Original Article

Trends in Pharmaceutical Sciences and Technologies 2025: 11(3):253-262.

Ivermectin inhibits acute chloroquine-induced scratching behavior by targeting p-NF-kB p65 and iNOS in mice

Tayebeh Noori[1];Ph.D, Antoni Sureda[2,3];Ph.D, Samira Shirooie[1*];Ph.D

[1]Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2]Research Group on Community Nutrition and Oxidative Stress (NUCOX) and Health Research Institute of Balearic Islands (IdISBa), University of Balearic Islands-IUNICS, Palma de Mallorca E-07122, Balearic Islands, Spain
[3]CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), 28029 Madrid, Spain

Abstract

Pruritus, or itching, is one of the most common skin complaints. The intensity of the itching is sometimes so uncomfortable and irritating that the patient injures himself by repeatedly scratching. Skin damage, inflammation through diverse chemical mediators, and pruritogens are among the agents capable of causing pruritus in the skin. Ivermectin (IVM), a broad-spectrum anti-parasitic agent, has also been evidenced to improve pruritus without histamine mediation. This study aimed to assess the ability of IVM to improve pruritus and histopathology alterations induced by chloroquine (CQ) in a mouse model. Thirty rats were divided into 5 groups of 6, including control group, CQ group, and IVM group (3 doses). IVM (1, 2, and 5 mg/kg, intraperitoneally (i.p.)) was injected 3 hours before CQ injection (200 µg/site, subcutaneously). Scratching behavior was recorded for 30 minutes after the CQ injection. The result showed that IVM (5 mg/kg) significantly reduced itching bouts and duration in the CQ group. In addition, Immunohistological analysis of the skin also showed that CQ significantly increased the expression of p-NF-kB p65, and iNOS, and these alterations were normalized in the IVM (5 mg/kg) groups. acute treatment with IVM reduces CQ-induced itching by ameliorating the inflammatory process.

Keywords: Pruritus, Ivermectin, Chloroquine, p-NFkB p65, iNOS

Please cite this article as: Noori T, Sureda A, Shirooie S. Ivermectin inhibits acute chloroquine-induced scratching behavior by targeting p-NF-kB p65 and iNOS in mice. Trends in Pharmaceutical Sciences and Technologies. 2025;11(3):253-262. doi: 10.30476/tips.2025.108187.1311

1. Introduction

Pruritus (itch) is an annoying and unpleasant feeling that makes a person scratch the skin (1). Pruritus is a consequence of some systemic disorders, and the most prevalent sign of skin diseases, which often does not reply to antihistamines (2). It acts as a protective mechanism to help protect the skin from damaging factors (3). Pruritogens stimulate receptors (G protein-coupled receptors (GPCRs)) on unmyelinated C nerve fibers, and consequently, scratch begins in the skin. These receptors are involved in the opening of ion channels contributing to the sensation of a quick shallow pain (4). C nerve fibers are classified as non-histaminergic or histaminergic based on the receptors they express. Histamines activate histaminergic neurons while pruritic agents (such as proteinase-activating

Corresponding Author: Samira Shirooie, Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran.
Email: shirooie@gmail.com, samira.shirooei@kums.ac.ir

Received: 16/01/2025; Accepted: 01/06/2025

receptor 2 (PAR2)) activate non-histaminergic neurons (4). Chloroquine (CQ) is a drug derived from quinoline utilized to cure malaria, but one of its intensive complications is itching (5). It has been evidenced that injection of CQ into the back of the neck of mice causes severe itching behavior (6). In pathophysiological studies of itching independent of histamine, the CQ model has been widely used (7). CQ activity is mediated by binding to the Mas-related G-protein receptor (Mrgpr), primarily expressed on sensory neurons. MrgprX1 is the human ortholog whereas MrgprA3 is the receptor of CQ in mice (8). MrgprA3 neurons mediate various forms of histamine-independent itch caused by various pruritogens and acute itch associated with dry skin, atopic dermatitis, and systemic disorders (9). These characteristics of CQ-induced pruritus make it a suitable model for investigating the mechanisms of pruritus in humans (2).

Nuclear factor-κB (NF-κB) is the primary driver of the inflammatory response and is necessary for the homeostasis of the immune system. NF-kB participates in the controls of the cell cycle, immune cell growth, proliferation, cell death, and inflammation through the regulation of gene transcription (10). p65 and p50 or p52 are subunits of NF-kB. p53 NF-κB and p65 NF-κB are essential transcription factors in apoptosis and the regulation of inflammatory response, respectively. NF-κB activation induces the transcription of pro-inflammatory molecules such as toll-like receptor 4 (TLR4), interleukin 1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α), as well as the inducible isoform of the nitric oxide synthase (iNOS) (11). iNOS is involved in producing of high amounts of nitric oxide (NO) with beneficial microbicidal, antiviral, antiparasitic, and antitumoral activities (12). Dysregulated iNOS leading to excessive NO production is frequently associated with inflammatory conditions. Macrophages, microglia, neutrophils, dendritic cells, smooth muscle cells, oligodendrocytes, astrocytes, and even endothelial cells of the brain are among the cells that express iNOS (13). Targeting these inflammatory pathways has been a strategy for reducing pathological inflammation, and several drugs with anti-inflammatory properties act through modulation of NF-κB and iNOS signaling. In this context, ivermectin (IVM), a semi-synthetic derivative of the macrocyclic lactone avermectin, has been reported not only for its antiparasitic effects but also for its ability to downregulate NF-κB activation and iNOS expression, thereby exerting anti-inflammatory effects in various models (14, 15). Mechanistically, IVM is involved in increasing neurotransmitter-gated gamma-aminobutyric acid (GABA) activity or binding to ion channels of glutamate (Glu-Cl), present in a wide range of parasites, but not in mammals (14). In addition to these antiparasitic effects, recent evidence suggests that ivermectin also possesses anti-inflammatory properties capable of reducing p-NF-kB p65 and iNOS (16, 17). In this sense, IVM is used topically in adults as a therapy for papulopustular inflammatory rosacea. Therapeutic efficacies of IVM can also be observed in other facial dermatoses due to its anti-inflammatory properties (16). Based on the effectiveness of topical IVM treatment in seborrheic dermatitis (SD), acne vulgaris (AV), and perioral dermatitis (PD), this compound can be considered an attractive potential candidate for use in other skin diseases (16). In the first stage, IVM suppresses the production of NO and prostaglandin E2 (PGE2) (inflammatory mediators) through inhibition phosphorylation of c-Jun N-terminal kinase (JNK), extracellular-signal-regulated kinase (ERK)n1/2, and mitogen-activated protein kinases (PKMA). In addition, IVM induces a decrease in the mRNA expression levels of cyclooxygenase-2 (COX2), and inducible NO synthase (iNOS) (18, 19). In the second step, IVM reduces the number of inflammatory lesions through its anti-inflammatory effects (20, 21). Animal studies have shown that IVM is able to downregulate the NF-kB expression and, consequently, has an immunomodulatory effect that ultimately leads to a reduction in itching sensation (22). Moreover, the rapid and permanent elimination of skin lesions and disappearance of prurience after healing with IVM allows the confirmation of a possible diagnosis of scabies in places where laboratory techniques or dermoscopy are not available (23) . So far, no side effects have been reported after oral therapy with IVM, confirming the

safety of IVM (23).

The aim of the present study was to investigate the anti-itch effect of IVM in a mouse model of CQ-induced pruritus and the potential implication of the p-NF-kB p65 and iNOS signaling pathways as mediators of the therapeutic effects of IVM.

2. Materials and Methods

2.1. Reagents

Chloroquine hydrochloride (CQ) and ivermectin (IVM) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals

In the study, 30 female rats (170-180 g and 8-week-old) were used. After purchase from Elm Bavaran Aftab Company, the animals were kept in cages under standard laboratory conditions at 25 °C and a 12-h light/dark cycle according to the standard instructions for working with animals of Kermanshah University of Medical Sciences, Iran.

2.3. Induction of CQ-scratch and IVM treatment

Before starting the experiment, the fur on the neck of the rats was shaved. Then, animals were divided into 5 different experimental groups with 6 animals each: 1) Control group: received a standard diet; 2) CQ group: Subcutaneous injection of CQ (200 µL/site); 3-5) IVM group: Intraperitoneally injection of IVM (1,2, and 5mg/kg), 3hr before CQ injections (24).

2.4. Itching Behavioral Tests

CQ-induced scratching behavior was investigated in rats for 30 minutes. Immediately after the induction of scratching, the number and time of scratching were recorded. Scratching was defined as a quick and frequent movement of the mouse's hind limb, rubbing the injection area and neck (5).

2.5. Skin tissue preparations

After carrying out behavioral tests, rats were sacrificed with xylazine (10 mg/kg) and ketamine (50 mg/kg) i.p. injection. Then, the skin of animals was dissected and fixed in formalin (10%) for immunohistochemistry (IHC) analysis (5). It should be noted that only the 5 mg/kg IVM group was used for histochemistry.

2.6. Immunohistochemistry staining

The expression levels of p-NF-kB p65 and iNOS were determined in the skin by IHC. Skin samples were deparaffinized with xylene and progressively rehydrated in a series of graded alcohols to distilled water and, finally washed with PBS. Antigen was retrieved in trisodium citrate buffer (pH 7.4, 100 °C, 20 min). Then, samples were washed with TBS plus 0.03% Triton X-100 and blocked with blocking solution (3% BSA) for 2 h. Afterward, the paraffin-embedded skin slides (5 µm) were incubated overnight at 4 °C with primary antibody rabbit monoclonal (rabbit monoclonal p-NF-kB p65 antibody and rabbit monoclonal antibody iNOS). Slides were washed (3 times for 5 min) with TBS plus 0.03% Triton X-100 and incubated 3% H_2O_2 in RT for 10 min and washed with PBS 4 times, and incubated with the goat biotinylated polyvalent secondary antibody for 10 minutes. After washing 3 times with TBS plus 0.03% Triton X-100, sections were incubated with horseradish peroxidase (HRP)-conjugated streptavidin for 10 minutes, washed with PBS and 3,3′-Diaminobenzidine (DAB) solution as a chromogen was finally added. Slides were assessed for antigen with an optical microscope (MDOB3- U, Olympus, Japan). The results of IHC were quantified with ImageJ® software version 1.43 (25). Finally, the mean optical intensity of positive cells in the control group was measured then each data in other groups was divided by that and the fold changes were reported.

2.7. Statistical analysis

Statistical analysis was carried out using the GraphPad Prism 9 software, and the

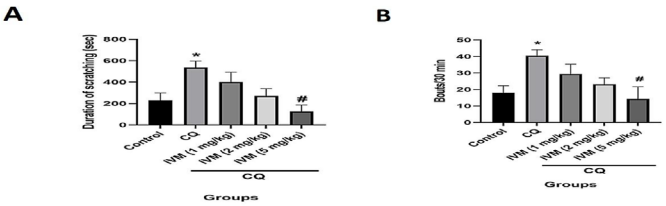

Figure 1. Scratching behavior: (A) shows the duration of scratching and (B) shows the bouts of mice at 30 min. *P < 0.05 vs the control group, #P < 0.05, vs the CQ group. CQ: Chloroquine; IVM: Ivermectin.

data were expressed as mean ± SEM. The statistical significance of the data was checked by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P value < 0.05 was considered statistically significant.

3. Results

3.1. The Effect of ivermectin on CQ-Induced Scratching Behavior

Figure 1A shows that the duration of scratching in the CQ group significantly increased compared to the control group (P <0.05). The outcomes revealed that the administration of IVM (5 mg/kg) to CQ-exposed rats led to lower scratch with respect to the CQ group (P<0.05). In contrast, at the doses of 1, 2 mg/kg, the effects were not statistically significant. Also, the treatment with IVM 5 mg/kg significantly reduced the scratching time compared to the CQ group (Figure 1B, P< 0.05).

3.2. Immunohistochemistry staining of p-NF-kB p65 and iNOS

Immunohistochemical staining of p-NF-kB p65 and iNOS antibodies to evaluate the effects of CQ and IVM are shown in Figure 2 and 3. Based on the significant difference between IVM 5 mg/kg with CQ group in scratching behavior, this dose was considered in IHC tests. In the group receiving CQ, a significant increase in p-NF-kB p65 levels was observed compared to the control group (Figure 2A-B, P<0.05). The administration of IVM (5 mg/kg) significantly recovered the increased levels of p-NF-kB p65 observed in the CQ group (P<0.05). Fig. 3 A-B shows that after injection of CQ, the levels of iNOS significantly increased compared to the con-

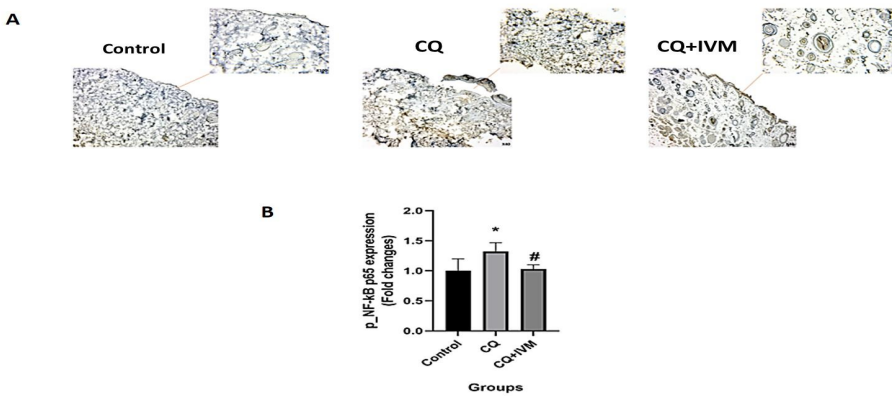

Figure 2. A: p-NF-kB p65 immunohistochemistry staining (IHC) (× 100), of skin, B: the optical intensity of IHC assay results using ImageJ. *P < 0.05 vs the control group; #P < 0.05 vs the CQ group. CQ: Chloroquine; IVM: Ivermectin (5 mg/kg).

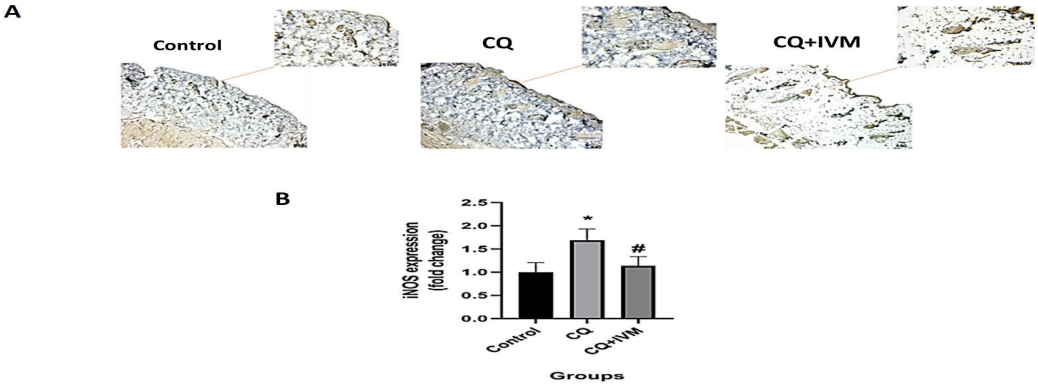

Figure 3. A: iNOS immunohistochemistry staining (IHC) (× 100), B: the optical intensity of IHC assay results using ImageJ. *P < 0.05 vs the control group; #P < 0.05 vs the CQ group. CQ: Chloroquine; IVM: Ivermectin (5 mg/kg).

trol group (P < 0.05). On the other hand, the treatment with IVM (5 mg/kg) significantly reduced the levels of iNOS compared to the CQ group (P< 0.05).

4. Discussion

Itching is an uncomfortable sensation that significantly affects everyday aspects such as sleep, mood, ability to concentrate, and affects the quality of life more than other skin conditions (26, 27). Skin damage, inflammation through diverse chemical mediators, and pruritogens are among the causative agents of pruritus in the skin (28). CQ is a drug that has been used for a long time in treating malaria, rheumatoid arthritis, and systemic lupus erythematosus (29). Itch is the main side effect of CQ, which leads to decreased patient compliance and, consequently, can favor the appearance of Plasmodium falciparum resistant to CQ (2). Acute subcutaneous or intradermal CQ injection has been observed to induce itching behavior in both humans and rodents (3, 30). CQ induces itch by activating the MrgprE3 homologous receptor in mouse and Mrgpr X1 in human. Then, coupling with modulating transient receptor potential channels of Gβγ (subfamily A, member 1 (TRPA1)) channels (31). The results of an in vivo study showed that the treatment with localized IVM elevates the regeneration of peripheral nerves through the induction of fibroblasts to adopt glia-like a phenotype (32). In addition, it causes the upregulation of neuronal and glial markers in the site of skin wounds after healing (32). In a clinical trial, it has also been evidenced that IVM 1% could be useful for the treatment of scabies with a 91% remission after 4 weeks (33). In the present study, we evaluated whether IVM is effective versus non-histaminergic scratch by its ability to restrain scratching behavior induced by subcutaneous injection of CQ. Intraperitoneal pre-treatment (3 hours before) of rats with IVM at doses of 1, 2, and 5 mg/kg resulted in a dose-dependent restrain of the scratch response in CQ-injected rats. Specifically, the administration of IVM (5 mg/kg) to CQ-exposed rats resulted in lower bouts than the CQ group. Moreover, in IVM groups (5 mg/kg) the time of scratching was reduced compared to the CQ group. IVM has been shown to reduce inflammation by inhibiting the NFkB pathway(17) . The NF-κB signaling pathway has a vital role in the regulation of innate immunity, activation, and efficient performance of inflammatory cells, and differentiation, so its dysregulation is related to the pathogenesis of multitude inflammatory diseases (34). The NF-κB pathway upregulates the expression of proinflammatory mediators such as the cytokines IL-1, IL-2, IL-6 and other inflammatory molecules, including vascular endothelial growth factor (VEGF), cyclooxygenase 2 (COX-2), and iNOS. Blocking the

activity of p50 and p65 NF-κB subunits has been reported to suppress the expression of genes associated with inflammation, COX-2, and iNOS (35). Outcomes of an in vivo study carried out by Aryannejad et al., showed that IVM reduced the inflammation psoriasis (15). The treatment with IVM significantly reduced the activity of myeloperoxidase (MPO), and reduced the levels of TNF-α, iNOS, COX-2, and the expression p-NF-κB p65 (17). In a clinical trial with 20 patients with moderate to severe rosacea, treatment with 1% IVM cream exerted anti-inflammatory effects by reducing the expression of IL-8, TLR-4 and TNFα, and antiparasitic effects by killing Demodex spp. mites after 12 weeks (36).

In the present study, IVM treatment inhibited the activation of the p-NF-KB p65 pathway, and consequently, decreased the inflammatory response, indicating a potential anti-inflammatory effect, although they mainly reflect an association rather than a confirmed causal relationship. Exposure to CQ also leads to the expression of iNOS associated with activating the NF-κB pathway (37). In this sense, iNOS has a vital role in the pathophysiology of acute non-histaminergic scratch (26). The cellular distribution of iNOS expression indicates the role of NO in all process of wound healing, including effects on the regulation of cell proliferation, inflammatory phase, formation of granulation tissue and neovascularization, apoptosis, differentiation, and tissue regeneration to some extent (38). Previous studies have demonstrated that the iNOS pathway is involved in the development of the disease through increasing inflammation and pro-oxidative processes (11, 39, 40). Similar to the present research, IVM was found to decrease the iNOS levels in skin tissue of allergic rats .(12) Thus, the iNOS signaling pathway inactivation is an excellent therapeutic target for ameliorating pruritus. In this sense, the reduction of p-NFkB p65 and iNOS levels, suggests that IVM may be a promising agent for the treatment of pruritus. However, clinical trials are needed to corroborate its effectiveness.

5. Conclusion

The present results showed the itch-inducing effects of CQ as a study model for itch and to evaluate the protective effects of IVM. Behavioral tests showed that IVM reduced the duration and number of itching in rats. Among mechanisms that may be involved in improving pruritus by IVM is the inhibition of p-NFkB p65, and the iNOS signaling pathway. Therefore, IVM treatment can reduce the inflammatory response associated with p-NFkB p65 and the iNOS pathway. According to the results, it can be argued that IVM can be a promising candidate for treating skin diseases such as itching.

Ethics Statement

The study protocol was approved by the Animal Research Committee of Kermanshah University of Medical Sciences (IR.KUMS.AEC.1403.024).

Acknowledgments

This project was supported by the Kermanshah University of Medical Sciences with grant number 50003625.

Authors contributions

SS Conceptualization, Methodology, Writing - original draft, Writing - review & editing & Supervision. SS and TN Data curation, Writing- Original draft preparation. AS Validation, Investigation & Writing - review & editing. TN Visualization, Investigation & Writing - review & editing.

Data availability

Data will be made available on request.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Lavery MJ, Kinney MO, Mochizuki H, Craig J, Yosipovitch G. Pruritus: an overview. What drives people to scratch an itch? *Ulster Med J.* 2016 Sep;85(3):164-173. PMID: 27698518; PMCID: PMC5031103.
2. Haddadi NS, Shakiba S, Afshari K, Haj-Mirzaian A, Vesaghati S, Gharagozlou S, Foroumadi R, Shafaroodi H, Ostadhadi S, Dehpour AR. Possible Involvement of Nitric Oxide in the Antipruritic Effect of Metformin on Chloroquine-Induced Scratching in Mice. *Dermatology.* 2020;236(2):151-159. doi: 10.1159/000501583. Epub 2019 Aug 22. PMID: 31437844.
3. Foroutan A, Haddadi NS, Ostadhadi S, Sistany N, Dehpour AR. Chloroquine-induced scratching is mediated by NO/cGMP pathway in mice. *Pharmacol Biochem Behav.* 2015 Jul;134:79-84. doi: 10.1016/j.pbb.2015.04.016. Epub 2015 May 6. PMID: 25957523.
4. Golpanian RS, Smith P, Yosipovitch G. Itch in Organs Beyond the Skin. *Curr Allergy Asthma Rep.* 2020 Jun 19;20(9):49. doi: 10.1007/s11882-020-00947-z. PMID: 32561998.
5. Shi H, Yu G, Geng X, Gu L, Yang N, Wang C, et al. MrgprA3 shows sensitization to chloroquine in an acetone-ether-water mice model. *Neuroreport.* 2017 Dec 6;28(17):1127-1133. doi: 10.1097/WNR.0000000000000877. PMID: 29064954.
6. Ru F, Sun H, Jurcakova D, Herbstsomer RA, Meixong J, Dong X, et al. Mechanisms of pruritogen-induced activation of itch nerves in isolated mouse skin. *J Physiol.* 2017 Jun 1;595(11):3651-3666. doi: 10.1113/JP273795. Epub 2017 Mar 19. PMID: 28217875; PMCID: PMC5451712.
7. Tarrasón G, Carcasona C, Eichhorn P, Pérez B, Gavaldà A, Godessart N. Characterization of the chloroquine-induced mouse model of pruritus using an automated behavioural system. *Exp Dermatol*. 2017 Nov;26(11):1105-1111. doi: 10.1111/exd.13392. Epub 2017 Aug 15. PMID: 28605064.
8. Zhu Y, Hanson CE, Liu Q, Han L. Mrgprs activation is required for chronic itch conditions in mice. *Itch (Phila)*. 2017 Dec;2(3):e9. doi: 10.1097/itx.0000000000000009. PMID: 29577089; PMCID: PMC5860675.
9. Han L, Ma C, Liu Q, Weng HJ, Cui Y, Tang Z, et al. A subpopulation of nociceptors specifically linked to itch. *Nat Neurosci.* 2013 Feb;16(2):174-82. doi: 10.1038/nn.3289. Epub 2012 Dec 23. PMID: 23263443; PMCID: PMC3557753.
10. Christian F, Smith EL, Carmody RJ. The Regulation of NF-κB Subunits by Phosphorylation. *Cells.* 2016 Mar 18;5(1):12. doi: 10.3390/cells5010012. PMID: 26999213; PMCID: PMC4810097.
11. Nakazawa H, Chang K, Shinozaki S, Yasukawa T, Ishimaru K, Yasuhara S, et al. iNOS as a Driver of Inflammation and Apoptosis in Mouse Skeletal Muscle after Burn Injury: Possible Involvement of Sirt1 S-Nitrosylation-Mediated Acetylation of p65 NF-κB and p53. *PLoS One.* 2017 Jan 18;12(1):e0170391. doi: 10.1371/journal.pone.0170391. PMID: 28099528; PMCID: PMC5242494.
12. Hemmati S, Rahimi N, Dabiri S, Alaeddini M, Etemad-Moghadam S, Dehpour AR. Inhibition of ovalbumin-induced allergic rhinitis by sumatriptan through the nitric oxide pathway in mice. *Life Sci*. 2019 Nov 1;236:116901. doi: 10.1016/j.lfs.2019.116901. Epub 2019 Oct 11. PMID: 31610206.
13. Sonar SA, Lal G. The iNOS Activity During an Immune Response Controls the CNS Pathology in Experimental Autoimmune Encephalomyelitis. *Front Immunol.* 2019 Apr 4;10:710. doi: 10.3389/fimmu.2019.00710. PMID: 31019516; PMCID: PMC6458273.
14. Chen L, Bi S, Wei Q, Zhao Z, Wang C, Xie S. Ivermectin suppresses tumour growth and metastasis through degradation of PAK1 in oesophageal squamous cell carcinoma. *J Cell Mol Med.* 2020 May;24(9):5387-5401. doi: 10.1111/jcmm.15195. Epub 2020 Mar 31. PMID: 32237037; PMCID: PMC7205794.
15. Noori T, Sureda A, Shirooie S. Ivermectin decreases inflammation and imiquimod-induced psoriasis-like skin lesions in rat via targeting TLR4/p65 NF-κB. *Iran J Basic Med Sci.* 2025;28(6):808-814. doi: 10.22038/ijbms.2025.83254.18008. PMID: 40343294; PMCID: PMC12057755.
16. Barańska-Rybak W, Kowalska-Olędzka E. New indications for topical ivermectin 1% cream: a case series study. *Postepy Dermatol Alergol.* 2019 Feb;36(1):58-62. doi: 10.5114/ada.2019.82825. Epub 2019 Feb 22. PMID: 30858780; PMCID: PMC6409879.
17. Aryannejad A, Tabary M, Noroozi N,

Mashinchi B, Iranshahi S, Tavangar SM,et al. Anti-inflammatory Effects of Ivermectin in the Treatment of Acetic Acid-Induced Colitis in Rats: Involvement of GABAB Receptors. *Dig Dis Sci.* 2022 Aug;67(8):3672-3682. doi: 10.1007/s10620-021-07258-x. Epub 2021 Oct 21. PMID: 34674071.

18. Zhang X, Song Y, Xiong H, Ci X, Li H, Yu L, Zhang L, Deng X. Inhibitory effects of ivermectin on nitric oxide and prostaglandin E2 production in LPS-stimulated RAW 264.7 macrophages. *Int Immunopharmacol.* 2009 Mar;9(3):354-9. doi: 10.1016/j.intimp.2008.12.016. Epub 2009 Jan 23. PMID: 19168156.

19. Steinhoff M, Vocanson M, Voegel JJ, Hacini-Rachinel F, Schäfer G. Topical Ivermectin 10 mg/g and Oral Doxycycline 40 mg Modified-Release: Current Evidence on the Complementary Use of Anti-Inflammatory Rosacea Treatments. *Adv Ther*. 2016 Sep;33(9):1481-501. doi: 10.1007/s12325-016-0380-z. Epub 2016 Jul 18. PMID: 27432381; PMCID: PMC5020118.

20. Stein L, Kircik L, Fowler J, Tan J, Draelos Z, Fleischer A, et al. Efficacy and safety of ivermectin 1% cream in treatment of papulopustular rosacea: results of two randomized, double-blind, vehicle-controlled pivotal studies. *J Drugs Dermatol*. 2014 Mar;13(3):316-23. PMID: 24595578.

21. Taieb A, Ortonne JP, Ruzicka T, Roszkiewicz J, Berth-Jones J, Peirone MH, et al. Superiority of ivermectin 1% cream over metronidazole 0·75% cream in treating inflammatory lesions of rosacea: a randomized, investigator-blinded trial. *Br J Dermatol.* 2015 Apr;172(4):1103-10. doi: 10.1111/bjd.13408. Epub 2015 Feb 11. PMID: 25228137.

22. Ci X, Li H, Yu Q, Zhang X, Yu L, Chen N, et al. Avermectin exerts anti-inflammatory effect by downregulating the nuclear transcription factor kappa-B and mitogen-activated protein kinase activation pathway. *Fundam Clin Pharmacol*. 2009 Aug;23(4):449-55. doi: 10.1111/j.1472-8206.2009.00684.x. Epub 2009 May 6. PMID: 19453757.

23. Burkhart KM, Burkhart CN, Burkhart CG. Our scabies treatment is archaic, but ivermectin has arrived. *Int J Dermatol.* 1998 Jan;37(1):76-7. doi: 10.1046/j.1365-4362.1998.00404.x. PMID: 9522247.

24. Yan S, Ci X, Chen N, Chen C, Li X, Chu X, et al. Anti-inflammatory effects of ivermectin in mouse model of allergic asthma. I*nflamm Res.* 2011 Jun;60(6):589-96. doi: 10.1007/s00011-011-0307-8. Epub 2011 Jan 29. PMID: 21279416.

25. Khaledi E, Noori T, Mohammadi-Farani A, Sureda A, Dehpour AR, Yousefi-Manesh H, et al. Trifluoperazine reduces cuprizone-induced demyelination via targeting Nrf2 and IKB in mice. *Eur J Pharmacol.* 2021 Oct 15;909:174432. doi: 10.1016/j.ejphar.2021.174432. Epub 2021 Aug 17. PMID: 34416238.

26. Ajayi AAL. Itching, chloroquine, and malaria: a review of recent molecular and neuroscience advances and their contribution to mechanistic understanding and therapeutics of chronic non-histaminergic pruritus. *Int J Dermatol.* 2019 Aug;58(8):880-891. doi: 10.1111/ijd.14252. Epub 2018 Oct 26. PMID: 30362504.

27. Gadotti VM, Zamponi GW. Ethosuximide inhibits acute histamine- and chloroquine-induced scratching behavior in mice. *Mol Brain.* 2021 Mar 2;14(1):46. doi: 10.1186/s13041-021-00762-1. PMID: 33653383; PMCID: PMC7927410.

28. Lesslar OJL, Smith PK. Itch Beyond the Skin-Mucosal Itch. *Front Allergy.* 2021 Oct 28;2:700368. doi: 10.3389/falgy.2021.700368. PMID: 35386995; PMCID: PMC8974814.

29. Lei ZN, Wu ZX, Dong S, Yang DH, Zhang L, Ke Z, et al. Chloroquine and hydroxychloroquine in the treatment of malaria and repurposing in treating COVID-19. *Pharmacol Ther.* 2020 Dec;216:107672. doi: 10.1016/j.pharmthera.2020.107672. Epub 2020 Sep 8. PMID: 32910933; PMCID: PMC7476892.

30. Li R, Sun H, Zheng H, Zong Z, Li S, Meng T, et al. Intradermal Injection of Oxytocin Aggravates Chloroquine-Induced Itch Responses via Activating the Vasopressin-1a Receptor/Nitric Oxide Pathway in Mice. *Front Pharmacol*. 2019 Nov 15;10:1380. doi: 10.3389/fphar.2019.01380. PMID: 31824317; PMCID: PMC6881818.

31. Liu Q, Tang Z, Surdenikova L, Kim S, Patel KN, Kim A, Ru F, Guan Y, Weng HJ, Geng Y, Undem BJ, Kollarik M, Chen ZF, Anderson DJ, Dong X. Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell.* 2009 Dec 24;139(7):1353-65. doi: 10.1016/j.cell.2009.11.034. Epub 2009 Dec 10. PMID: 20004959; PMCID: PMC2989405.

32. Cairns DM, Giordano JE, Conte S, Levin

M, Kaplan DL. Ivermectin Promotes Peripheral Nerve Regeneration during Wound Healing. *ACS Omega*. 2018 Oct 31;3(10):12392-12402. doi: 10.1021/acsomega.8b01451. Epub 2018 Oct 1. PMID: 30411007; PMCID: PMC6210064.

33. Usman A, Shehzad A, Shahid M, Saleem Z, Khawaja A. Comparison between efficacy of topical 1% ivermectin and topical 5% permethrin in the treatment of scabies. *J Pak Assoc Dermatol.* 2021;31(4).

34. Park J, Choi K, Jeong E, Kwon D, Benveniste EN, Choi C. Reactive oxygen species mediate chloroquine-induced expression of chemokines by human astroglial cells. *Glia*. 2004 Jul;47(1):9-20. doi: 10.1002/glia.20017. PMID: 15139008.

35. Yahfoufi N, Alsadi N, Jambi M, Matar C. The Immunomodulatory and Anti-Inflammatory Role of Polyphenols. *Nutrients*. 2018 Nov 2;10(11):1618. doi: 10.3390/nu10111618. PMID: 30400131; PMCID: PMC6266803.

36. Schaller M, Gonser L, Belge K, Braunsdorf C, Nordin R, Scheu A, Borelli C. Dual anti-inflammatory and anti-parasitic action of topical ivermectin 1% in papulopustular rosacea. *J Eur Acad Dermatol Venereol*. 2017 Nov;31(11):1907-1911. doi: 10.1111/jdv.14437. Epub 2017 Aug 29. PMID: 28653460.

37. Chen TH, Chang PC, Chang MC, Lin YF, Lee HM. Chloroquine induces the expression of inducible nitric oxide synthase in C6 glioma cells. *Pharmacol Res*. 2005 Apr;51(4):329-336. doi: 10.1016/j.phrs.2004.10.004

38. Abo El-Noor MM, Elgazzar FM, Alshenawy HA. Role of inducible nitric oxide synthase and interleukin-6 expression in estimation of skin burn age and vitality. *J Forensic Leg Med.* 2017 Nov;52:148-153. doi: 10.1016/j.jflm.2017.09.001. Epub 2017 Sep 12. PMID: 28926822.

39. Mansourpour H, Ziari K, Motamedi SK, Poor AH. Therapeutic effects of iNOS inhibition against vitiligo in an animal model. *Eur J Transl Myol*. 2019 Aug 6;29(3):8383. doi: 10.4081/ejtm.2019.8383. PMID: 31579486; PMCID: PMC6767835.

40. Younas, Khan A, Shehzad O, Seo EK, Onder A, Khan S. Anti-allergic activities of Umbelliferone against histamine- and Picryl chloride-induced ear edema by targeting Nrf2/iNOS signaling in mice. *BMC Complement Med Ther.* 2021 Aug 27;21(1):215. doi: 10.1186/s12906-021-03384-1. PMID: 34452623; PMCID: PMC8394045.