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" *Crocin mitigates liver Impairment in diabetic rats by Upregulation of Nrf2/HO-1 signaling pathway* "

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Abstract

Background: The most prevalent kind of diabetes is type 2 diabetes mellitus. Chronic liver damage is a problem that frequently accompanies diabetes mellitus. Crocin is believed to possess antioxidant and anti-inflammatory properties.

Objective: to demonstrate the potential underlying processes and hepatoprotective impact of Crocin in DM.

Material and methods: Diabetic, Diabetic+Crocin, and control (10/group) were the three groups into which thirty male albino rats were divided. Assessments were made of the following: The measured serum glucose, serum insulin, HOMA-IR index, serum glycosylated Hb A1c, serum lipids, serum liver enzymes, hepatic MDA, hepatic SOD, hepatic TNF-α, hepatic IL-6, hepatic IL-10, hepatic Nrf2 gene expression and hepatic HO-1 gene expression. Furthermore, histological and immunohistochemical examinations of the liver were carried out.

Results: The measured serum glucose, serum insulin, HOMA-IR index, serum glycosylated Hb $A1_c$, serum cholesterol, serum triglyceride, serum ALT, serum AST, serum ALP, serum GGT, hepatic MDA, hepatic TNF-α and hepatic IL-6 were all markedly raised in Diabetic group compared to control, while the Diabetic 's hepatic SOD, hepatic IL-10 and hepatic gene expression of Nrf2 and HO-1 were substantially lower than control group. Additionally there was dramatically downregulated hepatic NF-kB and caspase-3 immunoreaction of Diabetic group compared to control. Crocin significantly mitigated diabetic induced hepatic changes.

Conclusion: By upregulating the hepatic Nrf2/HO-1 signaling pathway and exhibiting lipid-lowering, anti-inflammatory, antiapoptotic, and antioxidant properties, crocin guards against diabetes-induced liver damage.

KeyWords: Crocin, Diabetis Mellitus, HO-1, Liver, NF-kB, Nrf2

Introduction

About 95% of all forms of diabetes mellitus are type 2 diabetes mellitus (T2DM), making it the most prevalent kind of the disease (Ahmed et al., 2023).

One sign of diabetes mellitus is hyperglycemia. Chronic liver damage is one of the several consequences that diabetes mellitus frequently brings with it, and it has grown to be a major health risk. According to WHO data, the number of adults with diabetes has nearly tripled since 1980, reaching 422 million. By 2045, there will likely be 693 million people with diabetes globally, with 90–95% of those individuals having type 2 diabetes (T2DM) (Cho et al., 2018). Peripheral insulin resistance (IR), poor control of hepatic glucose synthesis, and diminished β-cell activity are the causes of type 2 diabetes (T2DM), which eventually results in the gradual degeneration of pancreatic β-cells. According to reports, IR brought on by T2DM might exacerbate hyperglycemia and result in several types of liver damage (Xia et al., 2020).

Hyperglycemia in diabetes mellitus is linked to metabolic dysfunctions of fat, protein, and carbohydrates that interfere with the regular functioning of several organs. Chronic hyperglycemia develops gradually and causes microvascular and macrovascular problems that impact the liver and other organs (Alqahtani et al., 2023)

The liver is IR's primary target organ, and it is vulnerable to hyperglycemia, which can cause metabolic irregularities as well as T2DM liver damage, which exacerbates IR. T2DM is closely associated with inflammation and oxidative stress. The main pathogenic processes causing liver damage are inflammation, oxidative stress, and peripheral IR (Ouyang et al., 2024). A significant number of ROS and inflammatory cytokines are generated as a result of rising peripheral blood glucose levels and fatty metabolites building up in the liver, which leads to oxidative stress and inflammation and ultimately liver damage (Farzanegi et al., 2019). A high level of inflammatory cytokines can exacerbate T2DM liver injury by promoting the polarization of Kupffer cells in the liver, which in turn can increase macrophage infiltration and proinflammatory cytokine release. and ROS, which further aggravates liver injury (Ouyang et al., 2024).

There is currently no proven cure for type 2 diabetes-related liver damage. Therefore, it is essential to investigate a safe and efficient way to treat and prevent liver damage brought on by type 2 diabetes.

Pro-inflammatory cytokine expression and the regulation of inflammation and innate immunity depend on NF-κB. According to a prior study, NF-κB activation is a critical step in the early pathophysiology of diabetes (Alqahtani et al., 2023). Moreover, hepatic insulin sensitivity is enhanced by NF-κB inactivation in hepatocytes (Ke et al., 2015).

A crucial transcription factor that controls oxidative stress responses and is essential in diabetes mellitus is Nrf2. In order to reduce oxidative stress injury, Nrf2 activation can then increase the expression of antioxidant factors such HO-1 (Krisnamurti et al., 2022).

Nrf2 activation was crucial in shielding the liver from oxidative damage. Additionally, it has been shown that Nrf2 pathway activation improves lipid metabolism in NAFLD and reduces oxidative stress and proinflammatory response (Zeng et al., 2021).

Redox hemostasis is primarily regulated in the nucleus in both normal and oxidative conditions, and the Nrf2/HO-1 signaling pathway is a key mediator for modulating these responses. The level of inflammatory mediators, such NF-κB, which are necessary for hepatocyte regeneration and repair, deviates when cellular redox equilibrium is compromised. Consequently, activation of (Nrf2/HO-1) must be

accomplished in order to prevent detrimental oxidative circumstances and to restore redox balance (Sedik & Amer, 2022).
Numerous natural substances that activate Nrf2 shielded animals from liver damage by reducing oxidative stress (Al-Amarat et al., 2021).

Some of the medications frequently used to treat type-2 diabetes include insulin, glitazones, linagliptin, metformin, and empagliflozin. Nevertheless, it is still unclear if these medications can treat β cell dysfunction. Additionally, they have been linked to a number of adverse effects, including lactic acidosis, weight gain, stroke, GI distress, and hypoglycemia. Therefore, phytochemicals derived from natural sources are now the main focus in the search for complementary and alternative treatments (Bathaie & Mousavi, 2010).

For a variety of severe illnesses, medicinal plants with biological selectivity and bioactive compounds are valuable sources of alternative therapeutic approaches. Researchers studying plants have been interested in nutraceuticals because of their special qualities, which hold promise for treating a number of illnesses, including diabetes. The major ingredient in saffron, crocin, has generated a lot of interest in scientific studies because of its antioxidant properties (Bathaie & Mousavi, 2010).

Crocus sativus L., or saffron, contains crocin, an abundant antioxidant that has a range of pharmacological effects on different tissues. Saffron and its main component, crocin, have been shown in numerous studies to have hypoglycemic effects (Ghorbanzadeh et al., 2016).
Crocin possesses antioxidant, anticancer, and antiulcer properties. The inflammatory reaction was reduced with crocin. Although there is little information on how Crocin affects type 2 diabetes, few experimental studies have shown that it lowers blood sugar levels and has antihyperlipidemic and antioxidant effects in STZ diabetic animals (Asdaq et al., 2024).

Crocin has strong antioxidative properties and pharmacologic actions. This chemical molecule has a strong scavenging ability that stops oxidative damage and neutralizes the actions of free radicals. Additionally, it has the ability to enhance the cellular antioxidants. (Yaribeygi et al., 2021).

This motivated us to carry out this investigation in order to show the possible hepato-protective impact of crocin in liver damage brought on by type 2 diabetes as well as the various underlying mechanisms associated with referral to the Nrf2/HO-1 signaling pathway.

Materials and methods

Experimental animals

The smallest sample size, is thirty rats. The study has a 95% confidence level and 80% power. ARRIVE guidelines were adhered to throughout the experimental procedures, and thirty mature Wister male rats weighing between 100 and 150 g were used in the study after obtaining the necessary approvals from the Faculty of Medicine's Research Ethics Committee at Menoufia University, Egypt, IRB No. 6/2025ANAT2. The rats were given regular access to food and water.

Experimental groups

1. Control group: For eight weeks, the rats were given ordinary chow along with a single intraperitoneal (i.p.) injection of 1 milliliter of citrate buffer and 1 milliliter of normal saline once daily.

2. Diabetic group: Rats were given free access to a high-fat diet for two weeks, consisting of 58% fat, 25% protein, and 17% carbohydrates as a percentage of total kcal. After 12 hours of fasting, they received a single i.p. injection of STZ at a dose of 35 mg/kg BW dissolved in citrate buffer. In order to avoid hypoglycemia, a 5% glucose solution was then administered. One touch glucose strips and a glucometer (ACCU-CHEK) were used to measure the fasting blood glucose level by tail prick 72 hours after the STZ injection. Rats chosen for the study had a blood sugar level of greater than 200 mg/dL, which was deemed diabetes (Srinivasan et al., 2005). For eight weeks, the rats in this group were given free access to a high-fat diet and were given 1 milliliter of normal saline intraperitoneally once a day.

3. Diabetic -Crocin-treated group (Diabetic+Crocin): As in the diabetic group, DM was induced. They received 100 mg/kg of Crocin intraperitoneally (i.p.) dissolved in I ml normal saline concurrently. A 99.9% pure powdered crocin was purchased from

the Tokyo Chemical Industry in Toshima, Tokyo, Japan (C1527) (Asdaq et al., 2024).

Rats were subsequently sacrificed by cervical elongation and dislocation after blood samples were taken from the retro-orbital venous plexus. Liver was removed. For histological and immunohistochemical evaluation a portion of the liver was preserved in 10% formalin saline. The RT-PCR and biochemical assays were performed on the other hepatic section.

Blood sampling

Fasting blood samples were obtained from the retro-orbital venous plexus. Four milliliters of blood were extracted and divided equally between two tubes. The first tube was centrifuged for 10 minutes at 4000 revolutions per minute (rpm) following 10 minutes clotting in a water bath. Until it was needed for additional analysis, the serum was stored frozen at -20 °C. The calculation of glycosylated hemoglobin (Hb A1 c) was carried out after the blood from the second tube was transferred into an EDTA tube.

Calculation of HOMA-IR index

HOMA-IR index= fasting serum insulin (µU/mL)X fasting serum glucose (FSG) mg/dL/405

(Khodir et al., 2020)

Biochemical analysis

Fasting serum glucose (Diamond Diagnostic, Egypt), serum HbA1c (Stanbio Glycohemoglobin, Egypt), were measured by colorimetric kits. The serum liver enzymes (ALT, AST, ALP, and GGT), serum cholesterol, and serum triglyceride (TG) were measured using colorimetric kits (Biodiagnostic Company, Dokki, Giza, Egypt) after the serum was collected and frozen at -80°C.Utilizing the appropriate rat ELISA kits, the levels of serum insulin was calculated (DRG Instruments GmbH, Marburg, Germany).

Tissue Homogenate Preparation

Each weighted hepatic tissue was homogenized separately using a tissue homogenizer. The crude tissue homogenate was centrifuged in an ice-cold centrifuge for 11 minutes at 11,000 rpm.

Following the manufacturer's instructions, the ELISA Kit was used to quantify hepatic TNF-α (Cat.: MBS2507393, MyBioSource, Sandiego, CA, USA), hepatic IL-6 (Cat.: MBS269892, MyBioSource, Sandiego, CA, USA), and hepatic IL-10 (IL-10: ERI3010-1, Assaypro LLC, Saint Charles, Missouri, USA). In compliance with the manufacturer's instructions, hepatic MDA and SOD were measured using calorimetric kits (Biodiagnostic Company, Dokki, Giza, Egypt).

Quantitative RT-PCR (qRT-PCR)

One sample of liver tissue was taken from each rat and placed in a falcon tube, where it was kept at -80 °C for RNA extraction and the Nrf2 and HO-1 assay. A 7500 real-time PCR machine (Applied Biosystems, CA, United States) was used to identify Nrf2 and HO-1. The first step of PCR was the synthesis of complementary DNA using the QuantiTect Reverse Transcription Kit (205311; Qiagen, Applied Biosystems, USA), and then the second step of PCR (the real-time PCR step) after RNA was extracted from hepatic cells using a direct—zol RNA miniprep kit (Cat. No. R2051; Zymo Research, USA). The following primers were used for the Nrf2 gene:
(1) Forward primer: 5- GGTTGCCCACATTCCCAAATC-3
(2) Reverse primer: 5- CAAGTGACTGAAACGTAGCCG-3

The following primers were used for the HO-1 gene:
(1) Forward primer: 5-AGGTGCACATCCGTGCAGAG-3
(2) Reverse primer: 5-CTTCCAGGGCCGTATAGATATGGTA-3

β actin works as an endogenous control, Ten microliters of SYBR Green (2× QuantiTect PCR Master Mix), three microliters of cDNA, one microliter of forward primer, one microliter of reverse primer, and five milliliters of RNase-free water were used in each PCR reaction, which was carried out in a final volume of 20 microliters. Denaturation at 94 °C for 30 s, annealing at 55 °C for 40 s, and extension at 72 °C for 31 s were the next 55 cycles. The data was processed using the Applied Biosystems 7500 software version 2.0.1. Gene expression was measured relative to one another using the comparative Ct technique (Khodir et al., 2025).

Histopathological Method

Sections of liver tissue were fixed at 10% formalin for histological investigations. They were then dried in ethyl alcohol, washed in xylol, and lastly placed with paraffin. H & E was used to stain the 4 μm-thick sections. The liver paraffin sections (4 μm) were stained with NF-kB (monoclonal, dilution 1:200, Abcam) and Caspase-3 (rabbit polyclonal antibody, Dako, Carpinteria, California, USA) for immunohistochemical investigations.

Statistical analysis

Following data collection and analysis, they were found to satisfy the parametric assumptions based on the results of the Shapiro-Wilk test. As a result, one-way ANOVA and post hoc Bonferroni's tests were applied to the data. The data was displayed using the mean ±SD. Significance was considered to exist when the p value was 0.05 or less. The data was analyzed using Graph-Pad Prism software (version 9.3.1, San Diego, CA, USA).

Results

In contrast to the control group, the diabetic group's hepatic SOD, hepatic IL-10, and hepatic gene expression of Nrf2 and HO-1 were dramatically lower. The measured serum glucose, serum insulin, HOMA-IR index, serum glycosylated Hb A1c, serum cholesterol, serum triglyceride, serum ALT, serum AST, serum ALP, serum GGT, hepatic MDA, hepatic TNF-α, and hepatic IL-6 were all dramatically higher in the diabetic group than in the control group. The Diabetic+Crocin group had dramatically higher levels of hepatic SOD, hepatic IL-10, and hepatic gene expression of Nrf2 and HO-1, but dramatically lower levels of hepatic glucose, serum insulin, HOMA-IR index, serum glycosylated Hb A1c, serum cholesterol, serum triglyceride, serum ALT, serum AST, serum ALP, serum GGT, hepatic MDA, hepatic TNF-α, and hepatic IL-6 when compared to Diabetic group. Table (1).

Table (1): The measured Serum Glucose, Serum Insulin, HOMA-IR index, Serum glycosylated Hb $A1_c$, Serum Cholesterol, Serum Triglyceride, Serum ALT, Serum AST, Serum ALP, Serum GGT, Hepatic MDA, Hepatic SOD, Hepatic TNF-α, Hepatic IL-6, Hepatic IL-10, Hepatic Nrf2 gene expression and Hepatic HO-1 gene expression in all studied groups

	Control group	Diabetic group	Diabetic +Crocin group
Serum Glucose (mg/dl)	75.3±3.2	320.9±7.9 *	**185.6±3.2** *#
Serum Insulin (µU/mL)	6.98±1.02	26.9±0.95 *	**18.6±1.80** *#
HOMA-IR index	1.3±0.22	20.23±1.25 *	**8.9±1.51** *#
Serum glycosylated Hb $A1_c$ (% of normal Hb)	1.65±0.22	12.9±0.61 *	**7.86±0.44** *#
Serum Cholesterol (mg/dL)	75±3.2	312±7.5 *	**184±3.21** *#
Serum Triglyceride (mg/dL)	40.6±2.1	168±3.44 *	**92.3±4.58** *#
Serum ALT (U/L)	35.9±3.6	135.8±2.44 *	**84.9±1.3** *#
Serum AST (U/L)	49.6±3.13	180.6±4.2 *	**132.9±3.9** *#
ALP (U/L) Serum	95.6±3.6	187±2.458 *	**130.6±2.58** *#
GGT (U/L) Serum	4.9±0.1	16.38±1.02 *	**10.9±1.09** *#
Hepatic MDA (nmol/ gm. Tissue)	4.98±0.85	25.6± 0.98*	**10.8± 0.81** *#
Hepatic SOD (U/gm. Tissue)	3.25±0.11	0.95±0.11*	**1.98±0.31** *#
Hepatic TNF-α (pg/ml)	100.5±2.13	250.6±3.15*	**154.2±6.98** *#
Hepatic IL-6 (pg/mL)	145.5±4.16	377.5±4.2*	**225.8±5.98** *#
Hepatic IL-10 (ng/mL)	10.52±0.55	6.1±0.23*	**8.65±0.14** *#
Hepatic Nrf2 gene expression	1	0.41±0.06*	**0.73±0.08** *#
Hepatic HO-1 gene expression	1	0.33±0.03*	**0.68±0.04** *#

* Significant compared with control, # Significant compared with Diabetic.

Histopathological results

H & E staining:

Sections from the control group showed normal hepatic parenchyma with intact hepatocytes with central vesicular nuclei (Fig. 1 A). The Diabetic group showed sever perivascular inflammatory cells infiltrates and showed also apparent degenerative changes of the hepatocytes with large nuclei and prominent nucleole (Fig. 1B,C). The Diabetic+Crocin showed apparent improvement of the hepatocytes with mild inflammatory cells infiltrates (Fig. 1D).

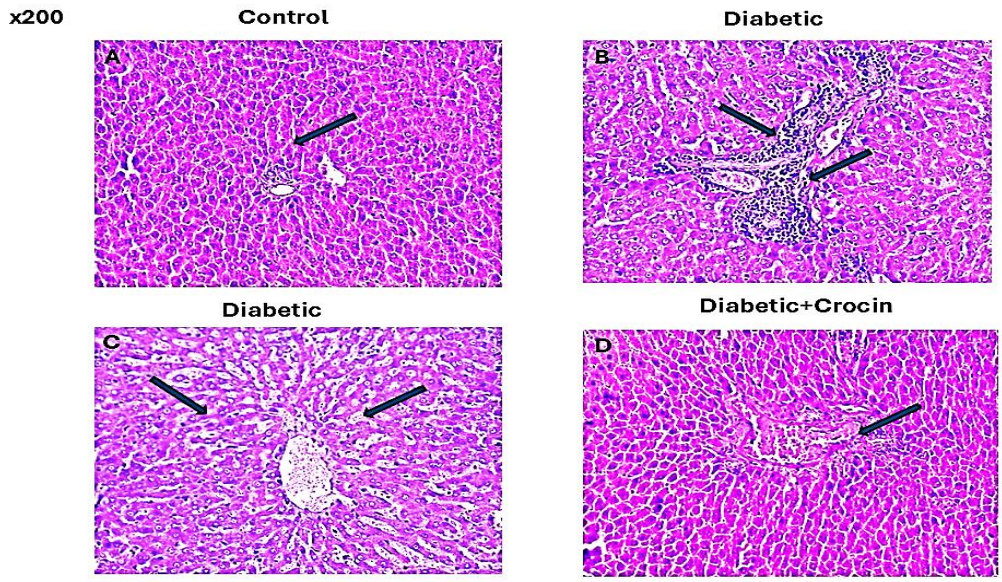

Fig. 1: H& E-stained Liver sections (H&E ×200): (A) is a photomicrograph of the control group showed normal hepatic parenchyma with intact hepatocytes with central vesicular nuclei (arrow) (B and C) is a photomicrograph of the Diabetic group showed sever perivascular inflammatory cells infiltrates and showed also apparent degenerative changes of the hepatocytes with large nuclei and prominent nucleole (arrows). (D) is a photomicrograph of the Diabetic+Crocin group showed apparent improvement of the hepatocytes with mild inflammatory cells infiltrates (arrow)

Immunohistochemical results

When compared to the control, the diabetic's percentage area of Caspase-3 increased significantly (81.5±0.02 vs. 5.8±0.15, respectively, $p<0.05$) in the Caspase-3 stain. While this proportion was remained higher than control, it was dramatically lower in the Diabetic + Crocin group compared to the Diabetic (27.4±0.33 vs. 81.5±0.02, $p<0.05$). (Fig. 2: A-D).

When compared to the control, the diabetic's percentage area of NF-kB increased significantly (68.5±0.05 vs. 5.2±0.03, respectively, $p<0.05$) in the NF-kB stain. While this proportion was dramatically lower in the Diabetic+Crocin group compared to Diabetic (19.4±0.12 vs. 68±0.05, $p<0.05$). (Fig. 2: E-H).

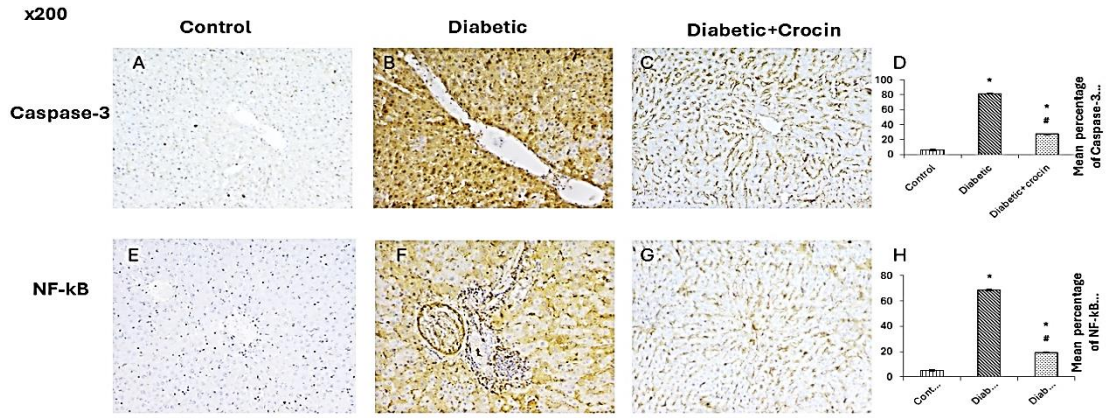

Fig (2): Representative micrographs of the different experimental groups showing significant increase of the Caspase-3(A-D) and NF-kB (E-H) immunoreaction in the Diabetic group and a substantial decrease in the Diabetic + Crocin group.

Discussion

The goal of our study was to look into the potential methods by which crocin protects the liver in DM, suggesting that the Nrf2/HO-1 signaling pathway may be involved. In comparison to controls, the T2DM rat model in this study displayed an impaired glycemic state with elevated blood glucose, HbA1c, and HOMA-IR levels. This outcome is comparable to what a prior study found (Ghiasi et al., 2015)

In comparison to the diabetic group, Crocin significantly improved glycemic status and lowered the HOMA-IR index. This is consistent with earlier research showing that Crocin's antihyperglycemic, antioxidant, and β cell-protecting qualities may aid in the development of alternative treatment plans for type 2 diabetes (Asdaq et al., 2024) .

Crocin's potent antioxidant qualities, activation of the Langerhans islets, or enhanced peripheral sensitivity to insulin could all be responsible for its capacity to lower blood glucose levels. Our findings instead imply that crocin's action stems from its capacity to scavenge free radicals (Emam et al., 2021)

Type 2 diabetes has dramatically increased in tandem with advances in living standards. Obesity-induced pancreatic β-cell dysfunction and alterations in lipid metabolism are linked to T2DM, a complicated metabolic disease that results in inadequate insulin production and insulin resistance. Liver damage is a serious side

effect of type 2 diabetes. T2DM-related metabolic problems, including inflammation, oxidative stress, hyperglycemia, and insulin resistance, will harm the liver and ultimately result in a number of liver illnesses (Ouyang et al., 2024).
AST and ALT, two serum or plasma aminotransferases, evaluate the intracellular hepatic enzymes that leak into the blood and act as indicators of liver damage (Ahmed et al., 2023).

Our findings showed that the diabetic group had higher liver enzymes than the control group, which suggests liver damage. This is consistent with other research and was supported by histological findings (Emam et al., 2021).

In accordance with earlier research, crocin significantly reduced liver enzyme levels as compared to the diabetic group, indicating its hepatoprotective effect (Karayakali et al., 2023).
Additionally, our result was consistent with earlier research that indicated that crocin dramatically decreased elevated levels of AST, ALT, and ALP in experimental liver injury caused by acetaminophen (Omidi et al., 2014). Crocin uses the antioxidant regulatory system to produce its hepatoprotective effects.

The pathophysiology of diabetes and NAFLD was known to be significantly influenced by oxidative stress, according to oxidative stress indicators (Rajaei et al., 2013). In this study, we discovered that the diabetic group had more MDA and lower SOD than the control group.consistent with earlier studies (Emam et al., 2021).

The research claims that when glucose is oxidized, superoxide radicals are created, which increase the risk of complications from diabetes. Therefore, macromolecules like proteins, lipids, carbohydrates, and DNA are destroyed when free radicals are produced in excess (Padiya et al., 2011). Furthermore, hepatic structural and functional problems are caused by ROS generation (Iskender et al., 2017).

Crocin dramatically ameliorated oxidative stress and this goes in line with previous study (Asdaq, et al., 2024).

Crocin's antioxidant effect is ascribed to its capacity to lower lipid peroxidation and raise antioxidant levels (Ghorbanzadeh et al., 2016)

All things considered, it appears that crocin's antioxidant properties are linked to both its capacity to scavenge radicals and its impact on increasing the concentration of antioxidant enzymes within tissues (Rajaei, et al., 2013).

ROS production and mitochondrial dysfunction are associated with diabetes mellitus (Koliaki & Roden, 2016). These results result in ß-cell dysfunction and glucolipotoxicity in rats with type 2 diabetes produced by HFD-STZ (Govindaraj & Sorimuthu Pillai, 2015).

Among the processes that lead to the overproduction of ROS in this scenario are oxidative stress, glucosetoxicity, and lipotoxicity (Ahmed et al., 2023). Rats with diabetes mellitus showed a significant increase in their lipid profiles in the study that was presented, which is consistent with earlier research (Emam et al., 2021).

Serum lipid levels were elevated in the diabetic rats in the current investigation. These findings are consistent with Ahmed's data (Ahmed, 2010).

Inflammatory processes have been linked to the development of diabetes mellitus. Inflammation and oxidative stress have recently been shown to interact in this illness. Insulin resistance and the advancement of T2DM are significantly influenced by inflammatory illness. The liver, and the pancreas are the primary locations of inflammation (Alqahtani et al., 2023).

According to our findings, the diabatic group's hepatic proinflammatory cytokines have sharply increased compared to control, which is consistent with prior research (Oguntibeju, 2019).

According to earlier reports, NF-κB activation causes cytotoxic cytokines to be produced, exacerbating liver damage [4]. In line with other research, our findings showed a dramatically higher NF-kB immunoreaction in the diabetic group as compared to the control group, indicating that T2DM significantly raises NF-κB expression (Alqahtani et al., 2023).

Crocin significantly reduced inflammatory cytokines in comparison to the group with diabetes. The anti-inflammatory effects of crocin have been documented before (Abou-Hany et al., 2018).

As demonstrated by a decrease in hepatic NF-kB immunoreaction as compared to the diabetic group, the anti-inflammatory effect of crocin may be due to NF-kB downregulation, which is consistent with earlier research (Chhimwal et al., 2020).

Crocin's anti-inflammatory and immunomodulatory properties are highlighted by the fact that it decreased kidney TLR4 and IL6 content (Abou-Hany et al., 2018).

Next, we investigated how the Nrf2/HO-1 pathway might mediate crocin's hepatoprotective effects in diabetic rats. It's interesting to note that Nrf2 activation has been sufficiently shown to protect against oxidative liver injury, inflammation, and apoptosis. Furthermore, Nrf2 deletion exacerbated hepatocyte damage. Therefore, Nrf2 activation leads to an increase in antioxidant enzymes that counteract excess ROS and shield the liver from harm. Nrf2 inhibits pro-inflammatory mediators and NF-kB (Al-Amarat et al., 2021)

By attaching to antioxidant response elements in promoter regions, Nrf2 controls a number of downstream genes, including HO-1, which stimulates the synthesis of antioxidant enzymes. According to our experimental findings, T2DM rats' livers had considerably lower levels of Nrf2 and HO-1 mRNA expression (Ouyang et al., 2024) And this goes in line with that of Ouyang et al. (2024), and the rat livers' Nrf2 and HO-1 mRNA expression levels were markedly elevated following crocin therapy. Additionally, this is consistent with (Zhang et al., 2022). Furthermore, in cellular models of diabetic nephropathy, crocin raises the expression of Nrf2 and hO-1 (Zhang et al., 2022)..

Proteolytic caspases are involved in the activation and execution of apoptosis in inflammation, and caspases are a family of protease enzymes essential for programmed cell death (Van Opdenbosch & Lamkanfi, 2019). By reducing beta-cell mass through apoptosis, caspase-3 activation is crucial to the development and initiation of type 2 diabetes in its early stages (Veluthakal et al., 2016).

Hepatic Caspase-3 immunoreaction was dramatically upregulated in diabetic group compared to control and this goes in line with previous study (Emam, et al., 2021).

In contrast to the diabetic group, crocin significantly reduced the hepatic caspase-3 immunoreaction. Crocin prevented apoptosis and parenchymal tissue damage brought on by CCl 4, according to a previous study (Cosgun et al., 2019)

According to (Thushara et al., 2013), crocin shields platelets from oxidative stress-induced apoptosis by lowering the activation of H2O2 caused by the apoptotic protein caspase 3.

Conclusion

By upregulating the hepatic Nrf2/HO-1 signaling pathway and exhibiting lipid-lowering, anti-inflammatory, antiapoptotic, and antioxidant properties, crocin guards against diabetes-induced liver damage.

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