

Morrisonide protect human granulosa cells against H₂O₂-induced oxidative damage via regulating Nrf2 and MAPK signaling pathway

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Research

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Abstract

Background

Morrnonside is the main ingredient of *Cornus officinalis*, which has an antioxidant effect. Ovarian granulosa cells (GCs) are responsible for regulating the development and atresia of follicles, which are susceptible to oxidative stress. In this study, we investigated whether morrnonside could inhibit oxidative stress of GCs induced by hydrogen peroxide (H_2O_2), thus leading to improve oocyte quality.

Methods

The study was divided into 5 groups: control group, H_2O_2 group, morrnonside (5 μM) + H_2O_2 , morrnonside (10 μM) + H_2O_2 , Morrnonside (20 μM) + H_2O_2 . Cell survival rate was determined by CCK-8, ROS fluorescence level was determined by DCFH-DA probe, MDA, 8-OHdG, T-AOC, SOD, NQO1 and caspase-3 were determined by ELISA, SOD, NQO1, Bax, Bcl-2, caspase-3, caspase-9, Nrf2 and MAPKs protein expression were determined by Western blot, and Nrf2 nuclear translocation level was determined by immunofluorescence method. SPSS21.0 was used for statistical data analysis.

Results

After pretreatment with morrnonside, the levels of ROS, MDA and 8-OHdG in ovarian GCs were significantly decreased. Morrnonside significantly upregulated the level of p-Nrf2 and promoted the nuclear translocation level of Nrf2, which transcriptionally activated antioxidant SOD and NQO1. In addition, the levels of apoptosis-related proteins Bax, Bcl-2, caspase-3 and caspase-9 were significantly regulated via p38 and JNK pathway by morrnonside.

Conclusions

These results suggested that morrnonside could reduce oxidative damage and apoptosis of ovarian GCs induced by H_2O_2 in multiple ways, which provided a new idea for clinical improvement of oxidative stress in female reproductive system.

Introduction

Ovarian granulosa cells (GCs) are located in the follicles and around the oocytes. They produce steroids, which are responsible for regulating the development and atresia of follicles. GCs are very important for oocyte maturation, oocyte quality and embryo development [1, 2]. Adenosine triphosphate in ovarian GCs can be directly transferred to oocytes through the gap of cumulus GCs [3]. Furthermore, the GCs can also convert glucose into pyruvate which is the energy substrate of oocytes, and transfer it to oocytes [4].

Evidence shows that the dysfunction of GCs is related to ovarian senescence, the fewer oocytes retrieved, poor oocyte and embryo quality, and low pregnancy rate of in vitro fertilization-embryo transfer (IVF-ET) [5, 6]. In addition, women with polycystic ovary syndrome and endometriosis have a higher rate of GCs apoptosis, and thus reduced fertility and pregnancy rates [5, 7]. Therefore, normal ovarian GCs are necessary to maintain reproductive function.

Physiological levels of reactive oxygen species (ROS) are necessary for follicular growth, oocyte maturation, normal ovulation and ovarian hormone synthesis [8]. Oxidative stress occurs with the generation of excessive ROS or when antioxidant defense mechanisms are weakened [9]. Oxidative stress is the basic pathogenesis of a variety of reproductive system diseases, which can damage fertility, decrease pregnancy and delivery rate, and result in recurrent abortion [10]. Eight-hydroxy-2'-deoxyguanosine (8-OHdG) is a sensitive indicator of DNA damage as the result of oxidative stress. The increase of 8-OHdG content in ovarian GCs is related to the low fertilization rate of oocytes and poor embryo quality during IVF-ET [11]. Nuclear factor erythroid 2 related factor 2 (Nrf2) is a key antioxidant transcription factors in response to ROS, and it can bind to antioxidant response element (ARE) and induces the expression of numerous antioxidant enzyme including superoxide dismutase (SOD) [12, 13], high level of SOD is positively correlated with IVF pregnancy rate [14]. In addition, excessive ROS generation can trigger GCs apoptosis through MAPK, AKT/FoxO3a and mTOR pathways and increased the apoptosis-related gene expressions, including caspase 9 and caspase 3, causing the GCs cycle arrest and reduces its supporting effect on oocytes, thus affecting oocyte development, ovarian reserve and reproductive potential [15, 16]. Therefore, in order to improve female reproductive function, the discovery of drugs that can improve GCs oxidative stress is an urgent problem to be solved.

Morrisonide is an important active ingredient in *Cornus officinalis* (Fig. 1A). It has a variety of biological activities, such as antioxidant, anti-apoptotic, anti-inflammatory [17–19], which can relieve nerve pain, improve cardiovascular and liver functions [20, 21]. Deng et al. have confirmed that morroniside inhibit autophagy in rats ovarian GCs by regulating the PI3K/AKT/mTOR pathway [22]. However, the effect of morroniside on oxidative stress of GCs induced by H_2O_2 is largely unclear. Therefore, the aim of this study was to investigate the effect of morroniside on GCs of oxidative stress induced by H_2O_2 and elucidated the molecular mechanisms by which morroniside protect human granulosa cells against H_2O_2 -induced oxidative damage which could improve oocyte development.

Materials And Methods

Cell Culture and Treatment

Ovarian GCs were obtained from patients undergoing IVF-ET due to fallopian tube factors in the Reproductive Department of the Second Hospital of Hebei Medical University, and were approved by the ethics committee (Shijiazhuang, China) and informed consent of the patients. The follicular fluid containing GCs were centrifuged at 4°C (2000rpm, 10min). Remove the upper follicular fluid, and 5ml PBS was added into the below sediment and mixed them. 5ml human lymphocyte separation fluid

(Lympholyte-H, Cedarlane Laboratories, Canada) was added to another 10ml centrifuge tube and inclined angle of 45°. PBS suspension was slowly added to the surface of the human lymphocyte separation solution, and then centrifuged at 4°C (2500rpm, 10min). The white floc in the middle layer is GCs.

The GCs were cultured in DMEM/F12 medium (Gibco, Thermo, USA) at 37°C in a 5% CO₂ incubator. After the cells adhered to the wall, the cells were incubated with H₂O₂ for 24 h after the different concentrations morroniside ranging from 5 to 20µM pretreated for 24 h.

CCK-8 assay

GCs were pretreated with different concentrations of morroniside and H₂O₂ which determined by CCK-8 assay kits (MCE, China). 96-well plate cells were added with 10µl CCK-8 reagent per well and incubated at 37°C for 2 h. The OD value of each hole was read at 450nm with a microplate reader (Vesar Max, Santak, USA).

Intracellular ROS Detection

Intracellular ROS levels in each group were detected with ROS assay kit (Beyotime, China). The culture medium containing serum was removed, the cells were incubated with diluted DCFH-DA (1:1000) for 20 min at 37°C in 5% CO₂ incubator and then washing three times with serum-free medium. ROS content was detected by fluorescence microscope (EVOS® FL, Thermo, USA) and fluorescence intensity was analyzed by Image J software.

ELISA assay

The ovarian GCs sample lysis fluid was diluted to the optimal concentration. The biomarkers related to oxidative stress and apoptosis, including ROS, malondialdehyde (MDA), 8-OHdG, total antioxidant capacity (T-AOC), SOD, NAD(P)H quinone oxidoreductase 1 (NQO1), and caspase-3, were detected by ELISA assay kit (Jianglai Biological Co., Ltd, Shanghai, China; Jiancheng Bioengineering Institute, Nanjing, China; Abcam, USA; Tongwei Industrial Co., Ltd, Shanghai, China) following the manufacturer's instructions. The absorbance values were measured by the microplate reader (VersaMax, Molecular Devices, USA).

Western blot analysis

The collected GCs were lysed to extract total protein and the protein concentration was determined using BCA protein assay kit (SolarBio, Beijing, China). The same amount of total protein in each well (10-15 µg) was separated by 10% SDS-PAGE and transferred to PVDF membrane (Millipore, Merck KGAA, Darmstadt, Germany) at the end of electrophoresis. The PVDF membrane was sealed in 5% skimmed milk powder for 2 h and incubated with different primary antibodies overnight at 4°C. The primary antibodies used in this study mainly included 1:1000 SOD (ab68155, Abcam, USA), 1:1000 NQO1(ab80588, Abcam, USA), 1:1000 Bax (ab32503, Abcam, USA), 1:1000 Bcl-2 (26593-1-AP, Proteintech, USA), 1:1000 caspase-3 (YT6113, Immunoway, USA), 1:1000 caspase-9 (ab202068, Abcam, USA), 1:500 p-Nrf2 (ab76026, Abcam, USA),

1:500 Nrf2 (ab62352, Abcam, USA), 1:500 p-ERK (YP0101, Immunoway, USA), 1:500 ERK (16443-1-AP, Proteintech, USA), 1:500 JNK (66210-1-Ig, Proteintech, USA), 1:500 p-JNK (YP0156, Immunoway, USA), 1:500 p38 (ab181602, Abcam, USA) and 1:500 p-p38 (ab4822, Abcam, USA). The PVDF membrane was washed with TBST for 3 times and incubated with the secondary antibody (SA00001-2, Proteintech, USA) and incubated for room temperature for 1 h. After the PVDF membrane was washed three times with TBST, antibody-antigen complexes were visualized using a Chemiluminescence Plus Western immunoblot analysis kit (Millipore, USA).

Immunofluorescence staining

The treated GCs were fixed with 4% paraformaldehyde for 20min, drilled with 1% Triton and sealed with 10% goat serum for 30min, and then incubated overnight with Nrf2 antibody (1:200) at 4°C. After the cells were incubated with fluorescence-labeled secondary antibodies at room temperature for 2 h, the cells were stained with DAPI for 10min. Finally, it was observed under a laser scanning confocal microscope (Leica, Germany).

Statistical analysis

All data were expressed as mean \pm standard deviation and statistical analysis was performed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Multiple groups comparison was performed by ANOVA and post-hot analysis. Values of $P < 0.05$ were considered statistically significant.

Results

Morrisonide increased GCs viability

CCK-8 assay were used to detect the survival rate of cells with different concentrations of morroniside and H₂O₂. Compared with the control group, there was no significant change in the survival rate of GCs after pretreatment with 1, 5, 10, 20 μ M morroniside except for the 50 μ M group (Fig. 1B). With the increase of H₂O₂ concentration, the survival rate of GCs treated with H₂O₂ decreased gradually in a concentration-dependent manner. 600 μ M H₂O₂ could significantly reduce the cell survival rate without causing more serious cell damage, so this concentration was used in our subsequent experiments (Fig. 1C). We tested the effect of different concentrations of morroniside on the cell survival rate induced by H₂O₂, and the results showed that after pre-incubated with morroniside, the survival rate of GCs induced by H₂O₂ increased significantly compared with H₂O₂ group (Fig. 1D).

Morrisonide inhibited GCs oxidative stress

To test the effect of morroniside on GCs ROS levels induced by H₂O₂, we performed with ROS assay kit and ELISA. As shown in Fig. 2A and B, compared with the control group, ROS level of GCs which were treated with 600 μ M H₂O₂ for 24 h increased significantly ($P < 0.05$). Compared with the H₂O₂ group, ROS levels of GCs which were pretreated with morroniside significantly decreased followed a concentration (5,

10, 20 μM)-dependent manner, expression peaked at 20 μM ($P < 0.05$). MDA, 8-OHdG and T-AOC content levels were detected by ELISA to evaluate the degree of oxidative stress. The oxidative damage products content of MDA and 8-OHdG in morroniside group were significantly reduced compared with H_2O_2 group ($P < 0.05$), which is consistent with the result of ROS level of GCs (Fig. 2C and D). In addition, the activity of T-AOC was detected to evaluate the antioxidant level of morroniside. The levels of T-AOC in GCs were significantly reduced after H_2O_2 treatment, while morroniside could significantly increase the activities of T-AOC ($P < 0.05$) (Fig. 2E). These results demonstrated that morroniside protects GCs by reducing oxidative damage induced by H_2O_2 .

Morroniside increased the expression of SOD and NQO1 in GCs inhibited by H_2O_2

NQO1 is believed to be partly the reduction of free radical load in cells and the detoxification of xenobiotics. SOD is one of the most important antioxidant enzymes, enabling organisms to survive in an oxygen containing atmosphere [23]. The activities of SOD and NQO1 in ovarian GCs were detected and found that H_2O_2 significantly reduced their activities, while morroniside could significantly increase the activities of them ($P < 0.05$) (Fig. 3A and B). The protein expression of SOD and NQO1 were also performed by Western blot, the results showed that H_2O_2 reduced the protein levels of SOD and NQO1, while different concentrations of morroniside significantly increased the protein levels in a dose dependent manner (Fig. 3C-E). These results demonstrated that morroniside upregulated the protein levels and activities of antioxidant enzymes to protect GCs against oxidative damage induced by H_2O_2 .

Morroniside inhibited GCs apoptosis induced by oxidative stress

High concentration of ROS can damage the structure of mitochondria, make the polar pores in the inner mitochondrial membrane in an expanded state which led to the outflow of calcium ions and cytochrome C, finally membrane potential disappeared and cells initiates apoptosis [24]. The expression of apoptosis-related proteins were detected by Western blot analysis. The results showed that the protein expressions of Bax, caspase-9 and caspase-3 in the H_2O_2 group were significantly increased, while the Bcl-2 expression was significantly decreased ($P < 0.05$). However, compared with the H_2O_2 group, the protein expression levels of Bax, caspase-9 and caspase-3 were significantly decreased, while Bcl-2 levels were significantly increased after pretreated with morroniside (Fig. 4A-E) ($P < 0.05$). Activity of casepase-3 were detected and showed that H_2O_2 increased its activity, while morroniside at different concentrations decreased its activity (Fig. 4F) ($P < 0.05$). These results suggested that morroniside can attenuate oxidative stress induced apoptosis of GCs.

Morroniside activates Nrf2, p38 and JNK signaling pathway in GCs

When the cell is stimulated by ROS, Nrf2 is activated, transported into the nucleus, and then combined with the antioxidant response element (ARE) to activate the express of anti-oxidase genes, thus play an important anti-oxidative damage effect [25]. In order to further study the mechanism of the anti-oxidation of morroniside, we observed the effect of morroniside on the nuclear translocation of Nrf2 in GCs cultured

in vitro. In the control and H₂O₂ group, Nrf2 was almost located in the cytoplasm. When GCs were pretreated with different concentrations morroniside, Nrf2 transported into the nucleus, and 20μM morroniside group almost located in the nucleus (Fig. 5A). Western blot results also showed that the level of Nrf2 in the nucleus in the morroniside group was significantly higher than that in the H₂O₂ group ($P < 0.05$) (Fig. 5B), which is consistent with Fig. 5A. Furthermore, phosphorylation level of Nrf2 was detected by Western blot analysis. Compared with the H₂O₂ group, phosphorylated Nrf2 (p-Nrf2) levels were increased in different morroniside groups ($P < 0.05$) (Fig. 5C). The results demonstrated that morroniside promoted p-Nrf2 expression, and the activated Nrf2 was transfer into the nucleus to regulate the expression of antioxidant enzymes and thus exert antioxidant effects.

Mitogen-activated protein kinases (MAPKs) are activated in response to oxidative stress. Several studies have demonstrated that ROS production and activation of MAPKs play a vital role in Aβ-induced apoptosis [26]. To further elucidate the signaling pathway that involved in morroniside preventing H₂O₂-induced apoptosis, we examined the effect of morroniside on activation of MAPKs in GCs. Our data showed that H₂O₂ upregulated phospho-JNK and phosphor-p38MAPK expression, but not ERK1/2 phosphorylation (Fig. 5D). Furthermore, morroniside significantly suppressed H₂O₂-induced upregulation of p-JNK and p-p38 MAPK in GCs. These results suggested that morroniside could inhibit JNK and p38 signaling pathway which induced cell apoptosis.

Discussion

In this study, we firstly investigated the protective effect of morroniside on ovarian GCs from the perspective of oxidative stress. We showed that morroniside increased the expression level of p-Nrf2, promoted the nuclear translocation of Nrf2, and then up-regulated the expression of antioxidant enzymes such as SOD and NQO1, and reduced the oxidative damage induced by H₂O₂. In addition, morroniside ameliorated p38 and JNK pathway-induced apoptosis by reducing ROS levels. Morroniside can be used as a potential drug to improve the quality of follicles by protecting GCs.

Oxidative stress is closely related to the injury of female reproductive function. With the increase of age, the antioxidant capacity of the ovary decreased, and the imbalance between oxidation and antioxidant in the ovary led to the apoptosis of oocytes and ovarian GCs [27]. The level of ROS and the expression of apoptotic proteins induced by ROS in ovarian GCs of patients with polycystic ovary syndrome (PCOS) were significantly higher than those of non-PCOS women [28]. Prieto L et al confirmed that the levels of ROS in the follicular fluid of infertility patients with endometriosis are increased, while the levels of T-AOC and SOD are generally decreased [29]. Morroniside could suppressed autophagy and apoptosis in rat ovarian GCs through the PI3K/AKT/mTOR pathway [22]. Our study showed that morroniside could inhibited the levels of ROS and 8-OHdG and MDA content and increased the expression of SOD and NQO1 in human ovarian GCs (Figs. 2 and 3). The oxidative damage of GCs caused by various reasons such as aging directly affects female reproduction, the results suggested that morroniside protected GCs from oxidative damage.

Nrf2 is important for antioxidant stress. When cells exposed to a variety of stimuli including antioxidants and xenobiotics, Nrf2 was activated and transferred into the nucleus, formed a complex with the maf protein and bound to ARE, and regulate ARE-mediated antioxidant enzyme gene expression such as SOD and NQO1 [12, 13]. The expression of Nrf2 detected in cumulus cells might be related to oocyte quality [30], while upregulation of Nrf2 in oocytes and cumulus cells might affect the GSH level in matured COCs [31]. Under the stimulation of harmful conditions (such as heat stress and heavy metals), the activation of Nrf2 pathway can affect the activity and proliferation of ovarian GCs [32, 33]. However, Nrf2 activators such as quercetin and dimethyl fumarate [34] can up-regulate the expression of Nrf2 and its downstream SOD and CAT in ovarian GCs to reduce the level of ROS, thus playing an antioxidant role [35]. Consistent with these studies, our results show that morroniside promoted the nuclear translocation of Nrf2, thereby regulating the expression of downstream antioxidant genes and playing a protective role on GCs. Thus, the morroniside through regulating the Nrf2 attenuating the ROS level (Fig. 5A and B).

Endogenous ROS as a second messenger is involved in multiple signaling pathways of cascading effect [36] and is an upstream activator of p38 and JNK which are the member of the MAPK family and are involved in the activation of apoptotic factor such as caspase 3 [28, 37, 38]. The release of cytochrome c (Cyt c) is the key to mitochondria-mediated activation of apoptosis protein, which activates caspase-3 together with its cofactor caspase-9 [39], and then activates the apoptotic signaling pathway. Bcl-2 and Bax are involved in this process. Bcl-2 inhibits the release of Cyt c while Bax promotes its release [40]. We got the same results that the morroniside reduced the phosphorylation level of p38 and JNK, decreased the expression of Bax, caspase-3 and caspase-9, and increased the level of Bcl-2 in ovarian GCs. ERK also belongs to the MAPK family and is widely expressed in the GCs. Different from the apoptotic effects of p38 and JNK, ERK is very necessary for oocyte maturation and embryo development [41]. Ma et al. found that the p-ERK level in GCs of women with low ovarian function was lower than that of women with normal ovarian function and confirmed that activation of ERK pathway could inhibit apoptosis and reduce the expression level of cleaved caspase-3 [42]. Our results showed that morroniside reduce H₂O₂-induced GCs apoptosis by regulating p38 and JNK pathway but not by ERK pathway (Figs. 4 and 5D).

In summary, our study suggested that the morroniside had a protective effect on GCs stimulated by H₂O₂. Morroniside increased the expression level of p-Nrf2, promoted the nuclear translocation of Nrf2, and then up-regulated the expression of antioxidant enzymes such as SOD and NQO1 which reduced the oxidative damage induced by H₂O₂. In addition, morroniside ameliorated p38 and JNK pathway-induced apoptosis by reducing ROS levels. This study also provides a new idea for the clinical treatment of reproductive diseases caused by oxidative stress.

Abbreviations

ARE: antioxidant response element; Cyt c: cytochrome c; GCs: Granulosa cells; H₂O₂: Hydrogen peroxide; IVF-ET: In vitro fertilization-embryo transfer; MDA: Malondialdehyde; MAPKs: Mitogen-activated protein kinases; NQO1: NAD(P)H quinone oxidoreductase 1; Nrf2: Nuclear factor erythroid 2 related factor 2; ROS:

Reactive oxygen species; SOD: Superoxide dismutase; T-AOC: total antioxidant capacity; 8-OHdG: 8-hydroxy-2-deoxyguanosine

Declarations

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Authors' contributions

YCM and GMH contributed equally to this work. YCM, GMH, MH and HLD jointly participated in the design of this study. XHL, ZMZ and AMY were responsible for collecting cell samples, YCM, XHL, AMY, YCC, SCZ, LJF, JRG, YZ and JWC performed the experiments, YCM, GMH and ZMZ were responsible for data statistics and wrote the paper. All authors have seen and approved the manuscript.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Hebei University of Chinese Medicine (Shijiazhuang, China). Cell samples were collected with the patient's informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no conflicts of interest.

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Figures

Fig. 1

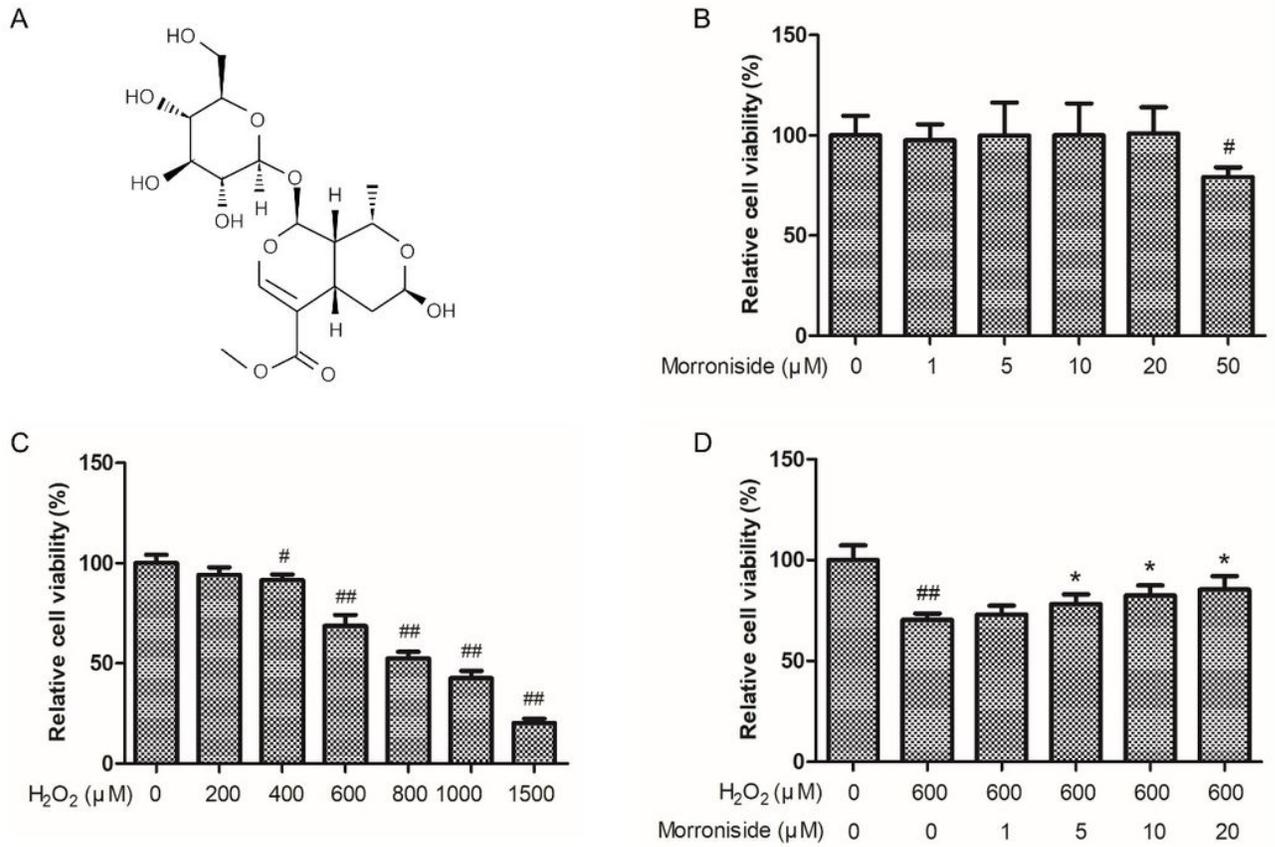


Figure 1

The effect of morroniside on GCs viability decreased by H₂O₂. (A) Structure of morroniside. (B) Ovarian GCs were treated with morroniside at different concentrations (including 1, 5, 10, 20, 50 μM) for 24 h. (C) GCs were treated with H₂O₂ at different concentrations (including 200, 400, 600, 800, 1000, 1500 μM) for 24 h. (D) GCs were pretreated with morroniside at different concentrations (including 5, 10, and 20 μM) for 24 h and then treated with 600 μM H₂O₂ for 24 h. The survival rates of GCs were determined by CCK-8 assay kit. [#]P < 0.01 versus the control group, ^{##}P < 0.001 versus the control group, ^{*}P < 0.01 versus the H₂O₂ group.

Fig. 2

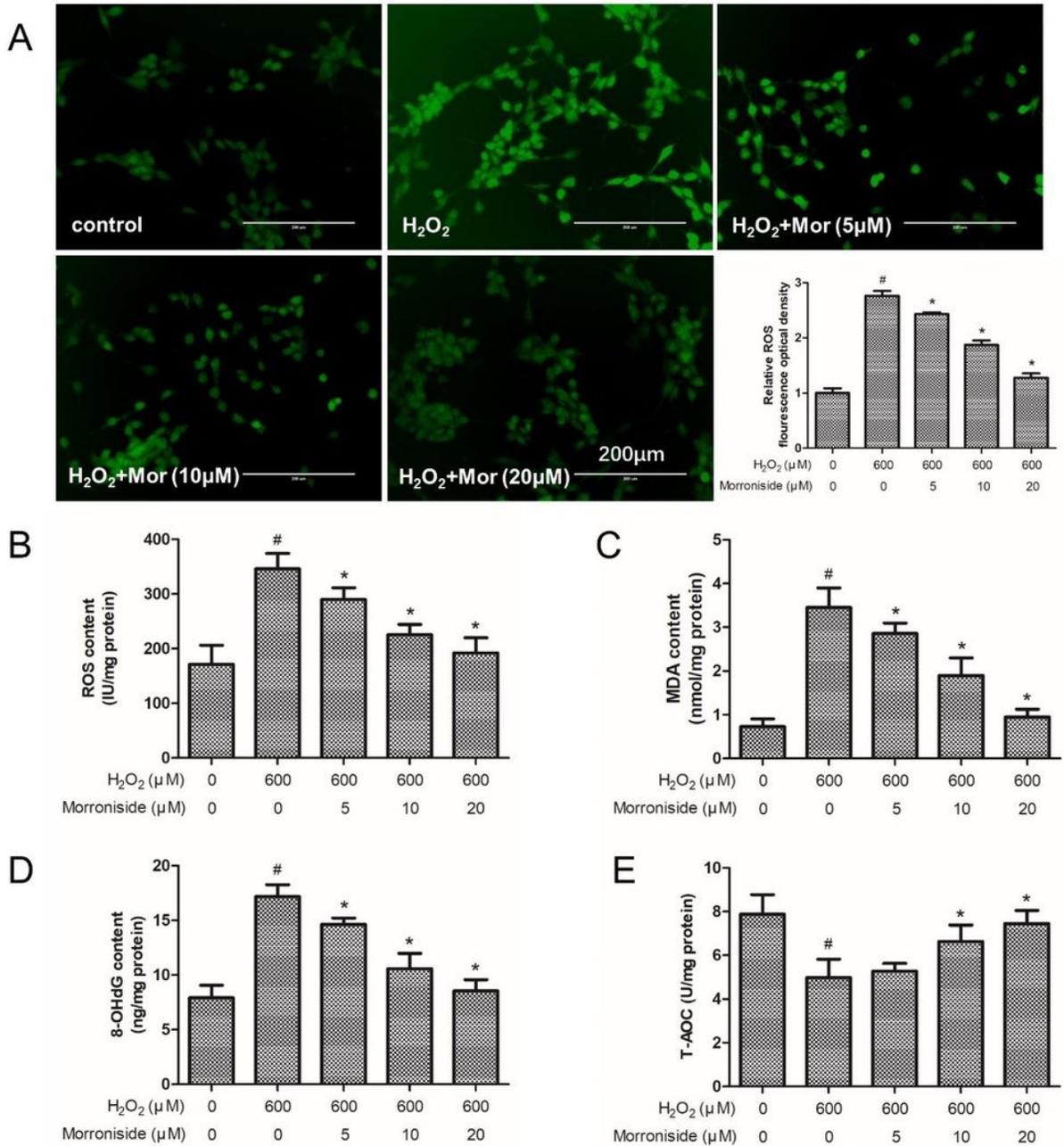


Figure 2

Morrisonide inhibited GCs oxidative stress induced by H₂O₂. (A) Intracellular ROS level and quantitative analysis of ROS. The oxidative stress biomarkers of (B) ROS, (C) MDA, (D) 8-OHdG and (E) T-AOC content levels were test with ELISA. #P < 0.05 versus the control group, *P < 0.05 versus the H₂O₂ group. Scale bar was 200µm.

Fig. 3

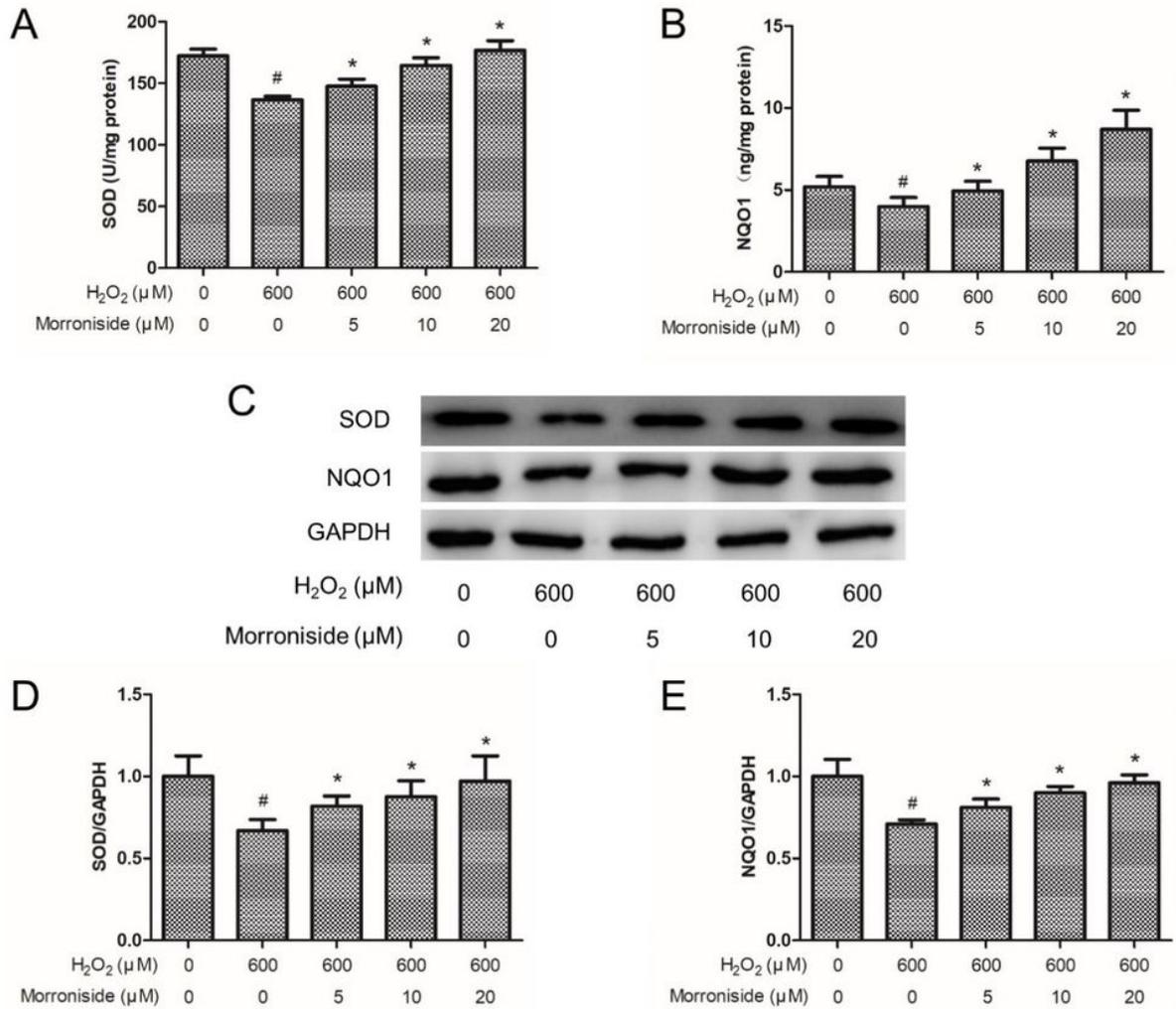


Figure 3

Effect of morroniside on the activity and protein expression of SOD and NQO1 in GCs inhibited by H₂O₂. The activity levels of antioxidant enzymes (A) SOD and (B) NQO1 were test with ELISA. (C) The protein expression levels of SOD and NQO1 were detected by western blot analysis. (D and E) The protein expression levels of SOD and NQO1 were quantitatively analyzed. #P < 0.05 versus the control group, *P < 0.05 versus the H₂O₂ group.

Fig. 4

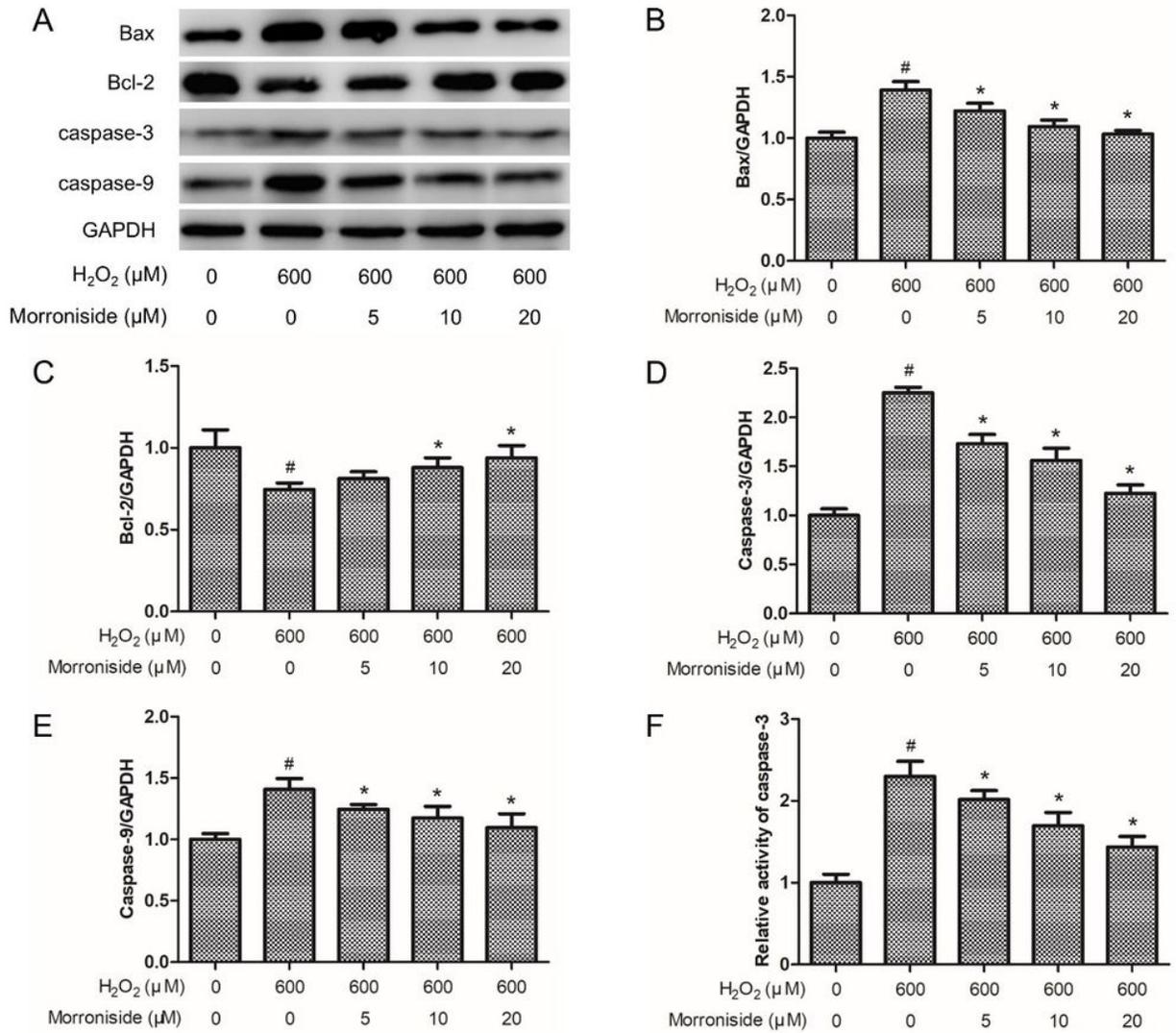


Figure 4

Effect of morroniside on GCs apoptosis induced by oxidative stress. (A) The protein expression levels of Bax, Bcl-2, caspase-3 and caspase-9, which were related to apoptosis, were detected by Western blot analysis. (B-E) The protein expression levels of Bax, Bcl-2, caspase-3 and caspase-9 were quantitatively analyzed. (F) The casepase-3 activity level. #P < 0.05 versus the control group, *P < 0.05 versus the H2O2 group.

Fig. 5

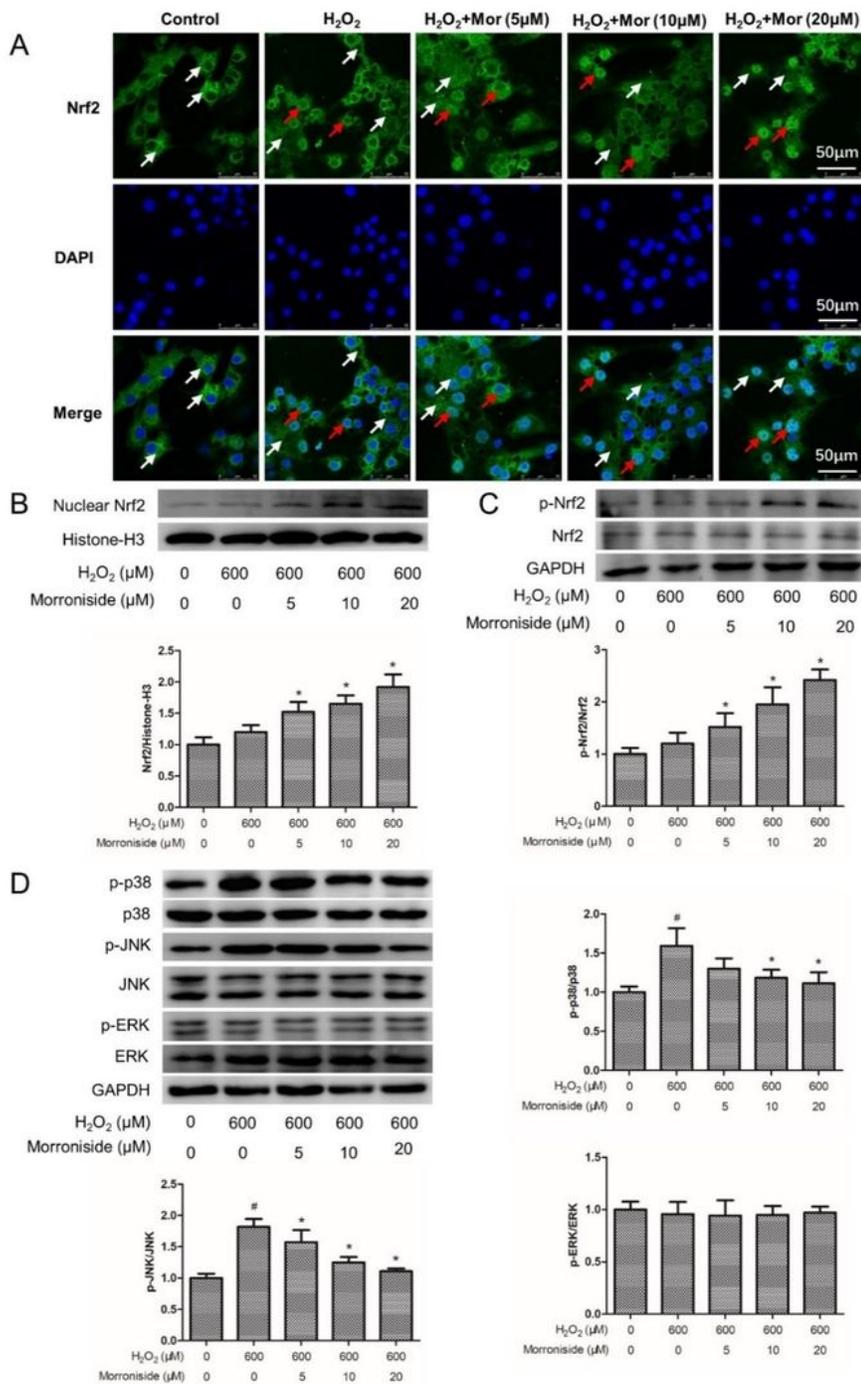


Figure 5

Effect of morroniside on the protein expression and nuclear translocation of Nrf2. (A) The green fluorescence represents Nrf2, and the nucleus is blue DAPI. The white arrows represent Nrf2 in the cytoplasm and the red arrows represent Nrf2 in the nucleus. Scale bars are 50μm. (B) The protein expression levels of nuclear Nrf2, (C) p-Nrf2, Nrf2, (D) p-p38, p38, JNK, p-JNK, ERK and p-ERK were detected by western blot analysis, and the protein expression levels of p-Nrf2 in nucleus, p-Nrf2/Nrf2, p-

p38/p38, p-JNK/JNK and p-ERK/ERK were quantitatively analyzed. #P < 0.05 versus the control group, *P < 0.05 versus the H2O2 group.

Fig. 6

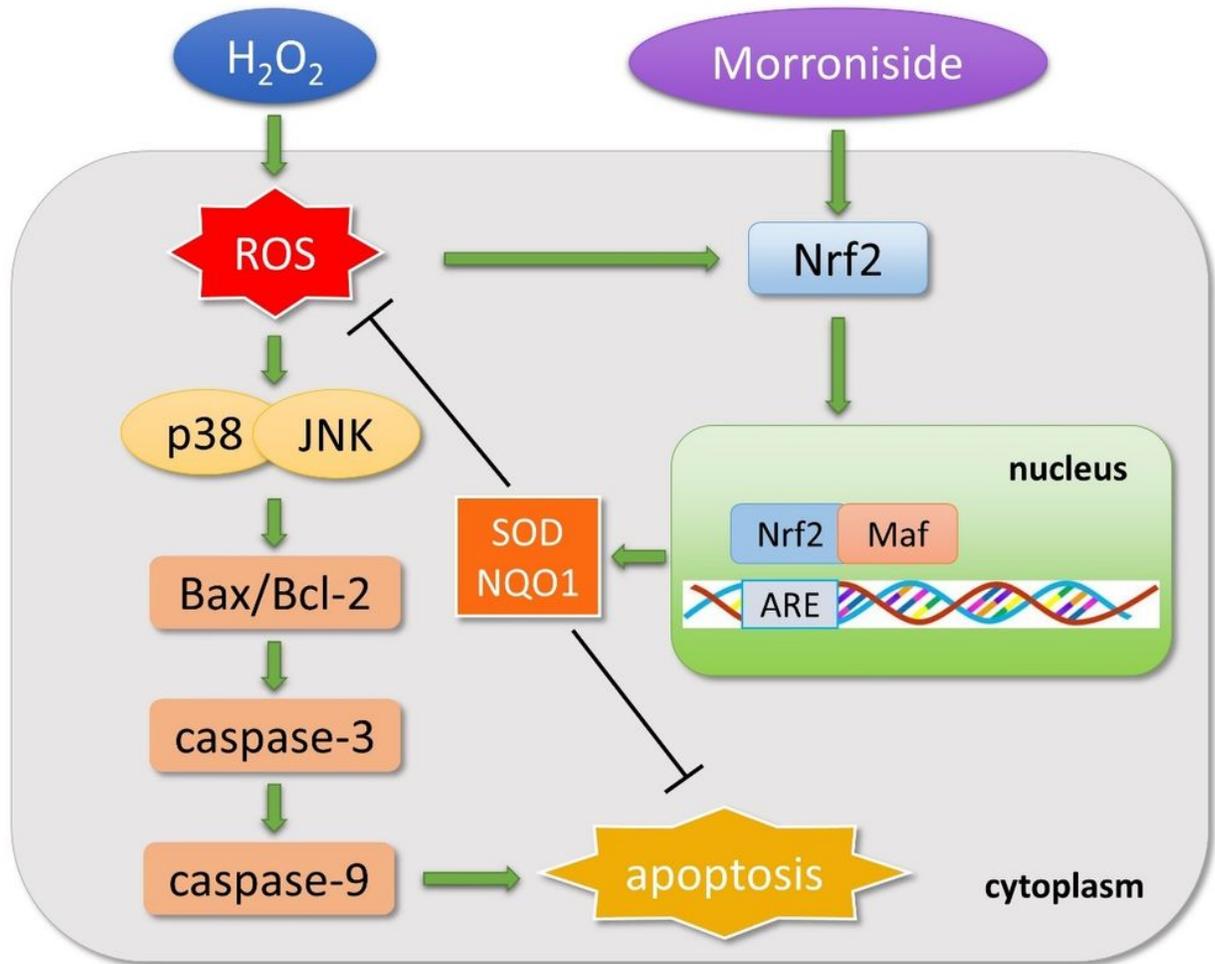


Figure 6

Schematic model of morroniside protecting human granulosa cells against H2O2-induced oxidative damage. Morroniside increased the expression level of p-Nrf2, promoted the nuclear translocation of Nrf2, and then up-regulated the expression of antioxidant enzymes such as SOD and NQO1 which reduced the oxidative damage induced by H2O2. In addition, morroniside ameliorated p38 and JNK pathway-induced apoptosis by reducing ROS levels.