frontiers

Mechanism of ~~moxibustion~~ inhibition~~ng~~ of p53-based regulation of ferroptosis by moxibustion to alleviate ~~improve~~ synovial inflammation injury in a rat rheumatoid arthritis model ~~rats~~

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Keywords: moxibustion, rheumatoid arthritis, inflammatory response, ferroptosis, tumor suppressor protein~~.~~

Abstract

Background: Moxibustion is an effective technique for treating ~~on~~ rheumatoid arthritis (RA), an autoimmune disease~~,~~; however, its ~~but the~~ mechanism is not yet fully understood. Inflammatory injury and ~~destruction of~~ cartilage and bone destruction are the primary ~~main~~ clinical manifestations of RA.

Here, we studied ~~This study explored~~ the mechanism through which ~~of~~ moxibustion alleviates ~~treatment on~~ inflammatory injury of synovial tissue in a ~~RA~~ rat ~~model~~ RA model by determining ~~observing the effect of~~ moxibustion's effect on ~~the regulation of~~ ferroptosis regulation by the tumor suppressor protein ~~(~~p53 and ~~)/~~ solute carrier family 7 member 11 (SLC7A11).

Methods: Sixty Sprague-Dawley~~SD~~ rats were randomly allocated to ~~divided into~~ five groups, with 12 rats per group: normal ~~group~~, model ~~group~~, agonist ~~group~~, moxibustion ~~group~~, and moxibustion + agonist ~~group, with 12 rats in each group~~. Except for rats in the normal group, rats in the remaining ~~other four~~ groups were developed ~~established~~ as RA models by exposure to the ~~combining wind, cold, and damp~~ environmental ~~factors~~ condition of wind, cold, and dampness together with the administration of Freund's complete adjuvant ~~(FCA)~~. In the moxibustion group, cigarette-like~~type~~ moxa strips were suspended near ~~used to suspend "~~"Shenshu~~"~~" and ~~"~~"Zusanli~~"~~" acupoints for 20 min/~~time~~time (both acupoints~~sides~~), and the two acupoints were stimulated ~~used~~ alternately~~tely~~ once ~~a day~~ daily for 15 days. ~~In the agonist group,~~ The p53 agonist NSC59984 was administered ~~injected~~ intraperitoneally (45 mg·mg·kg^{-1}·d-5, 3 injections) in the agonist group~~;~~. ~~the~~ The moxibustion + agonist group received ~~was given an~~ intraperitoneal ~~injection~~administration of ~~–~~NSC59984 and moxibustion treatment~~intervention~~. After 15 days of treatment~~intervention~~, histomorphological changes in the ~~of~~ knee synovial membrane were noted ~~observed~~ by transmission electron microscopy; ~~SLC7A11 and~~ GPX4 and SLC7A11 ~~protein expressions~~ expression was ~~were~~ detected by ~~W~~western blotting assay; serum levels of ~~GSH,~~ reactive oxygen species (ROS), glutathione (GSH), and Fe^{2+} ~~expressions~~ were estimated with~~detected by~~ the colorimetric method as well as ~~and~~ the fluorescent probe method~~,~~; and serum levels of ~~interleukin-1β (IL-1β) and~~ tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were quantified ~~detected~~ by ELISA~~lisa method expression~~.

Results: After ~~successful~~ the models were successfully established~~ing~~, ~~it was observed that mitochondrial damage in the cartilage of rats in both~~ the model and agonist group~~s~~ rats ~~was~~ showed evident mitochondrial damage in cartilage, while the moxibustion and moxibustion + agonist group~~s~~ rats showed varying degrees of reduction in mitochondrial damage after 15 days of treatment~~intervention~~. The ~~expression of~~ p53, ROS, Fe^{2+}, ~~IL-1β, and~~ TNF-α, and IL-1β levels ~~in the model group were significantly higher~~ in the model group were significantly higher ($P < .01$) than those ~~those~~ in the normal group ~~($P < 0.01$)~~, while ~~the expression of~~ SLC7A11, GPX4, and GSH levels were significantly lower ($P < 0.01$). In the agonist group, ~~the expression of~~ p53 ($P < 0.05$)~~,~~ and ROS, Fe^{2+}, ~~IL-1β, and~~ TNF-α, and IL-1β ($P < 0.01$) levels were significantly higher than those in the model

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group, while the expression of SLC7A11 ($P > 0.05$), GPX4 ($P < 0.05$), and GSH ($P < 0.01$) levels were markedly significantly lower. Additionally, in the moxibustion and moxibustion + agonist groups, the levels expression of p53 ($P < 0.05$, and $P > 0.05$ for the moxibustion and moxibustion + agonist groups, respectively), and ROS, Fe^{2+}, IL-1β, and TNF-α, and IL-1β ($P < 0.01$) were lower than those in the model group, while the expression of SLC7A11 ($P < 0.01$), GPX4 ($P > 0.05$), and GSH ($P < 0.01$) levels were markedly significantly higher. Moreover, the expression of p53, ROS, Fe^{2+}, IL-1β, and TNF-α, and IL-1β levels ($P < 0.01$) were significantly lower in the moxibustion and moxibustion + agonist groups than in compared to the agonist group, while the expression of SLC7A11, GPX4, and GSH ($P < 0.01$) levels were significantly higher.

Conclusion: Moxibustion may alleviate cartilage and synovial inflammation injury, and inhibit the expression of pro-inflammatory factors. Furthermore, and its mechanism of action is probably associated with may be related to the suppression inhibition of p53 protein expression, which activates the downstream gene *SLC7A11* to further suppress ferroptosis in knee joints.

1 Introduction

Rheumatoid arthritis (RA), is an autoimmune disease, manifests as characterized by chronic progressive arthritis (1). The pathogenesis of RA ishas a complex pathogenetic mechanism, with multiple cell death pathwaysmodalities involved, such as apoptosis, autophagy, necrosis, and ferroptosis (2-4). According to Rrecent studies, have found that p53, solute carrier family 7 member 11 (SLC7A11), p53, reactive oxygen species (ROS), and iron accumulation, which are important regulators of ferroptosis, are closely related to the development of RA development. This finding suggesting that RA and ferroptosis-related pathological processes are likely to convergeintersect; however, but their mechanisms of action remain unhave yet to be elucidated (5, 6). p53 is a potential regulatory target of ferroptosis and has a critical plays an important regulatory role in the development of RA disease development (7, 8).

RA belongs to the category of "Bi syndrome" iIn traditional Chinese medicine (TCM), RA is categorized as a “Bi syndrome”; this is mainly because of due to weakness of the body's upright Qi in the body; muscle invasion of by wind, cold, and dampness pathogens into the muscles; stagnation of Qi and blood circulation; and pain caused by obstruction of meridians by due to wind, cold, and dampness. The use of moxibustion, a characteristic therapy of TCM therapy, has shown excellent good therapeutic effects. Moxibustion is a therapy that involves direct or indirect burning and warming of

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acupoints on the body surface with mild heat generated by burning moxa cones~~, using the mild heat of fire~~ and using the ~~effect of~~ medicinal properties to ~~achieve the purpose of~~ promote~~ing~~ blood flow, remove~~ing~~ obstruction of meridians~~from channels~~, treat~~ing~~ diseases, and ~~health~~ preserve~~ation~~ health through meridian conduction. Modern clinical studies have shown ~~found~~ that moxibustion can effectively reduce inflammation in RA patients. Moxibustion treatment can regulate the immune function of RA patients, restore the dynamic balance of their humoral and cellular immunity, promote RF conversion to negative or decrease titers, and shows a ~~is~~ positive~~ly~~ correlation~~ed~~ with ~~the improvement of~~ clinical symptom improvement~~s~~ in patients (9).

Thus far, limited ~~There are few~~ studies have assessed ~~to explore~~ the mechanisms through which ~~of~~ moxibustion affects ~~for~~ RA from a ~~the perspective of~~ ferroptosis perspective.~~,~~ ~~and~~ ~~the~~ Our group~~'~~'s previous study demonstrated ~~found~~ that moxibustion ~~could~~ regulates ~~the expression of ferroptosis-related factors~~ p53 and SLC7A11 expression in the synovial tissue of rats with RA ~~model~~, inhibits ~~the occurrence of~~ ferroptosis occurrence, and mitigates ~~improve~~ the damage caused by ~~of~~ synovial inflammation (10). In the present ~~this~~ study, we used p53 as an entry point to determine ~~observe~~ whether moxibustion mediates SLC7A11 to regulate ferroptosis through p53 and thus ~~improve~~ ameliorate synovial inflammation and cartilage damage in RA rats~~,~~ and to investigate the underlying mechanism ~~of action of~~ through which moxibustion affects ~~in the treatment of~~ RA.

2 Materials and Methods

2.1 Experimental animal~~s~~ details and group~~ing~~s

Sixty clean-grade ~~male~~ Sprague-Dawley male ~~SD~~ rats, weighing ~~body mass~~ ~~(~~220 ± 20~~)~~ g, were supplied by ~~purchased from~~ Anhui Provincial Laboratory Animal Center ~~[production license number: SCXK (Anhui) 2017-001]~~. The animals were maintained under the following ~~L~~laboratory conditions: temperature~~:~~ ~~(~~27 ± 0.5~~)~~ °C, humidity~~:~~ ~~(~~60% ± 5~~)~~ %, a 12-h~~/12h~~ light/dark cycle, and unlimited ~~free~~ access to diet and water. Following ~~After 1 week of~~ adaptive feeding for 1 week, the rats were randomly allocated to five groups, with 12 rats per group: ~~divided into~~ normal, model, agonist, moxibustion, and moxibustion + agonist ~~groups, with 12 rats in each group~~. ~~The disposal of animals d~~During the experiments, animal disposal was performed strictly in compliance~~d~~ with the "~~"~~Guiding Opinions on the Good Treatment of Laboratory Animals"~~"~~ and other relevant regulations promulgated in 2006 by the Ministry of Science and Technology, ~~of the~~ People~~'~~'s Republic of China ~~in 2006~~.

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2.2 ~~Main~~ Important instruments and reagents

The following critical instruments and software were used in this study: ~~M~~microplate ~~R~~reader (Redu Life Sciences Ltd.); ~~U~~ultramicro spectrophotometer (Nanjing Wuyi Technology Co., Ltd.); ~~E~~electric ~~H~~heating ~~T~~thermostat (Shanghai Sanfa Scientific Instruments Co., Ltd.); e~~E~~lectrophoresis s~~S~~ystem/e~~E~~lectrophoresis t~~T~~ank/t~~T~~ransfer ~~I~~instrument (Shanghai Tennant Technology Co., Ltd.); ~~H~~horizontal shaker (Haimen Qilinbeier Instrument Manufacturing Co., Ltd.); a~~A~~utomatic exposure meter (Shanghai Peiqing Technology Co., Ltd.); h~~H~~igh-speed f~~F~~rozen c~~C~~entrifuge (Anhui Jiawen Instrument Equipment Co., Ltd.); and GraphPad Prism 6.0 s~~S~~oftware a~~A~~nalysis s~~S~~ystem (GraphPad Software, USA).

The following important reagents and kits were used in this study: Freund's c~~C~~omplete a~~A~~djuvant (Sigma, USA); GSH kit (Nanjing Jiancheng Institute of Biological Engineering); NSC59984 (Shanghai Blue Wood Chemical Co., Ltd.); ROS kit (Shanghai Bebe Biotechnology Co., Ltd.); GPX4/p53 kit (Abcam, UK); SLC7A11 kit (Beijing Boaosen Biotechnology Co., Ltd.); ~~F~~ferrous ion kit (Wuhan Elite Biotechnology Co., Ltd.); IL-1β/TNF-α kit (Wuhan Genome Biotechnology Co., Ltd.); RIPA cell lysate/ECL ultrasensitive luminescence kit (Shanghai Biyuntian Biotechnology Co., Ltd.); PAGE gel procoagulant (Beijing Solabao Technology Co., Ltd.); and g~~G~~oat anti-mouse IgG/goat anti-rabbit IgG/β-a~~A~~ctin antibody (Beijing Zhongsun Jinqiao Biotechnology Co., Ltd.).

2.3 Model preparation

The ~~wind-cold-humidity~~ environmental factors of wind, cold, and dampness combined with biological factors were used to develop ~~for~~ RA model~~ing~~ rats~~,~~. ~~and the~~ The modeled rats were placed in a self-made modeling chamber with ultrasonic nebulization to regulate ~~control~~ the humidity inside the chamber~~,~~; ~~with~~ an appropriate amount of ice was added, and the fan inside the chamber was set to high speed~~grade~~ to control the temperature at ~~(~~6 ± 2~~)~~ °C and humidity at 80~~%~~ ~ 90% for 20 d (12 h daily) (11, 12). On day 21 of the experiment, the right hind toe was injected with 0.15 mL of ~~Fever's~~ Freund's complete adjuvant per ~~(0.15 ml/~~injection~~only)~~ to induce ~~cause~~ inflammation, and the rats were kept under ~~modeling was~~ observation~~ed~~ for 3 days. The model~~ing~~ was considered to be successfully established based on ~~successful by~~ the appearance of acute inflammatory swelling in the toe accompanied with ~~by~~ secondary systemic polyarthritis~~,~~ or even erythema or inflammatory nodules in the forelimb or ear tail (13, 14).

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2.4 Intervention methods

The intervention was started on ~~the~~ day 24~~th day~~ of model~~ing~~ development. Based on ~~According to~~ the animal acupoint map ~~of~~ for experimental acupuncture, ~~"~~"Shenshu"~~"~~" and ~~"~~"Zusanli"~~"~~" acupoints were selected~~.,~~ ~~and~~ In the moxibustion group, ~~used~~ special cigarette-like~~type~~ moxa strips were suspended at 2 cm from the acupoints for 20 min/time (bilateral)~~;~~; the two ~~one~~ acupoints were alternately stimulated once per day~~, alternating between the two points, 20 min/time (bilateral)~~ (15). In the agonist group, NSC59984 (45 mg~~·~~·mg~~·~~·kg^{-1}~~·~~·d^{-5}) was administered ~~injected~~ intraperitoneally three times~~;~~. ~~in~~ In the moxibustion + agonist group, NSC59984 (45 mg·mg·kg^{-1}·d^{-5}~~mg·mg·kg^{-1}·d^{-5}~~) was administered ~~injected~~ intraperitoneally 30 min before moxibustion followed by the same intervention method as that used in the moxibustion group (16). ~~The rats in the n~~Normal and model group~~s~~ rats were placed on~~in~~ a special wooden frame for 20 min ~~only~~ according to the same grasping pattern. Each group was subjected to interven~~ed~~tion once daily for 15 d.

2.5 ~~Fetching methods~~Sample collection

On ~~The~~ the day after ~~the end of the~~ intervention completion, the rats in all groups were intraperitoneally anesthetized with a 20% uratan solution (0.3 mL/100 g) ~~was used to give anesthesia to each group of rats by intraperitoneal injection,~~. Subsequently, ~~and b~~blood samples were obtained ~~was collected~~ from the abdominal aorta, centrifuged using ~~in~~ a frozen centrifuge (4 °C, 3000 rpm~~/min~~) for 15 min with a centrifugal radius of 68 mm~~,~~, and the serum was separated; a part of the serum was ~~and~~ stored ~~partially and placed in a refrigerator~~ at -80 °C in a refrigerator. The right knee joint of each group of rats was incised longitudinally~~,~~. ~~and the~~ The skin and muscle were separated, the patella was exposed, and ~~the~~ synovial tissue was further separated~~,~~. ~~and the s~~Synovial tissue was peeled off with ophthalmic forceps and stored in a -80 °C freezer.

2.6 Determination of ~~C~~changes in the mitochondrial morphology ~~in~~ of knee cartilage ~~observed~~ by transmission electron microscopy

Several pieces of cartilage tissue of size 1~~–~~-3 mm^3 were immediately fixed in 2.5% glutaraldehyde for 24 h. Subsequently, the tissue pieces were ~~After~~ rinsed~~ing~~ in a buffer, ~~the tissue was~~ fixed in 1% osmotic acid fixative, dehydrated, soaked ~~through~~, and then embedded in an Epon 812 embedding solution. After localization, ~~ultrathin sections were stained with~~ lead citrate was used to stain ultrathin

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sections.~~,~~ ~~and~~ Transmission electron microscopy (TEM) was performed to assess changes in the mitochondrial morphology of knee cartilage ~~were observed by transmission electron microscopy~~.

2.7 Detection of the expression of ~~p53,~~ SLC7A11, p53, and GPX4 proteins ~~expression~~ in rat synovial tissue by western blotting assay

Briefly, 100 mg of synovial tissue was added ~~in~~to 600 μL RIPA lysis~~ate~~ buffer,~~,~~ and the mixture was centrifuged ~~at 12,000 rpm~~ for 10 min at 12,000 rpm.~~,~~ Subsequently, ~~and the~~ the supernatant was split into aliquots to extract ~~the~~ proteins. SDS-PAGE gels were prepared, sampled, and electrophoresed, and the separated proteins were transferred onto a PVDF membrane~~,~~. The membrane was ~~and~~ blocked with 5% skimmed milk powder ~~closed at room temperature for 2h by adding 5% skim milk powder.~~ at room temperature (RT) for 2 h and then i~~I~~ncubated overnight at 4 °C with primary antibodies~~y~~ (~~p53 1:1000,~~ anti-SLC7A11, 1:2000;~~,~~ anti-p53, 1:1000; anti-GPX4, 1:1500), ~~overnight at 4 °C;~~ followed by incubation~~e~~ overnight at 4 °C with secondary antibodies~~y~~ (1:20,000) ~~overnight at 4 °C~~. This was followed by ~~and~~ washing the membrane 3 times with PBST for 10 min each ~~time~~. ~~Add~~ The ECL ultra-sensitive ~~chemi~~luminescent~~ce~~ solution was then added uniformly to the membrane. ~~and analyze the~~ The ~~molecular weight and~~ net optical density value and molecular weight of the target bands were analyzed with~~by~~ a gel image processing system to quantitatively analyze the grayscale value of each protein band.

2.8 Determination of serum GSH and Fe^{2+} levels in rats by the colorimetric method

Briefly, ~~Mix~~ 0.05 mL~~l~~ of serum was mixed with the precipitant (0.2 mL~~l~~) in the kit, and the mixture was subjected to centrifugation~~e~~ ~~at 3,500 rpm~~ for 10 min at 3,500 rpm in a centrifuge with a centrifugal radius of 68 mm~~,~~. ~~take the~~ The supernatant was taken for ~~to be~~ measurement.~~d,~~ ~~add the~~ The corresponding reagents were added to the blank ~~wells~~, standard ~~wells~~, and test wells ~~respectively~~ according to the instructions ~~requirements~~ of the kit~~,~~. The contents were adequately mixed ~~well,~~ and left~~ave~~ undisturbed for 5 min~~,~~. ~~set the wavelength of the enzyme standard meter at 405 nm for~~ Ceolorimetric quantification~~,~~ was performed by measuring~~e~~ the absorbance ~~value~~ of each well at 405 nm, and ~~The absorbance of each well was measured and~~ the GSH content was then calculated. Next, ~~Add~~ 0.5 mL~~l~~ ~~of~~ sample was added to 5 mL~~l~~ EP tubes, and ~~add~~ 1.5 mL~~l~~ of the color developer was then added to each tube~~,~~. The contents were adequately mixed ~~well~~, boiled for 5 min, cooled, and centrifuged for 10 min~~,~~. Next, ~~take~~ 1.0 mL~~l~~ of the supernatant was taken~~,~~ from each tube for measuring~~e~~ the absorbance ~~of each tube~~ at 520 nm, and ~~calculate~~ the content of Fe^{2+} was estimated.

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2.9 Detection of serum ROS level in rats by the fluorescent probe method

Briefly, ~~Add~~ 100 μL of serum samples was added to a ~~the~~ 96-well plate ~~respectively,~~. Next, ~~using~~ 10 μL of the luminescent probe L-012 ~~012 probes~~ included in the kit was added to the plate~~,~~. The contents of the plate were mixed thoroughly~~,~~. ~~and~~ The plate was then incubated in dark at 37 °C for 15–~~-~~30 min ~~at 37°C protected from light~~. The fluorescence intensity of each well was estimated ~~measured~~ at ~~an~~ excitation and emission wavelengths of 488 and 530 nm, respectively, ~~and emission wavelength of 530 nm~~ in a fluorescence zymograph, and the ~~level of~~ ROS level was expressed as the fluorescence intensity.

2.10 Determination of serum IL-1β, TGF-β1, and TNF-α levels in rats by ELISA

~~Remove the~~ The refrigerated serum ~~from the refrigerator at -80 °C,~~ was thawed~~rewarm the serum in a gradient until it melts~~,. Next, the ~~equilibrate the~~ ELISA kit was equilibrated at RT~~room temperature~~ for 15–~~-~~30 min~~,~~. ~~then dilute the~~ The samples ~~standard~~ were then diluted with the standard~~sample~~, and this was followed by the addition of the enzyme~~,~~. The mixture was incubated~~, prepare the solution,~~ and washed, and the color was developed by following the protocol mentioned in the ~~the color according to the instructions of the~~ kit~~,~~. Next, the enzymatic reaction was terminated by adding ~~add 50 μL of~~ the termination solution (50 μL) ~~to terminate the reaction~~, and ~~set the enzyme standard to detect~~ the absorbance value (OD reading~~value~~) of each well was detected at 450 nm using the enzyme standard as the control. The standard curve and standard equation were plotted, and the OD reading~~value~~ of each well was substituted into the standard equation to ~~find~~ determine the actual concentration of each sample.

2.11 Statistical analysis

GraphPad Prism 6.0 software was utilized ~~used~~ for the ~~statistical analysis and~~ graphical representation and statistical analysis of the experimental results~~data~~. ~~Measurement data were expressed as m~~Mean ± standard deviation ($\bar{x} \pm s$) was used for expressing the measurement data. We used ~~One-way analysis of variance~~ (ANOVA~~) was used~~ for inter-group comparison~~,~~ and the least significant difference (LSD) test ~~was used~~ for inter-group difference. P ~~<~~< ~~0~~.05 indicated a ~~was considered statistically~~ significant difference.

3 Results

3.1 Comparison of mitochondrial morphology in rat knee cartilage ~~of rats in each group~~

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Morphological changes of mitochondria in rat knee cartilage were observed by tTEMransmission electron microscopy was performed to detect morphological changes in the mitochondria in rat knee cartilage. In the normal group, the mitochondria in the rat knee cartilage of rats were regular and intact, with clear outlines and apparent obvious mitochondrial cristae;. iIn the model and agonist groups, the mitochondria in the cartilage were was wrinkled and smaller (and showed vacuolation-like lesions),; the mitochondrial cristae were apparently was obviously reduced or disappeared, the mitochondrial membrane density was increased, and some of the outlinessides were was blurred and broken, thus suggesting that the rats in these model and agonist groups exhibited different extents of had obvious ferroptosis characteristics of different degrees. After 15 days of treatmentintervention, the mitochondrial structure in the cartilage of rats in the moxibustion group and moxibustion + agonist groups showed varying degrees of improvement, with a reduction in reduced membrane density and a remarkable significant increase in the number of mitochondrial cristae (Figure 1).

3.2 Comparison of p53 protein expression levels in the synovial tissue of rats in each group

The expression of p53 in the synovial tissue of rats in the The model group was exhibited significantly higher p53 expression in the synovial tissue than that in the normal group ($P < 0.01$). The expression of p53 in the The agonist group was showed significantly higher p53 expression than that in the model group ($P < 0.05$),; in contrast, and the moxibustion group showed a significant decrease in p53 expression in the moxibustion group was decreased significantly ($P < 0.05$). However, p53 expression did not differ significantly between there was no statistically significant difference in the expression of p53 in the moxibustion + agonist group compared to and the model groups ($P > 0.05$). Both the The moxibustion group and the moxibustion + agonist groups exhibited showed significantly lower p53 expression than the agonist group ($P < 0.01$), (Figures 2 (a) and (b)).

3.3 Comparison of SLC7A11 and GPX4 protein expression levels in the synovial tissue of rats in each group

The expression levels of The expression of SLC7A11 and GPX4 proteinss in the synovial tissue were significantly lower of rats in the model group was significantly lower than that in the normal group ($P < 0.01$). Compared to the model group, The expression of SLC7A11 in the the agonist group was showed a lower SLC7A11 expression levelthan that in the model group,; but however, the difference was nonwithout statistical significantce;. while the expression of GPX4 expression in the agonist group was significantly lower than that in the model group ($P < 0.05$). Furthermore, In the moxibustion group,

both SLC7A11 and GPX4 showed significantly higher expression levels in the moxibustion group than in the expression were significantly higher than those in the model group ($P < 0.01$)., These proteins showed increased expression in while in the moxibustion + agonist group, their expression showed an increasing trendthe moxibustion + agonist group,; however, this increase was nonsignificantbut without statistical significance. Both the The moxibustion group and the moxibustion + agonist groups showed significantly higher expression levels of SLC7A11 and GPX4 than compared to the agonist group ($P < 0.01$) (Figures 2 (c) and (d)).

3.4 Comparison of serum GSH, ROS, and Fe^{2+} levels expression in serum of rats in each group

The expression of serum GSH in rats in the The model group was showed significantly lower serum GSH level than that in the normal group ($P < 0.01$),; while however, a significant increase was noted in the serum ROS and Fe^{2+} levels in the model groupwere significantly increased ($P < 0.01$). Similarly, In the agonist group, showed the expression of GSH was significantly lower serum GSH level than that in the model group ($P < 0.01$), but significantly higher serum while ROS and Fe^{2+} levels were significantly increased ($P < 0.01$). Both the The moxibustion group and the moxibustion + agonist groups showed significantly higher serum expression of GSH level than compared to the model group and the agonist groups ($P < 0.01$), while the serum ROS and Fe^{2+} levels in the moxibustion and moxibustion + agonist groups were significantly reduced ($P < 0.01$) (Figure 3).

3.5 Comparison of serum IL-1β and TNF-α and IL-1β levels in of rats in each group

The levels of serum IL-1β and TNF-α in rats in the model group were significantly higher The model group showed significantly higher serum TNF-α and IL-1β levels than those in the normal group ($P < 0.01$). In the The agonist group, exhibited the levels of IL-1β and TNF-α were significantly higher serum TNF-α and IL-1β levels than those in the model group ($P < 0.01$). Both the The moxibustion group and the moxibustion + agonist groups showed significantly lower serum expression of IL-1β and TNF-α and IL-1β levels than compared to the model group and the agonist groups ($P < 0.01$) (Figure 4).

4 Discussion

The basic pathological changes of RA are sSynovitis and the formation of blood vessel plexus formation are the primary pathological changes of RA, which and these changes gradually destroy lead

~~to the destruction of~~ articular cartilage and bone. This may ultimately ~~eventually~~ cause ~~result in~~ joint deformity as well as ~~and~~ loss of function (17). Therefore, one of the important goals of RA treatment is ~~how~~ to reduce inflammatory damage and slow down the process of cartilage and bone destruction ~~has become one of the important goals of RA treatment~~. Currently, anti-rheumatic drugs are predominantly used in RA treatment ~~is still dominated by drugs that improve rheumatism,~~; however, the long-term use of these drugs results in ~~but there are~~ more adverse effects ~~on long-term use~~. As an important part of TCM~~Chinese medicine~~, moxibustion therapy has better anti-inflammatory, synovial membrane repair, and bone and cartilage protection effects on ~~in~~ RA ~~treatment~~, which precisely compensates for some of the deficiencies of mainstream Western medicine (18).

Ferroptosis ~~is a novel form of programmed cell death~~ was first proposed by Dixon et al. as a novel form of programmed cell death. It ~~can~~ triggers the body's intrinsic immunity, releases inflammatory mediators, and activates the body's inflammatory response ~~of the body~~, and ~~in which~~ RA ~~rheumatoid arthritis~~ is one of the common clinical inflammatory diseases (19, 20). ~~Meanwhile, a~~Abnormal levels of ~~expression of~~ ferroptosis-related factors, namely SLC7A11, GSH, GPX4, and ROS, are ~~all~~ closely associated with pathological changes in RA (11). In a previous study, ~~It has been reported that~~ ~~r~~resveratrol ~~(RES)~~ ~~can~~ increased ~~the content of~~ GSH as well as ~~and~~ glutathione peroxidase~~GSH-PX~~ content, lowered ~~decrease the content of~~ malondialdehyde~~MDA~~ content, and reduced ~~the level of~~ lipid peroxidation level~~,~~; these changes ~~which in turn~~ significantly alleviated ~~improves~~ the degree of joint inflammation and kidney damage in AA rats (21). In 2015, Jiang et al. first linked p53 to ferroptosis, suggesting that p53 can inactivate GPX4 by reducing intracellular GSH synthesis through transcription-dependent repression of the downstream gene *SLC7A~~#~~11*, leading to ROS accumulation and thus inducing ferroptosis (22, 23). p53 is a potential regulatory target of ferroptosis and~~,~~ also ~~at the same time,~~ plays an important regulatory role during ~~in the development of~~ RA development~~disease course~~. Therefore, in our ~~this~~ study, we further examined ~~explores~~ some of the mechanisms of action of moxibustion for treating ~~in the treatment of~~ RA from the perspective of p53 regulation of ferroptosis.

The results ~~of this study~~ showed that the p53 protein level and serum ROS and Fe^{2+} levels ~~was~~ were significantly elevated~~,~~ and SLC7A11 and GPX4 protein levels and serum GSH level were remarkably ~~significantly~~ decreased~~, GSH expression in serum was significantly decreased, and ROS and Fe^{2+} were significantly elevated~~ in the synovial tissue of ~~rats in the~~ model group rats~~,~~; these findings suggested~~ing the occurrence of~~ ferroptosis induction ~~process~~ in the RA model rats, which is consistent with the results of ~~the~~ a previous study. After intervention with the p53 agonist NSC59984, the p53 protein level

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and serum ROS and Fe^{2+} levels ~~was~~ were significantly elevated and ~~in synovial tissue,~~ SLC7A11 and GPX4 protein levels and ~~were significantly decreased,~~ serum GSH level were ~~expression in serum was~~ significantly decreased in synovial tissue~~, and ROS and Fe^{2+} were significantly elevated~~;~~,~~ these findings indicated that ~~suggesting that~~ the p53 agonist could suppress ~~inhibit the expressions of~~ SLC7A11 expression and further promote ~~the development of~~ ferroptosis development. Following the combined treatment of ~~After~~ moxibustion ~~intervention on the basis of the model group~~ and the p53 agonist, the rat synovial tissue showed a significant decrease in p53 expression and serum ROS and Fe^{2+} levels ~~protein,~~ and a significant increase in SLC7A11 and GPX4 proteins~~,~~ and ~~a significant increase in~~ serum GSH level~~expression in serum, and a significant decrease in ROS and Fe^{2+}~~;, these findings suggested~~ing~~ that moxibustion can enhance ~~the expression of~~ the downstream gene *SLC7A11* expression by inhibiting p53, promote the synthesis of GSH, and ~~thus~~ subsequently slow down the ferroptosis process.

A close relationship exists between ~~The development of~~ RA symptom development and ~~s is closely related to~~ the body's inflammatory response ~~of the body~~. Several ~~A large number of~~ inflammatory cells adhere to and accumulate ~~to~~ in the synovial membrane and are activated to exert their biological effects and secrete~~ion~~ of various cytokines, thus forming a complex cytokine network that is involved in the immune regulation and inflammatory response of RA. The expression of inflammatory factors is closely related to ~~the development of~~ RA ~~disease~~development, where~~in which~~ the overexpression of pro-inflammatory factors such as IL-1β~~TNF-α~~, ~~IL-6~~TNF-α, and ~~IL-1β~~IL-6 aggravates joint inflammatory lesions (24, 25).

~~The results of this~~ Our present study showed remarkably elevated ~~that the expression of~~ serum levels of ~~IL-1β and~~ TNF-α and IL-1β ~~was significantly elevated~~ in the model and agonist groups ~~of rats~~, thus suggesting an enhanced inflammatory response~~,~~; this finding is consistent with the ~~transmission electron microscopic~~ observations of TEM, which showed typical morphological changes of ferroptosis, such as mitochondrial atrophy, broken membrane structures, and reduced entoloph~~. The expression of s~~Serum ~~IL-1β and~~ TNF-α and IL-1β levels also decreased markedly~~significantly~~ in the moxibustion ~~group~~ and ~~the~~ moxibustion + agonist groups, thus indicating that moxibustion lowers ~~could reduce~~ the expression of pro-inflammatory factors. ~~Meanwhile, the~~ TEM~~electron microscopic~~ results showed a remarkable improvement in ~~that~~ the mitochondrial structure ~~was significantly improved~~, a reduction in the membrane density ~~was reduced~~, and a marked enhancement in the number

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of mitochondrial cristae ~~was significantly increased~~;~~,~~ these findings indicated~~ing~~ that moxibustion could reduce the cartilage damage in the knee joint of RA rats.

5 Conclusion

The present study assessed ~~explored~~ the impact of moxibustion treatment on RA from a ~~the perspective of~~ ferroptosis perspective and found that the mechanism of action of moxibustion for treating RA is probably ~~may be~~ closely associated with ~~related to the inhibition of~~ p53 suppression, which leads to increased ~~to enhance the~~ expression of the downstream gene *SLC7A11*;~~,~~ ~~which~~ this cascade subsequently ~~in turn~~ inhibits ~~the occurrence of~~ ferroptosis occurrence, attenuates the inflammatory response, and slows down ~~the destruction of~~ articular cartilage destruction. ~~The occurrence of F~~ferroptosis induction involves the expression and regulation of multiple genes and pathways, and it remains to be further studied whether moxibustion inhibits ~~the occurrence of~~ ferroptosis induction through other regulatory factors ~~still needs to be further explored~~.

6 Conflict of Interest

The authors declare that the present research does not have any ~~was conducted in the absence of any~~ commercial or financial relationships that could be construed as a potential conflict of interest.

7 Author Contributions

LH and CP proposed and designed the ~~this~~ study. CP and TW performed the experiments and wrote the manuscript. JW and QY assisted ~~helped~~ in conducting experiments~~ation~~. JS and CZ performed ~~evaluated the~~ data analysis and helped in writing. TW, FH, and RC designed the experiments~~,~~ and analyzed the data. All authors contributed to the article and approved the submitted version.

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9 Data Availability Statement

The raw data supporting the conclusions of this study ~~article~~ will be made available by the authors, without undue reservation.

10 Ethics statement

The animal study was reviewed and approved by the Institutional Animal Ethics Committee of Anhui University of Chinese Medicine.

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Figure captions

Figure 1~~:~~. Mitochondrial morphology of knee cartilage of rats in each group ~~of rats~~. (A) normal group; (B) model group; (C) agonist group; (D) moxibustion group; (E) moxibustion + agonist group.

Figure 2~~:~~. ~~Expression levels of p~~Protein expression levels in the synovial tissue of rats in each group. (A) Western blotting assay~~analysis~~ of p53, SLC7A11, GPX4, and β-a~~A~~ctin. (B) p53. (C) SLC7A11.

(D) GPX4. Compared ~~with~~ to the normal group, $^{\#\#}P < 0.01$; compared ~~with~~ to the model group, $^{*}P < 0.05$ and $^{**}P < 0.01$; compared ~~with~~ to the agonist group, $^{\Delta\Delta}P < 0.01$.

Figure 3~~:~~. Serum GSH, ROS, and Fe^{2+} levels ~~expression in serum~~ of rats in each group. (A) GSH; (B) ROS; (C) Fe^{2+}. Compared to ~~with~~ the normal group, $^{\#\#}P < 0.01$; compared to ~~with~~ the model group, $^{**}P < 0.01$; compared to ~~with~~ the agonist group, $^{\Delta\Delta}P < 0.01$.

Figure 4.~~:~~ Serum IL-1β and TNF-α levels of rats in each group. (A) IL-1β; (B) TNF-α. Compared to ~~with~~ the normal group, $^{\#\#}P < 0.01$; compared to ~~with~~ the model group, $^{**}P < 0.01$; compared to ~~with~~ the agonist group, $^{\Delta\Delta}P < 0.01$.