Hepatoprotective effect of morin against methotrexate-induced hepatotoxicity via targeting Nrf2/HO-1 and Bax/Bcl2/Caspase-3 signaling pathways

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Abstract

Background Organ toxicity limits the therapeutic efficacy of methotrexate (MTX), an anti-metabolite therapeutic that is frequently used as an anti-cancer and immunosuppressive medicine. Hepatocellular toxicity is among the most severe side effects of long-term MTX use. The present study unveils new confirmations as regards the remedial effects of morin on MTX-induced hepatocellular injury through regulation of oxidative stress, apoptosis and MAPK signaling.

Methods Rats were subjected to oral treatment of morin (50 and 100 mg/kg body weight) for 10 days. Hepatotoxicity was induced by single intraperitoneal injection of MTX (20 mg/kg body weight) on the 5th day.

Results MTX related hepatic injury was associated with increased MDA while decreased GSH levels, the activities of endogen antioxidants (glutathione peroxidase, superoxide dismutase and catalase) and mRNA levels of HO-1 and Nrf2 in the hepatic tissue. MTX treatment also resulted in apoptosis in the liver tissue via increasing mRNA transcript levels of Bax, caspase-3, Apaf-1 and downregulation of Bcl-2. Conversely, treatment with morin at different doses (50 and 100 mg/kg) considerably mitigated MTX-induced oxidative stress and apoptosis in the liver tissue. Morin also mitigated MTX-induced increases of ALT, ALP and AST levels, downregulated mRNA expressions of matrix metalloproteinases (MMP-2 and MMP-9), MAPK14 and MAPK15, JNK, Akt2 and FOXO1 genes.

Conclusions According to the findings of this study, morin may be a potential way to shield the liver tissue from the oxidative damage and apoptosis.

1. Introduction

Methotrexate (MTX), a folate analog and antagonist, is broadly used for the treatment of a variety of malignant and non-malignant disorders (1). Due to the beneficial anti-inflammatory and immunomodulatory activities it has, MTX is now the firstline disease modifying antirheumatic drug in the treatment of rheumatoid arthritis (2). MTX was first designed to block the synthesis of purines and pyrimidines, which is essential for synthesis of genetic material as well as the proliferation of a variety of malignant and nonmalignant cells. Even though this mechanism may contribute to the mechanism by which MTX suppresses inflammation, other mechanisms of action including increased adenosine release, inhibition of transmethylation reactions essential for some cellular functions, decreased polyamine accumulation and NO synthase uncoupling have been proposed (3). Despite its beneficial effects, MTX has several major side effects and can cause toxicities in organs such as the liver, kidneys, testes and brain (4–7). Protecting the organs from undesired side effects could be one way to mitigate the negative consequences of MTX in therapeutic applications. As a result, the protective effects of natural biomolecules with antioxidant activities that may reduce the severity of MTX-induced toxicities could be useful for therapeutic purposes.

Flavonoids are dietary bioactive chemicals obtained from plants that have significant impacts on health. Morin is an important flavonoid derived mostly from the fruits, stems, and leaves of Moraceae plants. Numerous studies have shown that morin hydrate is effective against a variety of chronic and life-threatening degenerative disorders (8). It has been revealed to have anti-inflammatory and antioxidant properties in a range of diseases including cancer (9), hepatotoxicity (10), nephrotoxicity (11) and testicular damage (12).

The current study was planned to examine the ameliorative effects of morin against MTX induced liver toxicity, based on serum biochemical parameters, oxidative stress and apoptotic alterations.

2. Material Method

2.1. Chemicals

Methotrexate (50 mg/5 mL injectable solution) was obtained Koçak Farma (İstanbul, Turkey). All of the chemicals used in this study, including morin hydrate, were procured from Sigma–Aldrich chemicals (St. Louis, MO, USA) and were of analytical grade.

2.2. Animals

Experiments were conducted on 35 male Wistar albino rats (weighing between 280 and 300 g, 11–12 weeks old) obtained from Bingol University's Experimental Research Center. The rats were housed in plastic cages and fed under standard laboratory conditions (12 hour light/dark cycle, 24 ± 1°C, and 45 ± 5% humidity). They were fed a commercial chow meal and had unrestricted access to water. All animal-related treatments were approved by Bingol University's Animal Experimentation Ethics Committee (Protocol No: 2022-E.66052).

The rats were separated into five groups of 7 male rats each at random:

Control group: The animals received 0.9% saline via oral gavage for 10 days and a single intraperitoneal injection of saline on day 5 only.

Morin group: The animals were given 100 mg/kg morin hydrate orally for 10 days and intraperitoneal saline injection was given on the 5th day of the experiment.

MTX group: The animals were administered saline orally for 10 days and on the 5th day of the experiment, a single dose of 20 mg/kg MTX was injected intraperitoneally.

MTX + Morin 50 group: Rats were given 50 mg/kg morin hydrate orally for 10 days and a single dose of 20 mg/kg MTX was injected intraperitoneally on the 5th day of the experiment.

MTX + Morin 100 group: Rats were given 100 mg/kg morin hydrate orally for 10 days and a single dose of 20 mg/kg MTX was injected intraperitoneally on the 5th day of the experiment.

Following day, the rats were sacrificed under mild sevoflurane anesthesia. Blood serum was separated by centrifugation at 3000×g for 10 minutes, and the serum samples were then tested for liver function analysis. Livers were immediately removed and washed with ice-cold physiological saline solution for biochemical and molecular analysis and then stored at -20°C.

2.3. Measurement of liver function markers

Mindray Perfect Plus 400 was used to measure the activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) in the serum. The results were given in units of U/L.

2.4. Oxidative stress indicators

To obtain the homogenate, liver tissue was homogenised in a homogeniser (Tissue Lyser II, Qiagen, Netherlands) in a solution of 1.15% KCl. The activity of glutathione peroxidase (GPx) was measured using Lawrence and Burk (13) procedure. The activity of superoxide dismutase (SOD) was determined using the method reported by Sun, Oberley (14). The activity of both enzymes has been presented as U/g protein. The activity of catalase (CAT) was measured using the Aebi (15) protocol and has been presented as Katal/g protein. The content of glutathione (GSH) has been analysed using the Sedlak and Lindsay (16) technique. MDA level was measured as described by (17). The GSH and MDA levels were measured in nmol/g tissue. The protein content of liver tissue was determined using the (18).

2.5. Gene expression analysis

In the first stage, total RNA was isolated from liver tissues of rats treated with MTX and morin. Total RNA isolation was performed using QIAzol Lysis Reagent (Qiagen, Cat: 79306, Germany). In the next step, cDNAs were synthesized from total RNAs with the iScript™ cDNA Synthesis Kit (BIO-RAD, United States). At the last stage, the relative mRNA transcript levels of the genes [nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase-1 (HO-1), Bcl-2-associated x protein (Bax), B-cell lymphoma-2 (Bcl-2), cysteine aspartate specific protease-3 (caspase-3), metalloproteinase-2 (MMP2), metalloproteinase-9 (MMP9), apoptotic protease activating factor-1 (Apaf1), forkhead box transcription factor O1 (FOXO1), c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase 14 (MAPK14), MAPK15 and Akt2] whose sequences are given in Table 1 were analyzed by RT-PCR method. For this, a mix was prepared with the primers of the relevant genes, cDNAs and iTaq Universal SYBR Green Supermix (BIORAD) and the reaction was started in the ROTOR-GENE Q (Qiagen, Germany) device. According to Livak et al. 2001, the acquired qRT-PCR data was analyzed using the comparative threshold (Ct) method. GAPDH was used as internal control.

Table 1

Primer sequences

Gene	Sequences (5'-3')	Length (bp)	Accession no
Bax	F: TTTCATCCAGGATCGAGCAG R: AATCATCCTCTGCAGCTCCA	154	NM_017059.2
Bcl-2	F: GACTTTGCAGAGATGTCCAG R: TCAGGTACTCAGTCATCCAC	214	NM_016993.2
Caspase-3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	270	NM_012922.2
Apaf-1	F: ACCTGAGGTGTCAGGACC R: CCGTCGAGCATGAGCCAA	192	NM_023979.2
Nrf2	F: TTTGTAGATGACCATGAGTCGC R: TCCTGCCAAACTTGCTCCAT	161	NM_031789.2
HO-1	F: ATGTCCCAGGATTTGTCCGA R: ATGGTACAAGGAGGCCATCA	144	NM_012580.2
Akt2	F: GAGTACTTGCACTCGACGGA R: CCATGAGGATGAGCTCGAAG	304	NM_017093.1
FOXO1	F: CAGCCAGGCACCTCATAACA R: TCAAGCGGTTCATGGCAGAT	143	NM_001191846.3
MMP2	F: CTCTAGGAGAAGGACAAGTG R: CTCAAAGTTGTACGTGGTGG	158	NM_031054.2
MMP9	F: AGCTGGCAGAGGATTACCTG R: ATGATGGTGCCACTTGAGGT	230	NM_031055.2
MAPK14	F: GTGGCAGTGAAGAAGCTGTC R: GTCACCAGGTACACATCGTT	170	NM_031020.2
MAPK15	F: TGTTTGAGTCCATGGACACC R: GCATCCAATAGAACGTTGGC	169	NM_173331.2
JNK	F: GAATCAGACCCATGCTAAGC R: CCATGAGCTCCATGACTATG	149	NM_053829.2

Gene	Sequences (5'-3')	Length (bp)	Accession no
GAPDH	F: GAGTATGTCGTGGAGTCTAC	179	NM_017008.4
	R: CAGGATGCATTGCTGACAAT		

2.6. Statistical analysis

The mean and standard error of the mean were used to express the findings (SEM). To compare groups, a one-way analysis of variance was used, followed by a Tukey's post-hoc test. The significance level was set at $p < 0.05$. For statistical analysis of the data, the package program SPSS version 20.0 (SPSS, Chicago, IL) was used.

3. Results

3.1. Liver function and oxidative stress parameters

In comparison to the control group, MTX caused a substantial increase in activity of serum AST, ALT and ALP ($p < 0.05$). In contrast to the only MTX injected group, morin treatment significantly reduced the activities of AST, ALT, and ALP in the MTX + Morin 50 and MTX + Morin 100 groups ($p < 0.05$). There was no discernible change between the control and the morin only treated group (Table 2).

Table 2
Effect of Morin on hepatic serum markers and oxidative stress biomarkers in MTX-induced hepatotoxicity

Parameters	Control	Morin	MTX	MTX + Morin-50	MTX + Morin-100
ALP (U/L)	39.12 ± 1.96^a	37.78 ± 1.39^a	74.32 ± 2.56^d	63.15 ± 2.19^c	50.06 ± 2.04^b
ALT(U/L)	41.03 ± 1.88^a	40.22 ± 1.59^a	77.15 ± 2.67^d	60.63 ± 2.38^c	52.96 ± 2.17^b
AST (U/L)	55.11 ± 2.23^a	54.34 ± 2.52^a	109.76 ± 3.91^d	90.39 ± 3.65^c	73.08 ± 4.03^b
MDA(nmol/g tissue)	29.55 ± 1.17^a	30.43 ± 3.88^a	47.02 ± 1.67^d	40.32 ± 1.16^c	34.87 ± 2.07^b
GSH (nmol/g tissue)	7.15 ± 0.22^d	7.30 ± 0.15^d	3.52 ± 0.11^a	4.46 ± 0.13^b	5.38 ± 0.12^c
CAT (katal/g protein)	56.20 ± 2.57^d	58.80 ± 1.55^d	33.97 ± 2.05^a	41.39 ± 2.11^b	47.72 ± 0.99^c
SOD (U/g tissue)	39.88 ± 1.41^d	40.21 ± 1.36^d	25.91 ± 1.26^a	30.00 ± 0.98^b	34.47 ± 0.94^c
GPx (U/g tissue)	34.63 ± 1.73^d	36.05 ± 1.59^d	19.95 ± 1.07^a	23.32 ± 0.98^b	28.40 ± 0.89^c
Different superscripts (a−d) in the same row indicate significant difference ($p < 0.05$) among groups.					

Table 2 shows that MTX significantly reduced SOD, CAT, and GPx activities, as well as GSH levels, as compared to the untreated control ($p < 0.05$). In contrast to the MTX group, different oral doses of morin (50 and 100 mg/kg) effectively reversed MTX-induced reductions in GSH content and SOD, CAT, and GPx activities ($p < 0.05$). MDA was used to analyse lipid peroxidation in liver and the results are reported in Table 2. When comparing the MTX-induced group to the control and just morin supplemented groups, there was a substantial increase in levels of hepatic MDA. When comparing to the MTX group, the MDA levels in the MTX + morin 50 and MTX + morin 100 groups were considerably lower ($p < 0.05$).

3.2. The expression profiles of Nrf2 and HO-1

By using qRT-PCR, the expression profiles of the Nrf2 and HO-1 genes in the hepatic tissue in the control and treatment groups were assessed. Only the group that received MTX had reduced expression levels of Nrf2 and HO-1 when compared to the control group (Fig. 1A and B). The mRNA transcript levels of Nrf2 and HO-1 were found to be at the same level as the control in the morin-treated group. In comparison to the MTX group, Nrf2 and HO-1 gene expression levels were higher in the MTX + morin 50 and MTX + morin 100 groups. In contrast to the MTX group, these expression levels were considerably lower ($p < 0.05$).

3.3. Morin alleviates the expression of apoptotic and anti-apoptotic genes triggered by MTX.

The effects of MTX injection and morin treatment on mRNA transcript levels of *Bax, Bcl2*, *Caspase-3* and *Apaf1* in rat hepatic tissue were examined. When compared to the untreated control group, MTX supplementation significantly ($p < 0.05$) elevated mRNA levels of *Bax, Caspase-3* and *Apaf1* while their expression was downregulated in the MTX + morin 50 and MTX + morin 100 groups (Fig. 2A-D). Furthermore, MTX treatment decreased Bcl-2 mRNA transcript levels, whereas supplementation with different dosages of morin increased its expression, suggesting that morin has an anti-apoptotic role in MTX-induced hepatic injury ($p < 0.05$).

3.4. Morin has an effect on the levels of MAPK14 and MAPK15 mRNA transcripts in hepatic tissue

The effects of MTX treatment and morin supplementation on mRNA transcript levels of MAPK14 and MAPK15 in rat hepatic tissue were examined. When compared to the untreated control group, MTX injection significantly elevated mRNA levels of MAPK14 and MAPK15 while their expression was down-regulated in the MTX + morin 50 and MTX + morin 100 groups (Fig. 3A and B) suggesting that morin has an ameliorative role in MTX-induced hepatic injury.

3.5. Morin has an effect on the levels of JNK Akt2 and FOXO1 mRNA transcripts in hepatic tissue

Figure 3C-E shows the effects of MTX and/or morin treatments on JNK, Akt2 and FOXO1 mRNA expression levels in hepatic tissue. The mRNA expression levels of JNK, Akt2 and FOXO1 were significantly up-regulated in the liver of MTX-induced rats according to our RT-PCR data. Morin (50 and 100 mg/kg) treatment, oppositely, reduced the mRNA transcripts levels of these markers in liver.

3.6. Morin has an effect on the levels of MMP-2 and MMP-9 mRNA transcripts in hepatic tissue

The expression profiles of MMP2 and MMP9 genes in the hepatic tissue were evaluated by qRT-PCR in the control and treatment groups. Figure 4A and B shows that MTX significantly up-regulated expression levels of MMP2 and MMP9 in the liver as compared to the control group. In contrast to the MTX group, supplementation of morin (50 and 100 mg/kg) effectively reversed MTX-induced upregulations in MMP2 and MMP9 levels.

4. Discussion

MTX is an antimetabolite therapeutics that is extensively used as an anticancer and immune-suppressive drug, although its therapeutic efficacy is hampered by organ toxicities (19). One of the most dangerous side effects of long-term MTX treatment is hepatotoxicity, which is caused by the accumulation of 7-hydroxymethotrexate, the drug's primary metabolite (20). Because of the importance of drug-induced hepatic toxicity in clinical medicine, researchers are increasingly interested in gaining a better understanding of the toxicity, specifically the processes and ways to reduce the occurrence of this nasty

side effects. In this study, the ameliorative impacts of morin on MTX-induced hepatocellular toxicities were assessed.

Hepatic dysfunction has been linked to MTX intoxication, as evident by raised serum activity of the ALT, ALP and AST enzymes (21, 22). In this study, serum marker enzymes of hepatocellular injury were evaluated. Under normal circumstances, these enzymes are mostly found in the hepatic tissue. They are frequently released into the circulation for the duration of hepatocyte necrosis or membrane damages. AST and ALT are found in periportal hepatocytes and engage in transamination reactions during amino acid metabolism (23). However, their serum activity have been shown to be enhanced following cellular membrane injury and leaking. As a result, the considerable rise in levels of these enzymes following MTX administration is revealing the disturbed membrane permeability in the treated rats, which is associated with hepatic injury. However, the noteworthy reductions in serum AST, ALT and ALP levels after morin administration to MTX-treated rats suggests that morin provides hepatoprotection against MTX-induced liver injury. In agreement with our findings, morin therapy has been shown to normalize blood biochemical indicators (10, 11).

Hepatic impairment caused by MTX have been linked to oxidative damage. MTX causes oxidative stress at the cellular level by producing free radicals and ROS (24, 25). LPO, alterations in antioxidant machinery, DNA damage, alteration in gene expressions and apoptosis are all symptoms of oxidative stress (26). The organism has evolved numerous mechanisms to defend itself against oxidative stress. These processes include antioxidant enzymes like SOD, CAT, and GPx, as well as the non-enzymatic antioxidant glutathione (27, 28). Several independent studies have been conducted on antioxidants and their importance in preventing oxidative injury and the cellular damage that comes with it, as well as the critical roles of SOD, CAT and GPx (24, 29, 30). The effects of morin supplementation on oxidative stress indicators in MTX-induced hepatotoxicity were studied in this work. The findings demonstrated that MTX administration increased MDA levels and decreased GSH levels as well as SOD, CAT, and GPx activities in hepatic tissues, while morin therapy mitigated these effects. MTX has also been reported to decrease Nrf2 binding activity and suppresses Nrf2 expression in the livers of MTX-treated mice (31). This decrease in Nrf2 activity may be linked to a decrease in the liver's anti-oxidant status. We measured Nrf2-HO-1 pathway mRNA expression to further understand the processes underlying morin's protective impact against MTX-induced liver damage. Nrf2 has been reported to be activated by cytoplasmic Kelch-like ECH-associated protein 1 and stimulates the production of antioxidant genes in response to oxidative stress (32). Nrf2 regulates the transcription of HO-1, an important cellular antioxidant enzyme (33). HO-1 can reduce inflammation, reduce oxidative stress, and slow the rate of apoptosis all at the same time. Colectively, our findings suggest that HO-1 induction through Nrf2 activation may play a role in morin's cytoprotection against MTX-induced oxidative stress.

Apoptosis is a type of biological cellular death that is vital for the formation and maintenance of homeostatic balance in human and animals. Pro-apoptotic proteins like Bax, caspase-3 and anti-apoptotic factors like Bcl-2 strictly regulate it (34). According to the reports, MTX causes liver damage, which may be directly related to pro-apoptotic protein activation. In the current study, we use the

quantitative real time PCR method to assess three apoptotic markers: Bax, caspase-3 and Apaf-1 and anti-apoptotic Bcl-2. The mitochondrial apoptosis process is started by Bax, which also damages DNA and activates caspase-3 (35). The intrinsic or mitochondrial mechanism of apoptosis uses the protein Apaf-1, which oligomerizes in response to the release of cytochrome c and creates the huge complex known as an apoptosome. The apoptosome recruits and activates procaspase-9, a mitochondrial pathway initiator caspase, which results in the processing of caspase-3 downstream (36). According to the literature, MTX treatment causes liver apoptosis through effecting modulating levels of Bax, caspase-3, Apaf-1 and Bcl-2 (37, 38). The findings of the current study are consistent with previous findings regarding Bax, caspase-3, Apaf-1 and Bcl-2 regulation. Morin dramatically mitigated Bax, caspase-3, Apaf-1 and Bcl-2 expressions to a level that is comparable to the control.

One of the key elements of the signaling cascade that controls a variety of cellular functions, including cell division, developmental programs, hormone responses, and biotic and abiotic stress responses, is the MAPK pathway (39). The most lately discovered atypical MAPK and one that has received the least attention is called MAPK15. Studies on the function of MAPK15 in many different cells and model organisms show that MAPK15 is involved in a wide range of cellular processes, including stimulating cell division and protein secretion; controlling cell division, cell transformation and apoptosis (40). MAPK14 is a osmoregulatory protein JNK, an important branch of the MAPKs, plays an important function in the apoptosis triggered by TNF-α that is induced via exposure to a variety of cellular stresses (41). One of the biochemical mechanisms leading to the adverse effects of MTX is p38 MAPK-signaling pathway is especially associated with a pulmonary inflammatory response (42). The findings of the current study are consistent with previous findings regarding MAPK signaling regulation (43). Morin dramatically mitigated effects of these to a level that is comparable to the control in parallel to literature (12, 44).

The Akt/PKB isoforms have different roles in animals, with Akt2 primarily regulating metabolic signaling and Akt1 regulating growth and survival (45). FOXO1 is a member of the forkhead transcription factor family, which is involved in a variety of biological functions such as DNA repair, apoptosis, cell cycle arrest, and resistance to oxidative stress (46). The Akt-FoxO1 signaling pathway plays an essential role during liver regeneration (47). The mRNA expression levels of Akt2 and FOXO1 were significantly upregulated in the liver of MTX-induced rats according to our RT-PCR data. Morin (50 and 100 mg/kg) treatment, on the other hand, reduced the levels of these markers. In a different study, morin inhibits glucose production in cultured HepG2 cells inactivating FOXO1 and Akt (48).

Matrix Extracellular matrix proteins are broken down and synthesized more quickly by metalloproteinases (MMPs), a class of zinc-dependent endopeptidases. Gelatinase MMP-2 and MMP-9 are two examples of this category of enzymes. High levels of these gelatinases in the bloodstream are linked to an inflammatory process (49). MMPs have been associated with the pathophysiology of the MTX (50). In current study, we demonstrated that serum MMP-2 and MMP-9 levels increased after MTX administration in consistent with the literature. In contrast to the MTX group, co-supplementation of morin (50 and 100 mg/kg) effectively reversed MTX-induced upregulations in MMP-2 and MMP-9 levels. The impacts of

morin on MMP-2 and MMP-9 expressions were investigated in other studies as well (51, 52). In this study, we have demonstrated ability of morin in mitigating MTX-induced hepatic injury.

Conclusions

In this study, morin mitigated oxidative stress and apoptosis, down-regulated mRNA levels of MMP-2, MMP-9, MAPK14, MAPK15, JNK, Akt2 and FOXO1 genes, reduced ALT, ALP and AST levels in MTX-induced hepatic tissue. All data obtained from the study reveal the ameliorative effects of morin supplementation towards MTX-induced liver damage.

Declarations

Authors' contributions

All authors contributed equally to this work.

Data availability

The data that support the findings of this study are available from the corresponding author, [Cuneyt Caglayan], upon reasonable request.

Conflict of interest

There are no conflicts of interest to declare.

Ethical approval

Experimental and animal-care protocols were approved by the Animal Experimentation Ethics Committee of Bingol University (Protocol No: 2022-E.66052).

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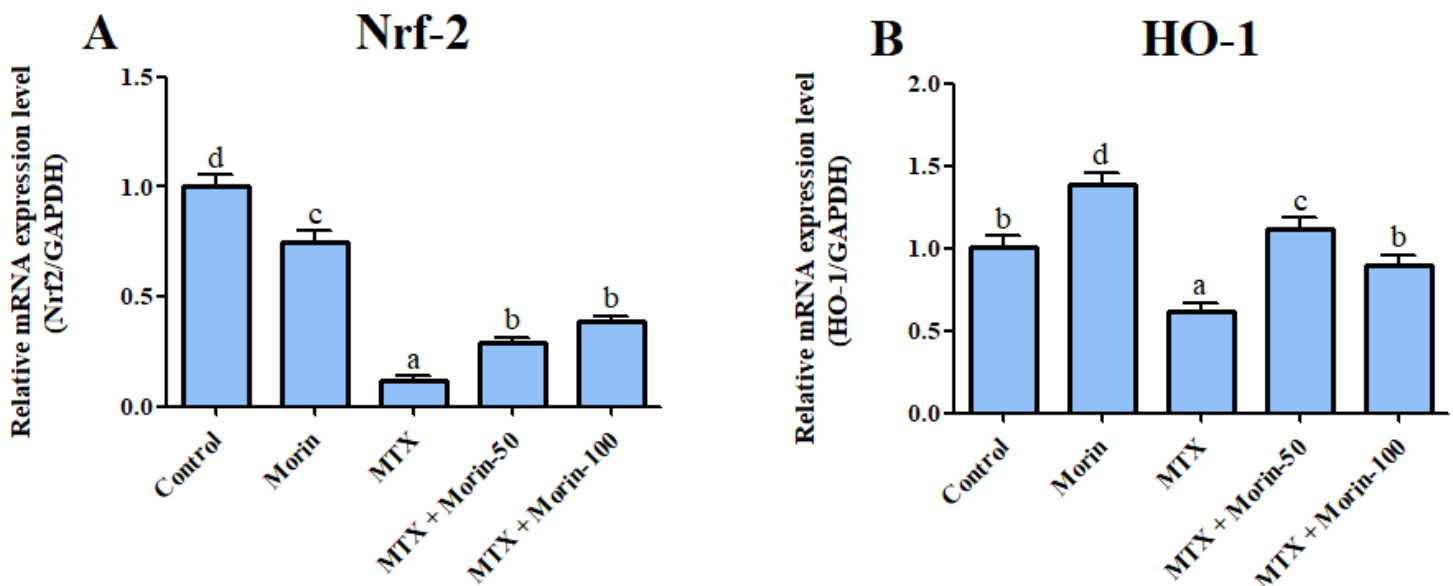
Figures

Figure 1

Effects of morin and MTX treatments on Nrf-2/HO-1 mRNA expression levels in liver tissue. A) Nrf-2 mRNA transcript levels, B) HO-1 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$).
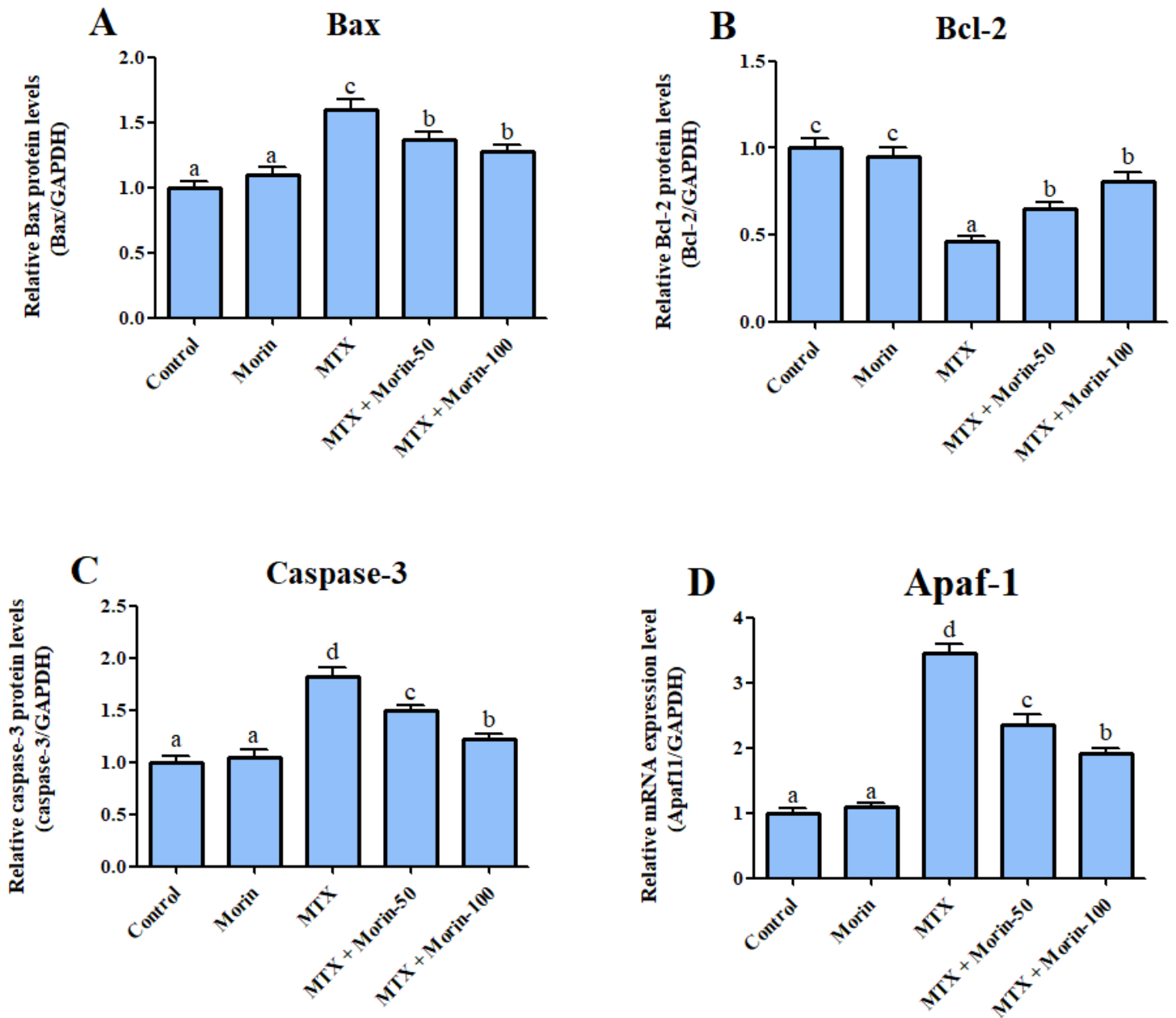
Figure 2

Effects of morin and MTX treatments on Bax, Bcl-2, Caspase-3 and Apaf-1 mRNA expression levels in liver tissue. A) Bax mRNA transcript levels, B) Bcl-2 mRNA transcript level, C) Caspase-3 mRNA transcript levels, D) Apaf-1 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a−d) on the columns show a statistical difference ($p < 0.05$).
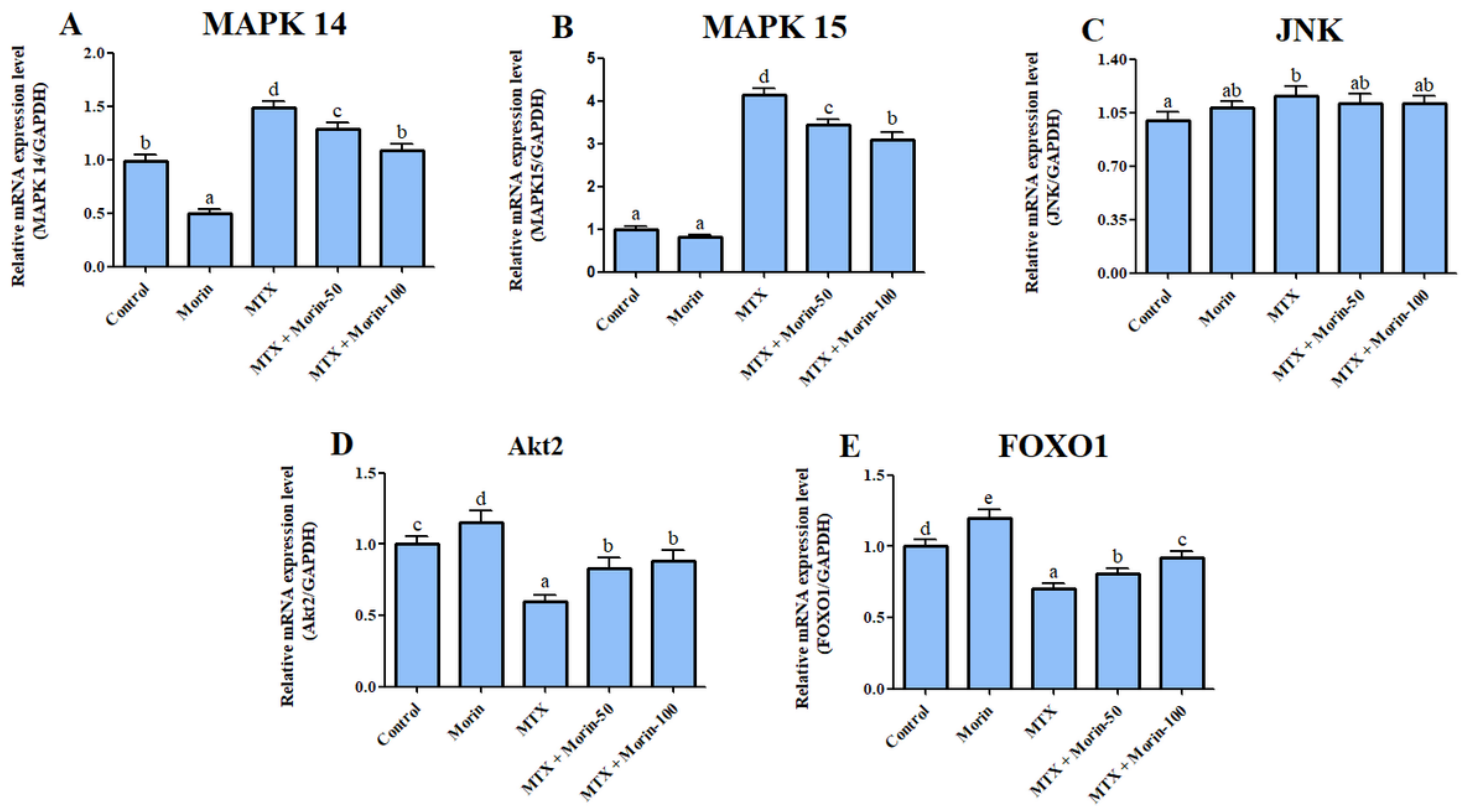
Figure 3

Effects of morin and MTX treatments on MAPK 14, MAPK 15, JNK, Akt2 and FOXO1 mRNA expression levels in liver tissue. A) MAPK 14 mRNA transcript levels, B) MAPK 15 mRNA transcript level, C) JNK mRNA transcript levels, D) Akt2 mRNA transcript levels, E) FOXO1 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a−d) on the columns show a statistical difference ($p < 0.05$).
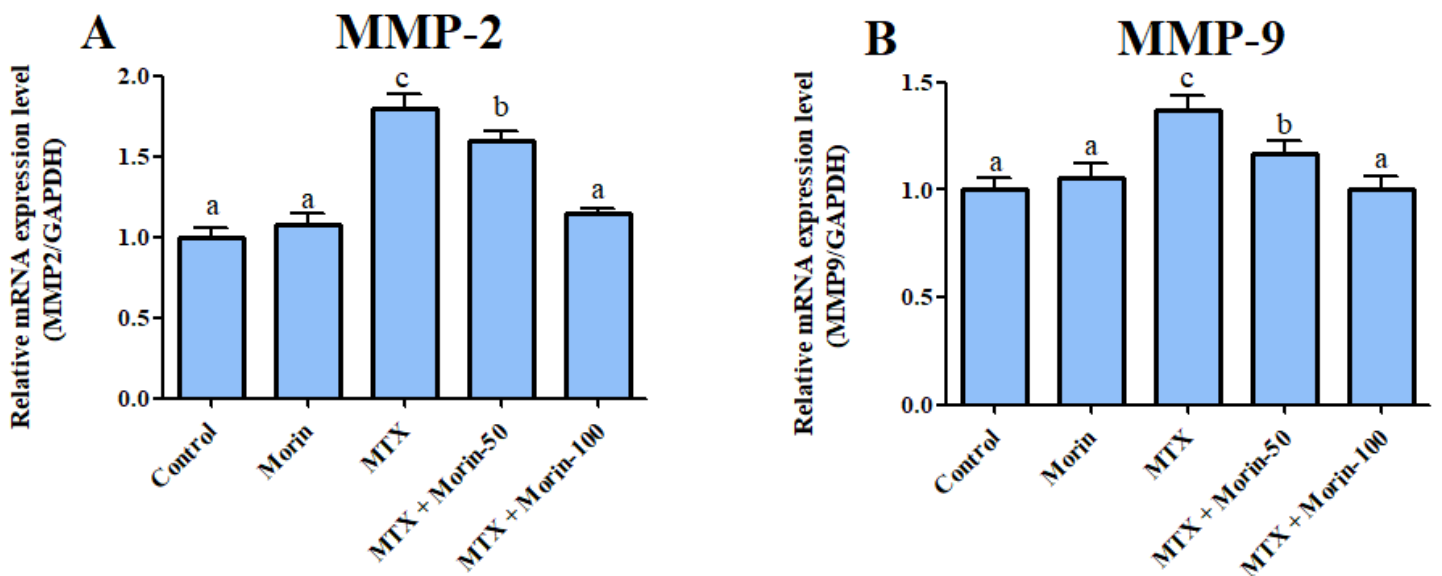

Figure 4

Effects of morin and MTX treatments on MMP2 and MMP9 mRNA transcript levels in liver tissue. A) MMP2 mRNA transcript levels, B) MMP2 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a−d) on the columns show a statistical difference ($p < 0.05$).