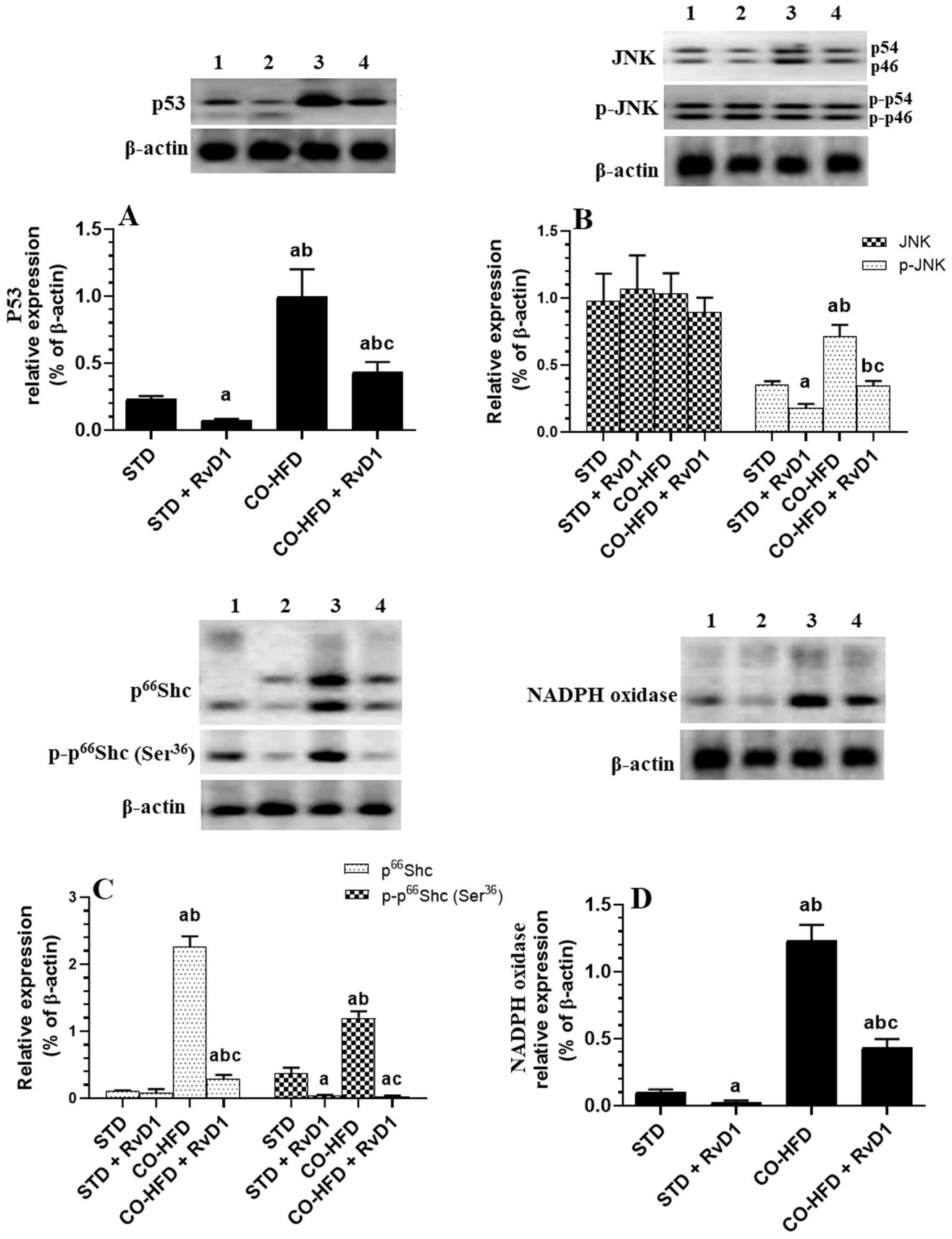
However, brain oxidative stress and inflammation and subsequent apoptosis remain the major key players behind HFD-induced hippocampal damage are believed to be major risk factors for cognitive deficits and development of dementia and AD in patients [3, 5, 7, 44–49]. Within this view, HFD-induced peripheral and brain IR is believed to be the major mechanism by which HFD affects our brain health and memory function [49]. Indeed, it has been suggested that HFD and through peripheral IR induces a peripheral inflammatory response in which the inflammatory cytokines and non-esterified fatty acids (NEFA) influx to the brain and cross the blood–brain barrier (BBB) to induce central inflammation and brain IR and exaggerate the production of ROS [3, 11]. Besides, the brain IR can accelerate the production of advanced glycation end products (AGEs) and stimulate the synthesis and accumulation of the ceramides and amyloid β-peptides, both of which can further increase the production of ROS through activation of NADPH [11].

Similar to these data, chronic feeding of rats with CO-HFD induced type 2 diabetes mellitus phenotype that is characterized by sustained hyperlipidemia, hyperinsulinemia, and peripheral IR. These effects were also associated with impaired memory retention, an increase in the hippocampal levels of ROS, pro-inflammatory cytokines (TNF-α and IL-6), and markers of intrinsic cell apoptosis including cleaved caspase-3, and cytochrome-c. Also, CO-HFD enhanced the hippocampal levels of NADPH oxidase, thus suggesting that activation of this enzyme is a major ROS-generating pathway in rats' hippocampi after chronic consumption of CO-HFD. Supporting these data, several authors have also shown that CO-HFD is associated with such metabolic phenotype and IR [40, 50, 51]. Besides, short or long term exposure to HFD impaired both short and long term hippocampal-dependent learning and memory in adult, juvenile and aged rats or mice [5, 6, 52, 53]. Furthermore, HFD-induced hippocampal and brain oxidative stress and apoptosis through activation of NADPH [54–57].

However, the mechanisms by which HFD induces oxidative stress and inflammation in different areas of the brain remain a matter of debate and still largely unknown. In addition to their effect on peripheral and central IR, numerous studies have shown that n-6 PUFA can incorporate into the cell membrane to dampen levels of n-3 PUFA, thus preventing their beneficial effects. Indeed, the adipogenic, obesity and inflammatory properties of n-6 PUFAs are believed to be mediated by decreasing cellular levels of n-3PUFA [58]. Also, the negative impact of n-6 PUFA on cognitive function is attributed to a decrease in the brain levels of DHA and eicosapentaenoic acid (EPA) [27, 28]. However, until now, studies on the effect of chronic administration of n-6 PUFA (e.g. CO) on hippocampal levels of n-3 PUFA are still lacking. In a single study, HFD reduced brain levels of DHA with a parallel reduction in the synaptic plasticity and impaired behavior [39]. Therefore, we have assumed that CO-HFD may also result in a similar effect in the hippocampi of the rats of this study.

One interesting observation in this study is that we have also found a significant decrease in levels of DHA and RvD1 in the brain of CO-HFD-fed rats. RvD1 is the most common class D-Series specialized pro-resolving mediators (SPMs) that is derived from the cellular catabolism of DHA [29, 59]. Therefore, the observed decrease in RvD1 in the hippocampi of CO-HFD-fed rats could be explained to be secondary to CO-HFD-induced decrease in DHA levels. However, previous studies have shown that the neuroprotective effect of RvD1 is mediated by the upregulation of Nrf2, a master transcription factor that acts as an antioxidant and anti-inflammatory factor that can stimulate the expression of many antioxidant genes and inhibit the activity of NF-κB [29, 31–35]. Of note, it was shown that the lower levels of RvD1 are associated with higher expression levels of NADPH oxidase, ROS generation, and lipid peroxidation in an animal model of atherosclerosis [36].

As expected and associated with the higher hippocampal levels of ROS, NADPH oxidase, TNF-α, IL-6, and lower



◂**Fig. 3** Protein levels of p53 (**A**), JNK/p-JNK (Thr^{183}/Tyr^{185}) (**B**), $p^{66}Shc/p\text{-}p^{66}Shc$ (Ser^{36}) (**C**) and NADPH oxidase (**D**) in the hippocampi of rats of all experimental groups. Data were considered significantly different at $P < 0.05$. Data are presented as mean ± SD of n = 6 rats/group. [a]Significantly different when compared with STD-fed rats (lane 1). [b]Significantly different as compared to STD + RvD1 (lane 2). [c]Significantly different when compared with CO-HFD-fed rats (lane 3). Lane 4 represents a protein sample taken from a CO-HFD + RvD1-treated rat. *STD* a standard diet, *RvD1* Resolvin D1, *CO-HFD* a high-fat diet rich in corn oil

levels of DHA and RvD1, the hippocampi of the CO-HFD-fed rats showed a concomitant decrease in the protein levels of Nrf2 and higher nuclear protein levels of p-NF-κB P65 (thus activation). Supporting these data, previous studies have shown that HFD induces hippocampal oxidative damage and decreases antioxidant levels through downregulation and inhibition of Nrf2 [1]. Also, HFD activated NF-κB and increased the production of the inflammatory cytokines in a variety of tissues including the brain [4]. Hence, it could be concluded that CO-HFD alters the activity of Nrf2 and NF-κB in rats' hippocampi by lowering the levels of RvD1.

To confirm this, we treated both the control and HFD-fed rats with exogenous RvD1, at a dose that has been previously shown to prevent neuroinflammation and motor function in a rat's model of Parkinson's disease [41]. Surprisingly, and independent of modulating body weights, fasting plasma levels of glucose and insulin, nor values of HOMA-IRI, RvD1 improved memory function in both control and HFD-fed rats and significantly increased the expression of Nrf2, and inhibited the activation of NF-κB and NADPH oxidase. This could explain why the hippocampi of these rats showed lower levels of ROS, MDA TNF-α, and IL-6 and higher levels of GSH, Bcl-2, and MnSOD. Besides, RvD1 significantly reduced cytochrome-c release in both the control and CO-HFD-fed rats. It also inhibited the activation of cleaved caspase-3 and improved the structural changes in the hippocampi of CO-HFD-fed rats. Based on these data, we became more confident that CO-HFD induces oxidative stress, inflammation, and apoptosis in rats' hippocampi, independent of obesity and IR, and directly, by lowering its content of DHA and RvD1, whereas exogenous administration of RvD1 is a neuroprotective agent that can reverse/prevent these events. In support, exogenous administration of RvD-1 inhibited hippocampal CA1 neural loss and prevented memory deficits in rats by inhibiting NF-κB [60]. Besides, nanogram (ng) doses of RvD1 exerted an antidepressing effect in rodents [37, 38].

On the other side, n-3 and n-6 PUFAs can directly regulate the activity of several transcription factors and the transcription of several genes [61, 62]. In this study, we have also aimed to investigate the effect of CO-HFD on the expression of one major pro-oxidant and apoptotic protein, named $p^{66}Shc$ [17]. We have chosen this protein rather than any other protein for many previous observations. First of all, it was shown that the brain levels of $p^{66}Shc$ are correlated negatively with the cognitive function [16, 17]. Also, it was shown that the activation of $p^{66}Shc$ is associated with neurodegeneration during different brain disorders and conditions including cerebral ischemia, diabetes-induced brain damage, and in AD animal model [14–16]. Genetic deletion of $p^{66}Sch$ in healthy animals or various animal models of oxidative stress-induced brain damage and memory loss (e.g. diabetes, stroke, AD) is associated with increased life span, less generation of ROS, neurogenesis, and improved memory function [13–17]. Furthermore, HFD-induced activation of NADPH oxidase and JNK in the cerebral cortex and hippocampi of animals [54–57]. Since NADPH is activated by $p^{66}Shc$ and JNK is a potent activator of $p^{66}Shc$ [12], it will be reasonable that HFD induces hippocampal injury and memory deficits through activation of JNK/$p^{66}Shc$-induced activation of NADPH oxidase.

However, $p^{66}Shc$ is indispensable for p53-induced mitochondria-mediated cell apoptosis [21]. Also, activation of $p^{66}Shc$ is induced by JNK and PKCβ through direct phosphorylation at its Ser^{36} [20, 21]. Once activated, $p^{66}Shc$ induces ROS and cell apoptosis by impairing mitochondria oxidative phosphorylation and membrane potential, the release of cytochrome-c, inhibition of antioxidant genes (SOD and CAT) through inhibition of FOXO-3a, and activation of NADPH oxidase [18, 19]. In this study, we are showing that CO-HFD can upregulate levels of p53 and $p^{66}Shc$ and increase the activation of both JNK and $p^{66}Shc$ (phosphorylation) in the hippocampi of rats. This data suggests that CO-HFD-induced activation of $p^{66}Shc$ is a possible mechanism by which CO-HFD upregulates NADPH oxidase and increases the cytoplasmic levels of cytochrome-c and the subsequent activation of caspase-dependent apoptosis.

In the same line, the deletion of $p^{66}Shc$ reduced systemic oxidative stress and vascular apoptosis and lowered the number of foam cells and atherosclerotic lesions in animal models of HFD and high sucrose-fed rats, as well as hypercholesterolemic apolipoprotein E Knockout mice [63]. Notably, the administration of RvD1 to control or CO-HFD significantly inhibited the activation of JNK and $p^{66}Shc$ and significantly lowered levels of p53 and $p^{66}Shc$. These findings could explain the previously reported data which failed to explain how RvD1 lower the activation of NADPH oxidase and further indicate an alternative anti-oxidant mechanism of RvD1 in the hippocampi of rats. However, further studies using transgenic animals or gene silencing are required to further support these findings.

In conclusion, the data presented in our hand indicate that the oxidant and inflammatory effects of CO-HFD in rats' hippocampi are associated with a reduction in the hippocampal levels of DHA and RvD1 with a concomitant increase in the levels/activity of Nrf2 and activation

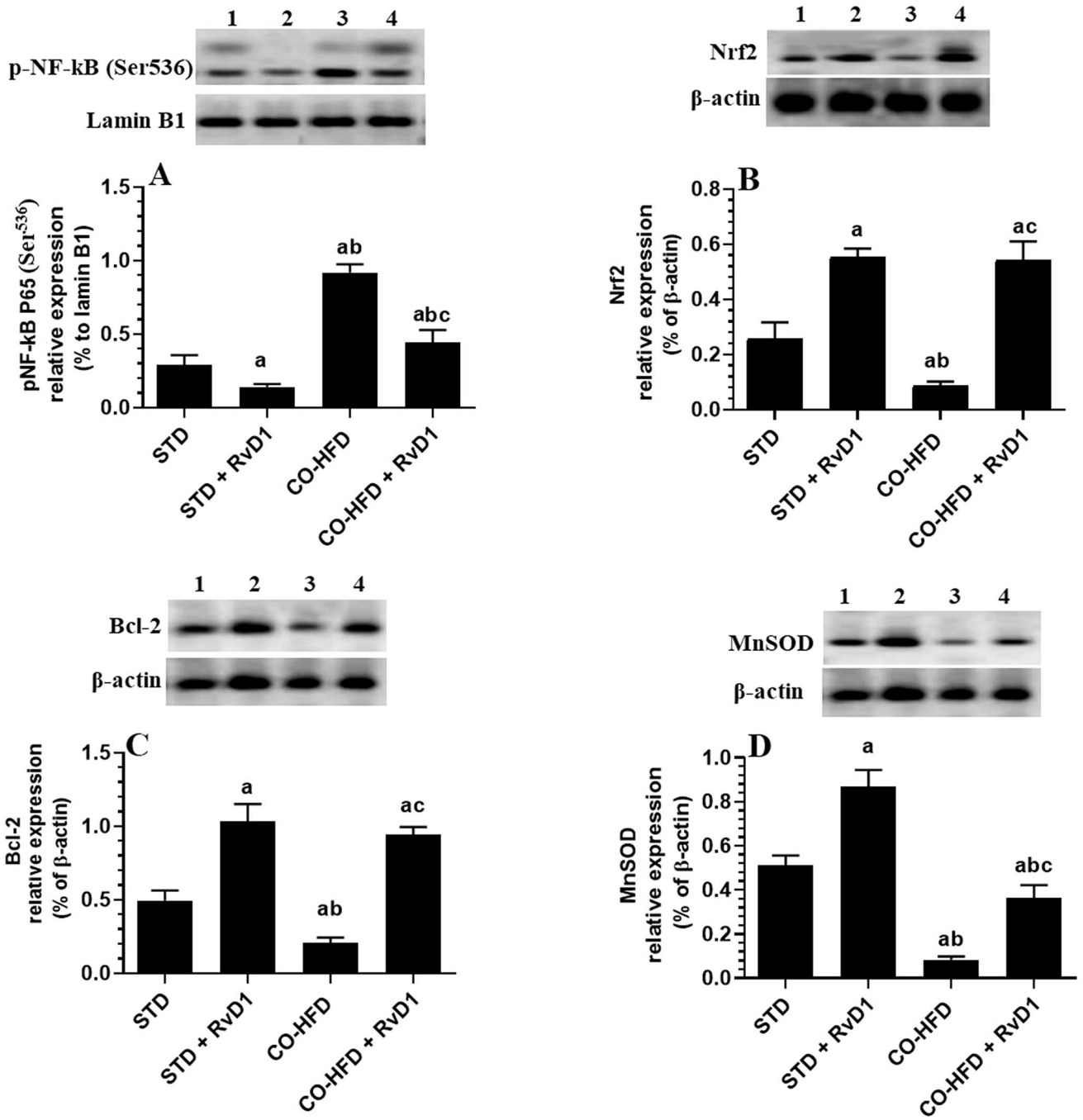


Fig. 4 Nuclear protein levels of p-NF-κB (Ser^{356}) (**A**) and total Nrf2 (**B**), Bcl-2 (**C**), and MnSOD (**D**) in the hippocampi of rats of all experimental groups. Data were considered significantly different at $P < 0.05$. Data are presented as mean ± SD of n = 6 rats/group. [a]Significantly different when compared with STD-fed rats (lane 1). [b]Significantly different as compared to STD + RvD1 (lane 2). [c]Significantly different when compared with CO-HFD-fed rats (lane 3). Lane 4 represents a protein sample taken from a CO-HFD + RvD1-treated rat. *STD* a standard diet, *RvD1* Resolvin D1, *CO-HFD* a high-fat diet rich in corn oil

of NF-Kb and p^{66}Shc. However, increasing hippocampal levels of RvD1 by exogenous administration could protect the hippocampi from the adverse effect of CO-HFD by reversing/preventing these events.

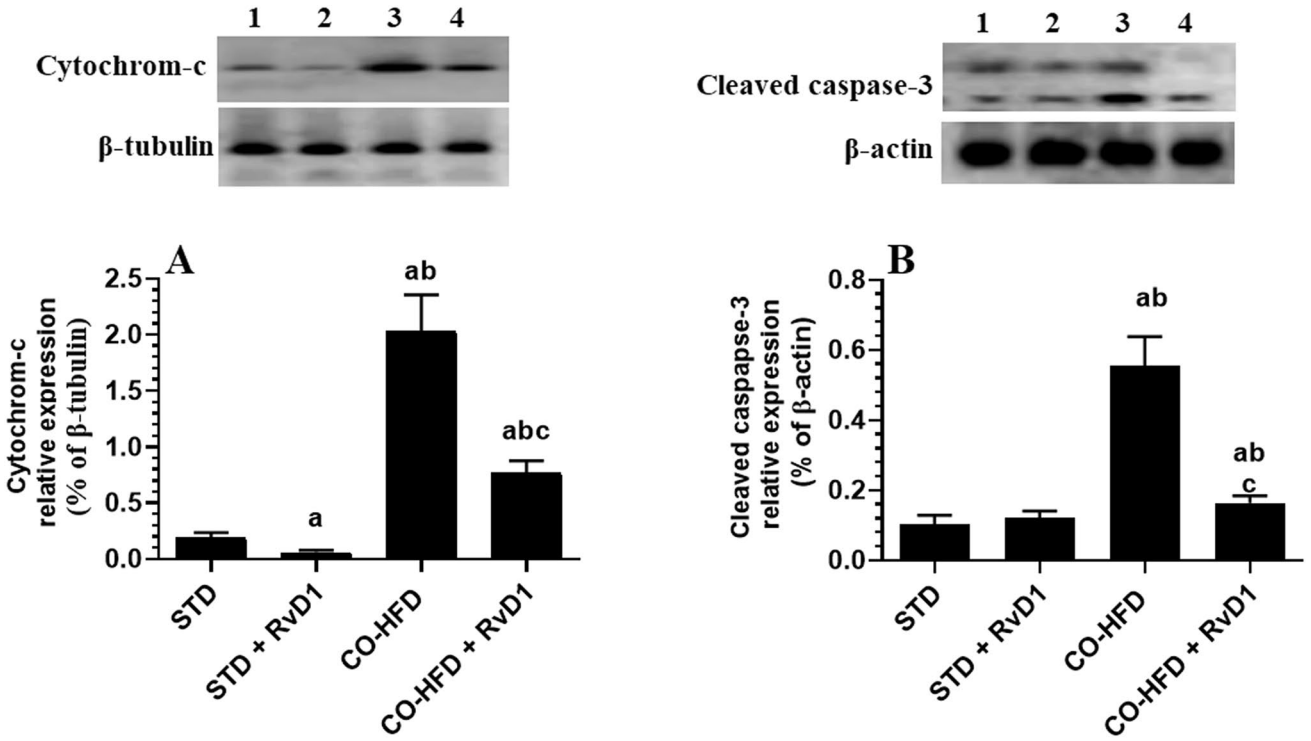


Fig. 5 Cytoplasmic protein levels of cytochrome-c (**A**) and total protein levels of cleaved caspase-3 in the hippocampi of rats of all experimental groups. Data were considered significantly different at $P < 0.05$. Data are presented as mean ± SD of n = 6 rats/group. [a]Significantly different when compared with STD-fed rats (lane 1). [b]Significantly different as compared to STD + RvD1 (lane 2). [c]Significantly different when compared with CO-HFD-fed rats (lane 3). Lane 4: represents a protein sample taken from a CO-HFD + RvD1-treated rat. *STD* a standard diet, *RvD1* Resolvin D1, *CO-HFD* a high-fat diet rich in corn oil

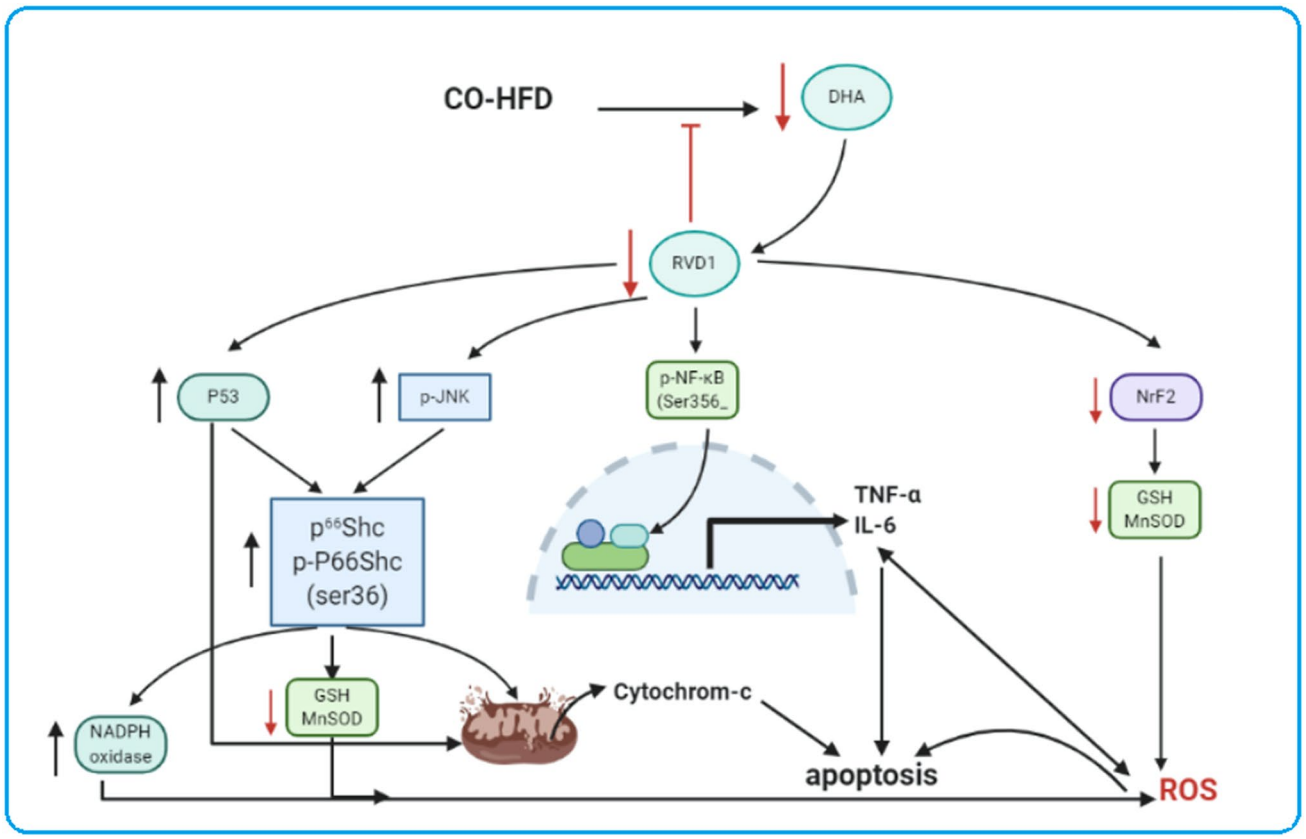


Fig. 6 A schematic presentation that outlines the possible mechanisms by which the high-fat diet rich in corn oil (CO-HFD) induces hippocampus oxidative stress, inflammation, and apoptosis and the protection role of Resolvin D1 (RvD1). In the hippocampus, CO-HFD reduces levels of docosahexaenoic acid. As a result, the conversion of DHA to RvD1 is inhibited. Accordingly, the decrease in RvD1 leads to decrease protein levels of Nrf2 which ultimately leads to decrease antioxidant synthesis and activation of NF-κB which stimulate the synthesis of inflammatory cytokines such as TNF-α and IL-6. Besides, the decrease in RvD1 upregulates and activates P66Shc by increasing levels of p53 and activation of JNK. In turn, P66Shc activates NADPH, suppresses antioxidants and stimulates the release of cytochrome-c. All these events together lead to the generation of ROS, inflammation, and apoptosis. However, the administration of RvD1 reverses all these events

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Author Contributions Dalia G. Mostafa, MD, is the principal investigator for the study who obtained the fund. She also participated in drafting and finalizing the manuscript and biochemical measurement. Huda H. Satti helped in material preparation, data collection, biochemical measurement, and analysis.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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