Original Research Article

The Anti-inflammatory and Anti-apoptotic Role of Crocin against Expression of MMp-9, TIMP-1, MCP-1, Caspase-3, PPARα in Heart Tissue, and Metamorphoses of MicroRNA 188 in Hyperhomocysteinemic Rats

The Anti-inflammatory and Anti-apoptotic Role of Crocin against Expression of MMp-9, TIMP-1, MCP-1, Caspase-3, PPARα in Heart Tissue, and Metamorphoses of MicroRNA 188 in Hyperhomocysteinemic Rats

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Abstract

Crocin (a water-soluble carotenoid) is the most abundant antioxidant among active constituents of *Crocus sativus*. Crocin has protective effects on brain, skeletal muscle, and kidney ischemia-reperfusion injury models via its antioxidant properties. The protective and anti-inflammatory effect of crocin against L-methionine-induced hyperhomocysteinemia (HHcy) in rats was evaluated. Thirty male albino rats were divided into three equal groups. Group I (control normal): rats fed ordinary diet without any treatment. Group II (L-methionine-induced HHcy): rats received L-methionine (1.7 g/kg body weight/day) orally for 8 weeks continuously. Group III (HHcy + crocin): rats received crocin in dose (50 mg/kg body weight/day, I.P.) for 4 weeks after induction of HHcy. The results obtained showed a significant increase in serum Hcy concentration and upregulation of NF-kβ gene expression in liver tissue and also in the gene expression level of TIMP-1, MMp-9, MCP-1, Caspase-3, and PPARα in heart tissues with significant downregulation of serum miRNA188 gene expression level in hyperhomocysteinemic (HHcy) rats. However, crocin treatment to HHcy induced in rats significantly improved all previous indicators to its normal ranges. The histopathological findings revealed that crocin treatment markedly reduced the HHcy-induced pathological changes and displayed marked improvement with normal histological architecture of the heart and brain tissues. These results suggested that crocin had a potentially ameliorating role with powerful anti-inflammatory and anti-apoptotic effects on cardiac tissue, as revealed by suppression of proinflammatory mediators and secretion through inhibition of NF-kB signaling pathways, which may lead to upregulated miR-188 gene expression, suppress proapoptotic Caspase-3, and may protect from heart tissue injury against HHcy.

Keywords: Hyperhomocysteinemia; Crocin; Proinflammatory mediators; MMp-9 and TIMP-1; MiR-188.

1. INTRODUCTION

Hyperhomocysteinemia is defined as a medical condition characterized by abnormally high levels (greater than 15 µmol/L) of homocysteine in the plasma [1]. Hyperhomocysteinemia can result from genetic impairment enzymes involved in homocysteine metabolism. The enzymes involved may be 5,10-methylene tetrahydrofolatereductase, methionine synthase, and cystathionine-β-synthase [2]. Homocysteine (Hcy) is an intermediate sulfhydryl-containing amino acid derived from methionine. Hcy has two fates: remethylation to methionine (with the ease of methionine synthase enzyme) or *trans* sulfurization to cysteine with cystathionine-β-synthase (CBS) [3]. An increase in reactive oxygen species (ROS) production caused by Hcy may cause subsequent oxidation of proteins, lipids, and nucleic acids [4], and may cause endothelial dysfunction and damage to the vessel wall with subsequent platelet activation and thrombus formation; the accumulation of oxidized biomolecules changes the biological functions of many cellular pathways. Hcy acts as powerful oxidizer -SH groups during the formation of reactive particles, such as superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2), mainly during its auto-oxidation [5].

Crocin is one of these components, which is a natural carotenoid found in saffron (*Crocus sativus* L.) and gardenia (*Gardenia jasminoides* J. Ellis) flowers. It is a compound formed by a disaccharide called gentiobiose and a carboxylic acid called crocetin, which is soluble in water, and is in diester form with high thermal stability [6]. The main active constituent of saffron is picrocrocin and its derivatives including safranal, flavonoid derivatives, and crocin [7]. Crocin pretreatment significantly prevented these increases. In agreement, it has been reported that crocin reduced the level of these enzymes

after nicotine-induced hepatic injury. Consequently, it has been reported that crocin reduces the level of these enzymes after nicotine-induced liver damage [8]. Therefore, crocin due to its antioxidant properties can protect liver cells from damage caused by oxidative stress. The action of crocin and crocetin in the manipulation of the inflammatory response has hardly been studied [9].

The treatment with crocin repaired the vascular injuries, inhibited MMP-9 expression, and reduced nitric oxide (NO) contents and nitric oxide synthase (NOs) activities. Consequently, it may be the mechanism by which crocin protects the brain against this reperfusion injury [10]. Moreover, pretreatment with crocetin resulted in a significant reduction of phospho-IκB expression and NF-κB activation by lipopolysaccharide (LPS), which is in accordance with reduced expression of TNF-α and MCP-1. This suggests that the suppressed expression of inflammatory cytokines might result from the reduced activation of NF-κB by crocetin [11].

Furthermore, crocin treatment to hyperhomocysteinemic rats resulted in a significant upregulation in serum gene expression level of miR-188; the preventive effects of crocin against ischemia-induced organ injuries could be led by its potent inhibition on NF-kβ activation, which has been extensively reported in studying the anti-inflammatory actions of crocin, based on the strong associations between ischemia, oxidative stress, and multiorgan injuries [12]. The purpose of this study was to explore the harmful effect of hyperhomocysteinemia and the possible cytoprotective effect of crocin and the molecular mechanism through which crocin results in the modulation of inflammatory responses, beneficial for the prevention and treatment of cardiovascular diseases. Moreover, its complications involved in hyperhomocysteinemia were to be clarified.

2. METHOD(S)

2.1. Experimental Animals
Thirty male albino rats aged 10-12 weeks and weighing 150-200 g were used in this study. The rats were kept in separate metal cages and kept in constant environmental and nutritional conditions throughout the experiment. The rats were fed a regular diet, and fresh clean drinking water was supplied *ad-libitum*. All rats were acclimated for a minimum period of 15 days before the start of the study. The experimental protocols were also approved by the Animal Care and Use Committee of the Benha University and comply with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Chemicals and Antioxidants
The chemicals and antioxidants used in this study were as follows:

(a) L-methionine was acquired from El Gomhouria for trading Chemicals and Medical Devices, Egypt. Methionine was dissolved in freshly prepared 1M HCL and orally administered at a dose of 1.7 g/kg b. wt/day for 8 weeks [13].
(b) Crocin was purchased from Sigma. It was dissolved in a physiological solution and administered daily at a dose of 50 mg/kg b. wt, I.P [14].

2.3. Experimental Design
The rats were randomly divided into three groups (10 rats each) placed in individual cages and were classified as follows:

Group I (normal control group): rats did not receive drugs, served as control, for all experimental groups.

Group II (L-methionine-induced hyperhomocysteinemia (HHcy): rats received L-methionine orally (1.7 g/kg b. wt/day) for 8 continuous weeks.

Group III (HHcy + crocin-treated group): rats received crocin once a day for 4 weeks at a dose of 50 mg/kg body weight/I.P after induction of hyperhomocysteinemia.

2.4. Sampling
2.4.1. Blood Samples
Blood samples were collected by puncture of the eye veins in dry, clean test tubes and allowed to clot for 30 minutes, and the serum was separated by centrifugation at 3,000 rpm for 15 minutes. The serum was taken using an automatic pipette in dry sterile tubes and then stored in a freezer at $-80°C$ until use for determination of Hcy and MicroRNA 188 gene expression.

2.4.2. Tissue Samples

a. **For molecular analysis (liver and heart):**
About 0.5 g of liver and heart tissue was put in eppendorf tubes and immediately kept in liquid nitrogen and stored at $-80°C$ till RNA extraction for determination of liver NF-kβ and heart tissue TIMP-1, MMp-9, Caspase-3, PPARα, and MCP-1 gene expression.
b. **For histopathological examination (heart and brain):**
A sample of each tissue was excised and fixed in 10% neutral buffered formalin solution and subjected to histo-pathological examination according to the technique described by Bancroft and Stevens [15].

2.5. Biochemical Analysis
Determination of serum homocysteine level:
Serum Hcy was determined according to the method described by rat homocysteine (Hcy) ELISA kit (Cat#MBS703069, MyBioSource, China).

2.6. Molecular Analysis
Determination of liver NF-kβ, and heart TIMP-1, MMp-9, Caspase-3, PPARα, and MCP-1 gene expression:
The mRNA expression contents of NF-kβ, TIMP-1, MMp-9, Caspase-3, PPARα, and MCP-1 were determined using real-time quantitative polymerase chain reaction analysis (real-time qPCR) in rat liver. β-actin was used as load control. Total RNA was isolated from the liver and heart using High Kit for isolation of pure RNA (iNtRON Biotechnology, easy-REDTM Total RNA Extraction kit) according to the manufacturer's instructions. With each cDNA sample was reverse transcribed using RevertAid TM First Strand CDNA synthesis kit (#EP0451, Thermo Scientific, Fermentas, USA). Then, real-time quantitative PCR amplification was performed on Faststart Universal SYBR Green Master (Roche, GER). The target gene was normalized with β-actin by the $2^{-\Delta\Delta Ct}$ method [16].
Determination of serum miRNA188 gene expression:
miRNA was extracted from serummirVana PARIS kits (Ambion, Life Technologies, USA, #AM1556). cDNA was synthesized from Quanti-Mir RT kit (SBI, System Biosciences, Cat. # RA420A-1). Real-time PCR with SYBR Green was used to measure expression of miRNA188 in the serum, with miRNA16 as an internal reference. The isolated cDNAs were amplified using 2X Maxima SYBR Green/ROX qPCR Master Mix following the manufacturer protocol (# K0221, Thermo scientific, USA) and miRNA specific forward primer (Table 2) and a universal reverse primer included with the purchase Quanti-Mir RT kit. To ensure primer sequence is unique for the template sequence; we checked similarity to other known sequences with BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi).

2.7. Statistical Analysis
Results were expressed as mean and standard error ($\pm$SE) using SPSS(13.0 software, 2009). Data were analyzed using one-way ANOVA [17] followed by Duncan's test. Values were statistically taken into account significantly at $p < 0.05$.

3. RESULTS

3.1. Molecular Analysis Results
The data presented in Table 3 showed a significant increase in serum homocysteine concentration and significant upregulation in heart tissue Caspase-3 and MCP-1 gene expression levels in L-methionine-induced HHcy in rats compared with the control

Table 1: Forward and reverse primers sequence for real-time PCR.

Gene	Forward primer (′5 ------ ′3)	Reverse primer (′5 ------ ′3)
PPARα	AATCCACGAAGCCTACCTGA	GTCTTCTCAGCCATGCACAA
MCP1	TCGCTTCTGACACCATGCA	TGCTACAGGCAGCAAATGTGA
Caspase-3	GGTATTGAGACAGACAGTGG	CATGGGATCTGTTTCTTTGC
MMP9	TCGAAGGCGACCTCAAGTG	TTCGGTGTAGCTTTGGATCCA
TIMP1	CGCAGCGAGGAGGTTTCTCAT	GGCAGTGATGTGCAAATTTCC
NF-kB	CCTAGCTTTCTCTGAACTGCAAA	GGGTCAGAGGCCAATAGAGA
β-actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

Table 2: Forward primers sequence for real-time PCR.

Gene	Primer sequence (′5 ------ ′3)
miRNA188	CATCCCTTGCATGGTGGAGGG
miRNA16	CGGTAGCAGCACGTAAATATTGGCGA

Table 3: Effect of crocin treatment on serum homocysteine concentration and heart tissue Caspase-3 and MCP-1 gene expression levels in HHcy induced in rats.

Parameters Exp. Groups	Homocysteine (nmol/ml)	Fold change in Caspase-3 gene expression	Fold change in MCP-1 gene expression
Group I: Normal control	10.24 ± 0.33[e]	1.00 ± 0.02[c]	1.00 ± 0.02[c]
Group II: (HHcy)	32.35± 0.72[a]	3.68± 0.12[a]	4.56± 0.12[a]
Group III: HHcy + Crocin	18.45±0.02[c]	2.10±0.10[b]	1.31±0.09[c]

Mean values with different superscript letters in the same column are significantly different at ($p \leq 0.05$).

Table 4: Effect of crocin treatment on liver tissue NF-kβ and serum miRNA188 gene expression levels in HHcy induced in rats.

Parameters Exp. groups	Fold change in NF-kβ gene expression	Fold change in micro RNA188 gene expression
Group I: Normal control	1.00 ± 0.02^e	1.00 ± 0.03^a
Group II: (HHcy)	13.55 ± 0.47^a	0.01 ± 0.001^e
Group III: HHcy + Crocin	3.34 ± 0.13^d	0.43 ± 0.02^c

Mean values with different superscript letters in the same column are significantly different at $P \leq 0.05$.

Table 5: Effect of crocin treatment on heart tissue TIMP-1, MMp-9, and PPARα gene expression levels in HHcy induced in rats.

Parameters Exp. groups	Fold change in TIMP-1 gene expression	Fold change in MMp-9 gene expression	Fold change in PPARα gene expression
Group I: Normal control	1.00 ± 0.03^d	1.00 ± 0.02^d	1.00 ± 0.03^d
Group II: (HHcy)	8.17 ± 0.26^a	4.14 ± 0.12^a	5.46 ± 0.26^a
Group III: HHcy + Crocin	2.31 ± 0.11^c	2.28 ± 0.11^c	$2.99 \pm 0.12^{b,c}$

Mean values with different superscript letters in the same column are significantly different at $p \leq 0.05$.

normal group. However, crocin treatment to HHcy induced in rats caused a significant decrease in elevated serum homocysteine level and significantly downregulated heart tissue Caspase-3 and MCP-1 gene expression levels compared with the L-methionine-treated group.

The results obtained in Table 4 revealed that rats with HHcy showed significant upregulation in liver tissue NF-kβ gene expression with downregulation of serum miRNA188 gene expression levels when compared with the normal control group. On the other hand, crocin treatment to HHcy male rats caused significant downregulation in liver tissue NF-kβ and significantly upregulated in serum miRNA188 gene expression when compared with HHcy group.

The current results presented in Table 5 exhibited a significant upregulation in heart tissue TIMP-1, MMp-9, and PPARα gene expression levels in L-methionine-treated rats when compared to the normal control group. Meanwhile, crocin treatment to HHcy male rats caused significant downregulation in heart tissue gene expression level of TIMP-1, MMp-9, and PPARα when compared with HHcy group.

3.2. Histopathological Examination
Various histopathological changes were demonstrated in the heart, blood vessels, and brain of rats treated with L-methionine (1.7 g/kg b. wt, orally) for 8 weeks. The microscopical examination of the heart showed severe congestion of the myocardial blood vessels (Figure 1a). Also, degenerative change in the wall of myocardial blood vessels that represented proliferation of their endothelial cell lining and vacuolation in the tunica media was demonstrated (Figure 1b). Moreover, focal areas of degeneration in the cardiac muscle with intermuscular hemorrhage were also detected (Figure 1c). Additionally, myomalcia of the myocardium in association with vacuolation in their sarcoplasm was observed (Figure 1d).

However, the microscopical examination of the blood vessels of rats showed a severe degree of degenerative changes on the blood vessels' wall with prevascular hemorrhage in association with vacuolation of the tunica media of the wall of blood vessels (Figure 1e). Additionally, rupture in the wall of the blood vessels was also noticed (Figure 1f). Meanwhile, the histopathological alteration in the brain of rat in this group represented mainly a severe degree of meningitis characterized by severe congestion of meningeal blood vessels infiltrated with few leucocytes in combination with degeneration, and hyalinization in the wall of the meningeal blood vessels was noticed (Figure 2a). Perivascular hemorrhage was also seen. Moreover, scattered areas of hemorrhage in association with encephalomalcia were also detected in the brain tissue (Figure 2b). Moreover, degenerated and necrotic neurons with apoptotic changes were also detected (Figure 2c). Additionally, the focal area of gliosis characterized by degenerated neurons in association with glial cells' infiltration in brain substance was observed (Figure 2d). In addition, neural degeneration characterized by the neuron becomes rounded, more esinophilic, and swollen with either pyknotic nucleus or entirely loss of nucleus (Figure 2e).

In the meantime, the microscopical examination of heart, blood vessels, and brain of rats treated with crocin for 4 weeks revealed marked improvement in comparison to L-methionine-treated rats. Histopathological examination of the heart obtained from rats in this group displayed congestion of the myocardial blood vessels (Figure 3a). Moreover, intermuscular hemorrhage was also detected in some examined rats in association with degeneration of myocardium (Figure 3b).

Moreover, extensive degeneration of the tunica intema with vacuolation of sarcoplasm of tunica media with myomalacia was demonstrated in the wall of the examined blood vessels (Figure 3c). The examined brains of the rats of this group

Figure 1: H&E stained sections of heart tissue (a–d) and blood vessels (e–f) taken from L-methionine-treated rat for 8 weeks (Group II) showing (a) severe congestion of the myocardial blood vessels (arrow, 200×), (b) degenerative change in the wall of cardiac blood vessels, represented by proliferation of endothelial lining (arrow) and vacuolation in its tunica media (zigzag arrow, 200×), (c) extensive degeneration of the cardiac muscle (arrow) with intermuscular hemorrhage (asterisk, 200×), (d) myomalcia of the cardiac muscles (asterisk) and vacuolation in their sarcoplasm (arrow, 400×), (e) marked degeneration (asterisk) and vacuolation (arrow) of the tunica media. Notice also perivascular hemorrhage (zigzag arrow, 200×), and (f) extensive degree of degeneration and rupture in the blood vessels wall (arrow, 400×).
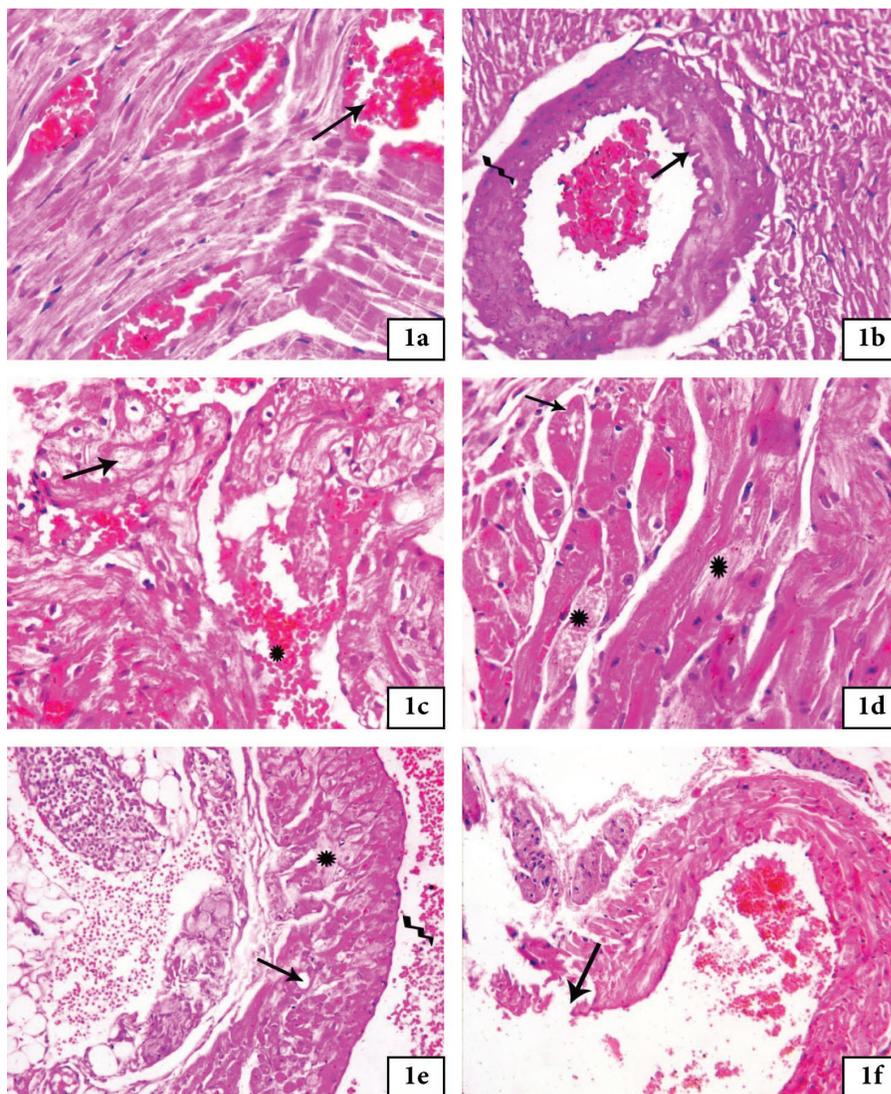
showed mild congestion of the blood vessels with degeneration of the blood vessels' wall. Moreover, neural degeneration and necrosis were demonstrated in some examined rats (Figure 3d, e).

4. DISCUSSION

Hyperhomocysteinemia can be caused by a genetic lack of methionine and homocysteine metabolism, including cystathion b-synthase, methionine synthase, and methylenetetrahydrofolate reductase (MTHFR) [18]. A significant increase in serum homocysteine and in heart Caspase-3 and MCP-1 gene expression levels were observed in L-methionine-induced HHcy in rats. Similarly, Kapoor and Sanyal [19] established that high concentrations of Hcy induced apoptotic molecules and activated Caspase-3

Figure 2: H&E stained sections of brain tissue taken from L-methionine-treated rat for 8 weeks (Group II) showing (a) severe degree of meningitis with degeneration and hyalinization of the wall of meningeal blood vessels (arrow, 400×), (b) scattered area of hemorrhage (arrow) and encephalomalcia (200×), (c) necrotic neurons (asterisk) and apoptosis (arrow, 200×), (d) focal area of gliosis in the brain substance (arrow, 200×), (e) tyrgylosis, as the neuron become rounded more esinophilic and swollen with loss nucleus (arrow, 200×)
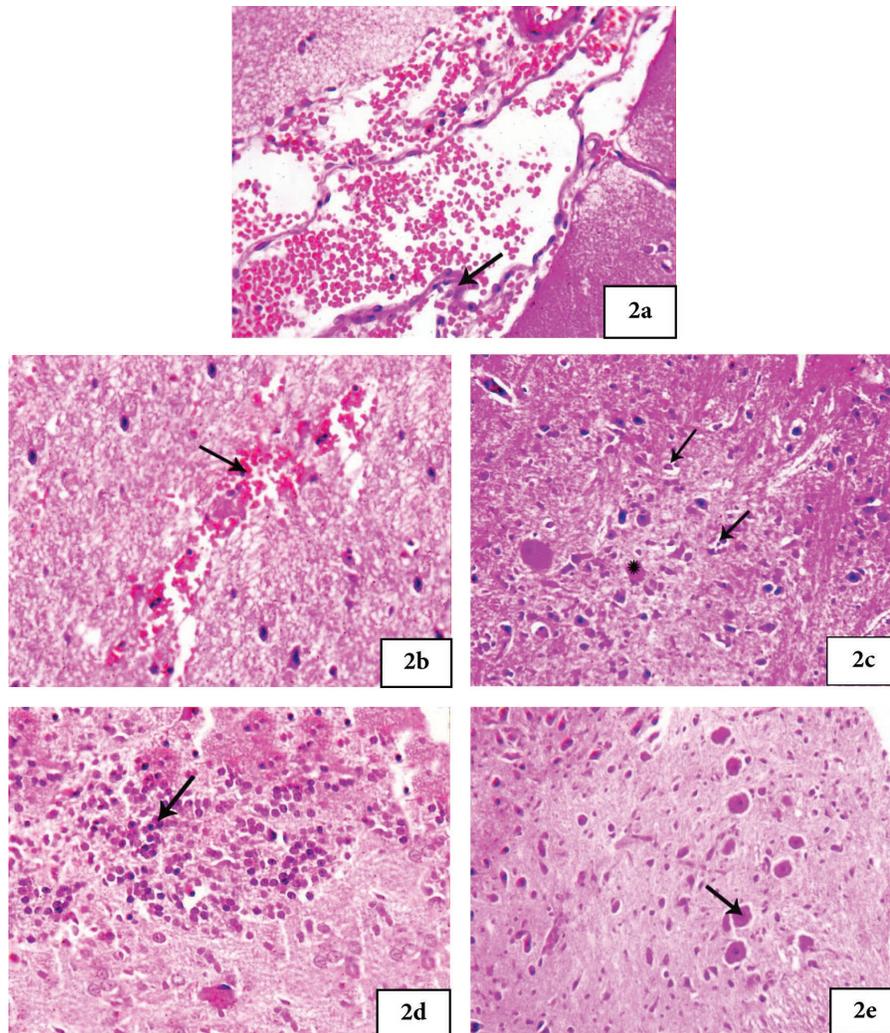
expression. Also, Chan and Mattson [20] observed that the Hcy-induced NF-kβ activation mediated the increase in early protein markers of apoptosis onset, such as Caspase-3. Moreover, Collins and Cybulsky [21] showed that homocysteine-induced expression of MCP-1 in monocytes and endothelial cells occur through activation of NF-kβ, a transcription factor involved in mediating downstream inflammatory processes. Ross [22] reported that homocysteine induces expression of MCP-1 through activation of NF-kβ, a transcription factor known to stimulate the production of chemokine, cytokines, hempoetic growth factors, and leukocyte adhesion molecules, which are considered to be involved in atherogenesis and vascular inflammation; the development and progression of atherosclerosis is considered a form of chronic inflammation, and endothelial dysfunction is the key process promoting inflammatory reactions. Furthermore, Poddar et al. [23] reported that homocysteine has been shown to increase the expression of IL-8; HHcy stimulated the expression of MCP-1 in rats leading to an increase in the adhesion of monocytes to the aortic endothelium. Such an effect may make a significant contribution to the development of atherosclerosis, contributing monocyte/macrophage infiltration into the arterial wall [24].

Crocin treatment to hyperhomocysteinemic rats resulted in a significant decrease in serum Hcy level and downregulation in gene expression level of Caspase-3 and MCP-1 in heart tissue. Nearly similar results were shown by Rinki [25] who stated that a significant decrease of homocysteine level was observed in saffron-treated HHcy rats. Crocin inhibited CCL4-induced inflammation indicated by abrogation of CCL4-induced elevation of plasma IL-6 and TNF-α levels and inhibition of Caspase-3

Figure 3: H&E stained sections of heart tissue (a–b), blood vessels (c), and brain (d–e) taken from crocin-treated rats after induction of HHcy by L-methionine for 4 weeks (Group III) showing (a) showing congestion of the myocardial blood vessels. H&E stain (200×), (b) showing hemorrhage in-between the cardiac muscles (arrow). H&E stain (200×), (c) marked degeneration of tunica intema (arrow) with vacuolation and myomalacia in blood vessels wall (asterisk). H&E stain (200×), (d) mild congestion of the blood vessels and degeneration of the blood vessels wall (arrow). H&E stain (200×), (e) neural degeneration and necrosis (arrow) H&E stain (200×).
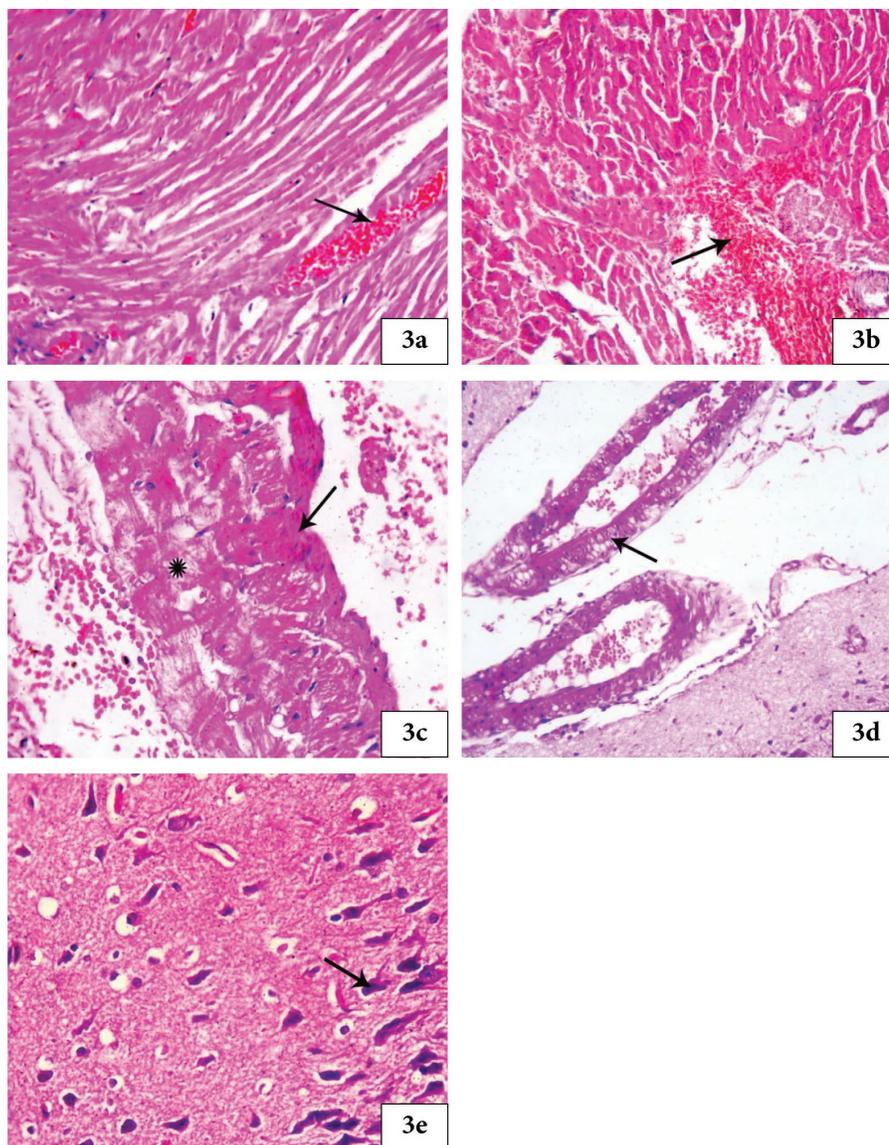
activity, an effect that protects liver cells from death [26]. Also, it is plausible that antioxidant, radical scavenging effects, and anti-inflammatory actions of crocin interfered with CCL4-induced elevation of Caspase-3 activity, and hence the liver cells were preserved, liver weight was normalized, and functions were restored [27].

A significant upregulation in liver tissue NF-kβ and downregulation in serum miRNA188 gene expression levels were observed in the HHcy group. These results are nearly similar to Jablonski et al. [28], who indicated that activation of NF-kβ has been shown to cause endothelial dysfunction in HHcy. Also, Mishra et al. [29] recorded the involvement of miRNAs and dicer in Hcy-mediated heart failure. The functional assay using miR-188 mimic for ameliorating Hcy-induced cardiac remodeling will provide concrete support to the present results considering the tremendous therapeutic potential of miRNAs. Additionally, Fang and Davies [25] demonstrated that, particularly, the importance of miRNA as a contributing risk factor in the pathogenesis of atherosclerosis has been well documented; in addition, miR188 has been shown to be involved in Hcy-induced cardiac remodeling. Furthermore, miR-188 in the smooth muscle cells of the human airway is downregulated by a proinflammatory stimulus

and is considered to act as a regulator of the inflammatory response [30]. Intriguingly, miR-188 could affect TNF-α related apoptosis-inducing ligand (TRAIL)-induced apoptotic pathways through blocking Caspase-3 activation [31].

Crocin treatment to hyperhomocysteinemic rats resulted in a significant upregulation in serum miR-188 gene expression level. Similarly, Hemshekhar et al. [12] showed that the preventive effects of crocin against ischemia-induced organ injuries could be led by its potent inhibition on NF-kβ activation, which has been extensively reported in studying the anti-inflammatory actions of crocin. As shown in this study, crocin treatment to HHcy caused upregulation of miR-188 level, as crocin caused downregulation of NF-kβ and TNF-α. The blockade of NF-kβ signaling by crocin may be correlated with a reduction of the early immediate response genes that facilitate the rapid production of NO and prostaglandins that initiate the lethal effects of hemorrhagic shock (HS) resuscitation [32]. The downstream induction of inflammatory cytokines, which can play a catalyzing role for tissue damage during the resuscitation phase, can be greatly attenuated by crocin via NF-kβ suppression [33]. Moreover, 2-arachidonoylglycerol (2-AG) and PPARγ activation suppressed NF-kβ reporter activity, suggesting that inhibition of NF-kβ by 2-AG would relieve the inhibitory effect of NF-kβ on miR-188-3p transcription. This assumption is supported by the results showing that expression of miR-188-3p was suppressed by amyloid-β (Aβ) and proinflammatory cytokines that increase phosphorylation of NF-kβ, and the suppression was reversed by 2-AG, PPARγ activation, or NF-kβ inhibition [34].

A significant upregulation in heart tissue TIMP-1, MMp-9, and PPAR-α was observed in HHcy rats. These results are nearly identical to Feng et al. [35] who demonstrated that HHcy increased the expression of MMP-9 and TIMP-1.The imbalance between MMPs and TIMPS may lead to vascular pathological remodeling by altering the collagen/elastin content ratio [36]. Also, Smeets et al. [37] showed that PPAR-α, the predominant PPAR-α isoform in the heart, has been implicated in hypertrophic signaling. The absence of PPAR-α results in a more pronounced hypertrophic growth response and cardiac dysfunction, which are associated with the increased expression of inflammatory markers and extracellular matrix remodeling. Furthermore, Hcy caused endothelial cell apoptosis in part by generating ROS; this, in turn, released cytochrome-c and activated the intracellular caspase proteolytic cascades, leading to TIMP-1, MMp-9, and PPARα activation and nuclear fragmentation. Elevated levels of Hcy are an independent risk factor for cardiovascular diseases [38]. Because endothelial cells are lacking the CBS [39], an enzyme responsible for Hcy clearance, endothelial cells are the prime target for Hcy toxicity. Previously, we have found that Hcy induced generation of ROS production by upregulation of NADPH oxidase and downregulation of thioredoxin [40].

Crocin treatment to HHcy rats caused significant downregulation in heart tissue TIMP-1, MMp-9, and PPARα gene expression levels. These results are almost the same with Zheng et al. [10] who documented that administration of crocin repaired the vascular injuries, inhibited MMP-9 expression, and reduced NO contents and NOs activity. Moreover, crocin has an important contribution to the ischemic blood brain barrier (BBB), which is due to the inhibition of the extracellular-signal-regulated kinase (ERK) pathway and ultimate downregulation of MMPs.

5. CONCLUSION

It could be established that a marked increase in heart tissues MMP-9, TIMP-1, PPAR-α, MCP-1, and Caspase-3 gene expression levels and downregulation in serum miRNA-188 gene expression were observed in a rat model of HHcy. This suggests the importance of miRNA as a contributing risk factor in the pathogenesis of atherosclerosis and the involvement of miR188 in Hcy-induced cardiac alterations. Moreover, crocin has a protective anti-inflammatory effect through inhibition of NF-kβ signaling pathways that may lead to upregulated miR-188 gene expression. With respect to cardiac tissue apoptosis, crocin suppressed proapoptotic marker Caspase-3 and successively depressed MMP-9, TIMP-1, PPAR-α, and MCP-1 gene expression and could be applicable as a cytoprotective and may alleviate the undesirable effects mediated by hyperhomocysteinemia and its complications.

Author Contributions
All authors contributed equally to this study.

Conflict of Interest
None.

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