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Thymoquinone and Melatonin Boost Both Sirt1 and Nrf2 Activities in a Complementary Way – A Strategy for Controlling Oxidative Stress and Inflammation

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Abstract

The oil of black cumin seed (Nigella sativa) has been employed therapeutically in Asian traditional medicine, and rodent studies with it or its most prominent and intriguing component, thymoquinone, suggest that it may indeed have the potential to provide benefit in a wide range of pathologies. There is reason to suspect that thymoquinone's versatile utility may reflect, in large part, its ability to boost the activity of the sirtuin 1 (Sirt1) deacetylase while concurrently activating the Nrf2 transcription factor. Increased Sirt1 activity promotes autophagy, mitochondrial biogenesis, and FOXO-mediated expression of antioxidant enzymes, while suppressing NF-kappaB-mediated inflammatory signaling; Nrf2 promotes expression of a wide range of antioxidant enzymes, as well as the enzyme rate limiting for glutathione synthesis. Thymoquinone is a substrate for NAD(P)H quinone oxidoreductase 1 (NQO1); this reaction gives rise to NAD^+ - obligate substrate for Sirt1 activity – while generating thymohydroquinone, a potent scavenging antioxidant. Thymoquinone, which is electrophilic, promotes Nrf2 activation through covalent reaction with its functional inhibitor Keap1. We suggest that supplemental melatonin may be an ideal complement to thymoquinone, as it can promote increased expression of both Sirt1and Nrf2 – likely via activation of the "clock" transcription factor Bmal1 – whereas thymoquinone boosts the biological activities of the pre-formed proteins.

Keywords: Thymoquinone; Sirt1, NAD(P)H quinone oxidoreductase 1; nrf2; Melatonin; NF-kappa B

Thymoquinone – Sirt1 Activation via NQO1-Mediated NAD^+ Generation

Thymoquinone is believed to be the chief bioactive agent in the oil of Nigella sativa (black cumin), an agent which has been employed therapeutically in Ayurvedic or Islamic traditional medicine. Black cumin seed oil and thymoquinone are now attracting increased research attention, and have been the subject of many recent reviews. In rodent or cell culture studies, thymoquinone or black cumin seed oil are reported to exert a wide range of protective effects; benefits have been reported in models of diabetes, atherosclerosis, hypertension, renal and pulmonary disorders, neurodegenerative disorders, rheumatoid arthritis, and wound healing, among others **[1-14]**.

A case can be made that these benefits are primarily reflective of thymoquinone-mediated activation of the sirtuin 1(Sirt1) deacetylase – increased activity of which has been linked to enhanced healthspan **[15-17]**– and of the transcription factor Nrf2, which enhances expression of a number of antioxidant enzymes and of the rate-limiting enzyme for glutathione synthesis **[18]**. Sirt1 is a particularly intriguing for its wide-ranging modulatory activities – enhancing autophagy, mitophagy, mitochondrial biogenesis, DNA repair, antioxidant enzyme expression, osteoblast generation, and endothelial nitric oxide synthase expression and activity, while inhibiting

apoptosis, senescence, de novo lipogenesis, and – via suppression of canonical NF-kappaB activity – inflammation **[19-23]**. In aggregate, these effects may account for the favorable impact of Sirt1 on health span.

Sirt1 activity is absolutely dependent on NAD^+ as a substrate. In high redox circumstances, in which the ratio of NAD^+/NADH is low, or when NAD^+ is degraded, as when PARP is activated by DNA damage or C38 activity is high, a paucity of NAD^+ can compromise Sirt1 activity **[24,25]**. Conversely, Sirt1 activity can be boosted by: fasting or calorie restriction – which tends to enhance the NAD^+/NADH ratio owing to a paucity of oxidizable substrate;[26] an increase in NAD^+ synthesis owing to AMPK activation – which boosts expression of nicotinamide phosphoribosyltransferase (NAMPT), rate-limiting for conversion of free nicotinamide (a Sirt1 inhibitor) to NAD^+; **[27-29]** or administration of NAD+ precursors, such as nicotinamide riboside or nicotinamide mononucleotide[30]. (Nicotinamide per se, while promoting NAD+ synthesis, directly inhibits Sirt1 activity – which is another reason why NAMPT induction aids Sirt1 activity **[31]**. And high intakes of nicotinic acid are problematic owing to flushing/itching side effects.)

NAD^+ availability can also be enhanced by enzymatic activities which oxidize NADH. In this regard, NAD(P)H quinone oxidoreductase 1 (NQO1) is of particular pertinence. This enzyme, inducible via Nrf2, can reduce a number of endogenous or exogenous quinones with NADH or NADPH – generating NAD^+ (or $NADP^+$) in the process **[32-34]**. Recent evidence indicates that NQO1 associates tightly with enzymatically active Sirt1 within cells; this association hence appears to be a homeostatic mechanism whereby NQO1 can generate NAD^+ within the microenvironment of Sirt1[35-36].A corollary is that administration of quinone substrates for NQO1 may have potential for boosting Sirt1 activity, particularly when cellular NAD^+ levels are relatively low. Indeed, this effect has been demonstrated with the phytochemical β- lapachinone **[37, 38]**. And the ability of the phytochemical pyrroloquinoline quinone (PQQ) to promote mitochondrial biogenesis has been traced to Sirt1 activation mediated by an increase in NAD^+ [39,40].PQQ has been found to bind to lactate dehydrogenase with high affinity; this bound form of PQQ readily oxidizes NADH to NAD+, and the resulting increase of NAD+ in the vicinity of lactate dehydrogenase tends to drive oxidation of lactate to pyruvate, which becomes available for mitochondrial oxidation **[41]**. (This mechanism may explain why dietary PQQ has been found to have a quasi-vitamin status in rodent studies **[42]**).

There is now evidence that thymoquinone can act in a comparable way – a finding which rationalizes several reports that thymoquinone is a Sirt1 activator **[43-48]**. Nonetheless, several of these studies also find a thymoquinone-mediated increase in Sirt1 expression, suggesting an additional and complementary mechanism for thymoquinone's positive effect on Sirt1 activity. Thymoquinone can act either as an antioxidant or a pro-oxidant in cells. When it interacts with NQO1, the thymohydroquinone that is generated can act as a potent scavenging antioxidant **[43, 49, 50]**. In circumstances of high cellular ROS production, the donation of electrons from this agent in quenching of radicals can return it to its oxidized form thymoquinone, which can then catalyze generation of another molecule of NAD^+ via interaction with NQO1. On the other hand, there are several enzymatic activities known to induce one-electron reductions of thymoquinone, converting it to a semi-quinone, while concurrently donating an electron to molecular oxygen, generating superoxide **[43]**. This latter effect appears to explain how thymoquinone can exert cytotoxic effects in certain tumor cell lines in which NQO1 activity is very deficient or absent **[43, 51]**. Fortunately, the low toxicity of thymoquinone in rodent studies (acute oral LD50 of about 800 mg/kg), and the apparent good tolerance of black cumin seeds as a component of diet or as a traditional herbal medicine, suggests that its net effect is antioxidant in most circumstances, at least when intake is moderate **[52]**.

Thymoquinone Activates Nrf2 via Interaction with Keap1

Moreover, thymoquinone can act as a Nrf2 activator, inasmuch as it is electrophilic and can interact covalently with the free acidic sulfhydryl groups in Keap1 **[53-57]**. Keap1 is a protein which binds to Nrf2 in the cytoplasm, preventing it from migrating to the nucleus where it can promote transcription; Keap1 also promotes the ubiquitination and proteasomal degradation of Nrf2 **[58, 59]**.The interaction of thymoquinone with Keap1 inhibits its binding to Nrf2, enabling the latter to modulate protein transcription **[53]**. Nrf2 activation would be expected to complement the direct oxidant-scavenging activity of thymohydroquinone. And thymoquinone-mediated Sirt1 activity also can exert antioxidant activity; Sirt1 alleviates mitochondrially generated oxidant production by promoting mitophagy and mitochondrial biogenesis via PGC-1α activation **[60]**. Also, via deacetylation of FOXO transcription factors, Sirt1 promotes expression of various antioxidant enzymes – notably catalase and the mitochondrial superoxide dismutase - at the transcriptional level **[61-63]**. Hence, any direct pro-oxidant activity of thymoquinone induced by its conversion to a semi-quinone appears likely to be counterbalanced by the direct scavenging activity of thymohydroquinone and the consequences of Nrf2 and Sirt1activation.Also of interest is the fact that, as noted about, Nrf2 promotes expression of NQO1; in this way, thymoquinone's ability to activate Nrf2 may amplify its ability to activate Sirt1.

Owing to the fact that Sirt1 can deacetylate the p65 component of the NF-kappaB transcription factor in a way that suppresses its ability to drive transcription, thymoquinone has anti-inflammatory potential that complements its antioxidant effects **[64]**. This suggests that adequate doses may have particular therapeutic potential in inflammatory pathologies associated with oxidative stress. The vast range of benefits conferred by thymoquinone in rodent studies may reflect the

fact that inflammation and oxidative stress play a role in the pathogenesis of a great number of health disorders.

Melatonin May be an Ideal Complement to Thymoquinone

Another natural compound with antioxidant and anti-inflammatory activity – the neurohormone melatonin, now commonly used as a nutraceutical – may have important clinical potential as a complement to thymoquinone, owing to the fact that it can promote the synthesis and protein expression of both Sirt1 and Nrf2 **[65-69]**. Melatonin's induction of Sirt1 and Nrf2 may be attributable to activation of the melatonin-regulated clock transcription factor Bmal1; this been shown to drive the transcription of the genes coding for Sirt1 and Nrf2 **[70-74]**. Bmal1 also supports Sirt1 activity by inducing nicotinamide phosphoribosyltransferase **[75]**. In at least certain tissues, melatonin signaling through its plasma membrane receptor MT1 up-regulates Bmal1 mRNA **[76-78]**. Hence, whereas thymoquinone can boost the biological activities of both Sirt1 and Nrf2, melatonin acts to increase their protein expression.

With respect to dosage considerations, mouse studies reporting protective effects of oral thymoquinone typically employ daily intakes in the range of 20-50 mg/kg. 20 mg/kg in a 25 g mouse amounts to 0.5 mg. If you extrapolate this to a 60 kg human, using the 2/3 power of relative body mass (i.e. ratio of body surface areas **[79]** as the correction factor, that equates to about 180, or an absolute dose of 90 mg thymoquinone daily. (Extrapolation by ¾ power would yield a value of about 170 mg thymoquinone.) Nigella sativa oil preparations for nutraceutical use are now widely available, as gel caps or oil, standardized at 2% thymoquinone content. A teaspoon (5 ml/ 5 g) of 2% oil would provide 100 mg. So, a teaspoon or two of high-quality Nigella sativa oil daily might be a worthwhile and a practical dose for assessing the potential clinical benefits of thymoquinone. With respect to the limited clinical literature already availability, several controlled clinical studies from Iran have found that 2.5 g of Nigella sativa oil twice daily, or 3 g of the oil once daily, lowered systolic and diastolic and diastolic blood pressure in healthy volunteers, and, in patients who were overweight with metabolic syndrome, decreased LDL cholesterol, fasting blood sugar, and serum levels of C-reactive protein and tumor necrosis factor-α, while potentiating the weight loss achieved with a reduced-calorie diet **[80-83]**. As to melatonin, bedtime doses of 3-20 mg have typically been employed clinically for effective treatment of sleep disorders, post-surgical pain, and cancer **[84]**.

Conflicts: The authors have no conflicts of interest to declare.

References

1. Hannan MA, Rahman MA, Sohag AAM, Uddin MD, Dash R, et al. Black Cumin (Nigella sativa L.) (2021) A Comprehensive Review on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety. Nutrients 13: 1784.
2. Sarkar C, Jamaddar S, Islam T, Mondal M, Islam MT, et al. (2021) Therapeutic perspectives of the black cumin component thymoquinone: A review. Food Funct 12: 6167-213.
3. Fatima SK, Soubra W, Cordato DJ (2021) The role of thymoquinone, a major constituent of Nigella sativa, in the treatment of inflammatory and infectious diseases. Clin Exp Pharmacol Physiol 48: 1445-53.
4. Al-Gabri NA, Saghir SAM, Al-Hashedi SA, El-Far AH, Khafaga AF, et al. (2021) Therapeutic Potential of Thymoquinone and Its Nanoformulations in Pulmonary Injury: A Comprehensive Review. Int J Nanomedicine 16: 5117-31.
5. Majdalawieh AF, Yousef SM, Abu-Yousef IA. (2021) Thymoquinone, a major constituent in Nigella sativa seeds, is a potential preventative and treatment option for atherosclerosis. Eur J Pharmacol 909:174420.
6. Hannan MA, Zahan MS, Sarker PP, Moni A, Ha H, et al. (2021) Protective Effects of Black Cumin (Nigella sativa) and Its Bioactive Constituent, Thymoquinone against Kidney Injury: An Aspect on Pharmacological Insights. Int J Mol Sci 22: 9078..
7. Burdock GA (2022) Assessment of black cumin (Nigella sativa L.) as a food ingredient and putative therapeutic agent. Regul Toxicol Pharmacol 128: 105088.
8. Maideen NMP, Balasubramanian R, Ramanathan S (2021) Nigella Sativa (Black Seeds), A Potential Herb for the Pharmacotherapeutic Management of Hypertension - A Review. Curr Cardiol Rev 17: e230421187786.
9. Sallehuddin N, Nordin A, Bt HI Fauzi MB (2021) Nigella sativa and Its Active Compound, Thymoquinone, Accelerate Wound Healing in an In Vivo Animal Model: A Comprehensive Review. Int J Environ Res Public Health 17: 4160.
10. Bule M, Nikfar S, Amini M, Abdollahi M (2020) The antidiabetic effect of thymoquinone: A systematic review and meta-analysis of animal studies. Food Res Int1 27:108736.
11. Hamdan A, Haji IR, Mokhtar MH (2019) Effects of Nigella Sativa on Type-2 Diabetes Mellitus: A Systematic Review. Int J Environ Res Public Health 16: 4911.
12. Uddin MN, Hoq MI, Jahan I, Siddiqui SA, Clinton CD, et al. (2021) The Mechanistic Role of Thymoquinone in Parkinson's Disease: Focus on Neuroprotection in Pre-Clinical Studies. Curr Mol Pharmacol 14: 1083-1092.
13. Isaev NK, Chetverikov NS, Stelmashook EV, Genrikhs EE, Khaspekov LG, et al. (2020) Thymoquinone as a Potential Neuroprotector in Acute and Chronic Forms of Cerebral Pathology. Biochemistry (Mosc) 85: 167-176.
14. ZieliÅ„ska M, DereÅ K, Polak-SzczybyÅ OE, Stępień AE (2021) The Role of Bioactive Compounds of Nigella sativa

in Rheumatoid Arthritis Therapy-Current Reports. Nutrients 13: 3369.

15. Satoh A, Stein L, Imai S (2011) The role of mammalian sirtuins in the regulation of metabolism, aging, and longevity. Handb Exp Pharmacol 206: 125-162.
16. Baur JA, Ungvari Z, Minor RK, Le Couteur DG, de CR (2012) Are sirtuins viable targets for improving healthspan and lifespan? Nat Rev Drug Discov 11: 443-461.
17. Houtkooper RH, Pirinen E, Auwerx J (2012) Sirtuins as regulators of metabolism and healthspan. Nat Rev Mol Cell Biol 13: 225-238.
18. Jung KA, Kwak MK (2020) The Nrf2 system as a potential target for the development of indirect antioxidants. Molecules 15: 7266-291.
19. Di Nicolantonio JJ, McCarty MF, Iloki-Assanga SB, Lujan LL, O'Keefe JH (2022) Ferulic acid and berberine, via Sirt1 and AMPK, may act as cell cleansing promoters of healthy longevity. Open Heart 9: e001801.
20. Ponugoti B, Kim DH, Xiao Z, Miao J, Zang M, et al. (2010) SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. J Biol Chem 285: 33959-33970.
21. Mattagajasingh I, Kim CS, Naqvi A, Yamamori T, Hoffman TA, et al. (2007) SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci USA 104:14855-14860.
22. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, et al. (2004) Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 23: 2369-2380.
23. Kim HN, Ponte F, Warren A, Ring R, Iyer S, et al. (2021) A decrease in NAD (+) contributes to the loss of osteoprogenitors and bone mass with aging. NPJ Aging Mech Dis 7: 8.
24. Guarente L Linking (2014) DNA damage, NAD (+)/SIRT1, and aging. Cell Metab 20: 706-707.
25. Aksoy P, Escande C, White TA, Thompson M, Soares S, et al. (2006) Regulation of SIRT 1 mediated NAD dependent deacetylation: a novel role for the multifunctional enzyme CD38. Biochem Biophys Res Commun 349: 353-359.
26. Bordone L, Guarente L (2005) Calorie restriction, SIRT1 and metabolism: understanding longevity. Nat Rev Mol Cell Biol 6: 298-305.
27. Costford SR, Bajpeyi S, Pasarica M, Albarado DC, Thomas SC, et al. (2020) Skeletal muscle NAMPT is induced by exercise in humans. Am J Physiol Endocrinol Metab 298: E117-E126.
28. Fulco M, Cen Y, Zhao P, Hoffman EP, McBurney MW, et al. (2008) Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. Dev Cell 14: 661-673.
29. Brandauer J, Vienberg SG, Andersen MA, Ringholm S, Risis S, et al. (2013) AMP-activated protein kinase regulates nicotinamide phosphoribosyl transferase expression in skeletal muscle. J Physiol 591:5207-5220.
30. Imai S, Guarente L (2014) NAD+ and sirtuins in aging and disease. Trends Cell Biol 24: 464-471.
31. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA (2002) Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. J Biol Chem 277: 45099-45107.
32. Ross D, Siegel D (2021) The diverse functionality of NQO1 and its roles in redox control. Redox Biol 41: 101950.
33. Lin X, Yang H, Zhou L, Guo Z (2011) Nrf2-dependent induction of NQO1 in mouse aortic endothelial cells overexpressing catalase. Free Radic Biol Med 51: 97-106.
34. Elangovan S, Hsieh TC (2008) Control of cellular redox status and upregulation of quinone reductase NQO1 via Nrf2 activation by alpha-lipoic acid in human leukemia HL-60 cells. Int J Oncol 33:833-838.
35. Tsvetkov P, Adler J, Strobelt R, Adamovich Y, Asher G, et al. (2021) NQO1 Binds and Supports SIRT1 Function. Front Pharmacol 12: 671929.
36. Qiu D, Song S, Wang Y, Bian Y, Wu M, et al. (2022) NAD (P)H: quinone oxidoreductase 1 attenuates oxidative stress and apoptosis by regulating Sirt1 in diabetic nephropathy. J Transl Med 20: 44.
37. Hwang JH, Kim DW, Jo EJ, Kim YK, Jo YS, et al. (2009) Pharmacological stimulation of NADH oxidation ameliorates obesity and related phenotypes in mice. Diabetes 58: 965-974.
38. Oh GS, Kim HJ, Choi JH, Shen AH, Choe SK, et al. (2014) Pharmacological activation of NQO1 increases NADâ• º levels and attenuates cisplatin-mediated acute kidney injury in mice. Kidney Int 85: 547-560.
39. Saihara K, Kamikubo R, Ikemoto K, Uchida K, Akagawa M (2017) Pyrroloquinoline Quinone, a Redox-Active o-Quinone, Stimulates Mitochondrial Biogenesis by Activating the SIRT1/PGC-1Î± Signaling Pathway. Biochemistry 56: 6615-6625.
40. Zhang H, Li J, Cao C, Zhang B, Yang W, et al. (2020) Pyrroloquinoline quinone inhibits the production of inflammatory cytokines via the SIRT1/NF-ÎºB signal pathway in weaned piglet jejunum. Food Funct 11: 2137-253.
41. Akagawa M, Minematsu K, Shibata T, Kondo T, Ishii T, Uchida K (2016) Identification of lactate dehydrogenase as a mammalian pyrroloquinoline quinone (PQQ)-binding protein. Sci Rep 6: 26723.
42. Jonscher KR, Chowanadisai W, Rucker RB (2021) Pyrroloquinoline-Quinone Is More Than an Antioxidant: A Vitamin-like Accessory Factor Important in Health and Disease Prevention. Biomolecules11: 1441.
43. Al-Hayali M, Garces A, Stocks M, Collins H, Bradshaw TD (2021) Concurrent Reactive Oxygen Species Generation and Aneuploidy Induction Contribute to Thymoquinone Anticancer Activity. Molecules 26: 5136.
44. Yang Y, Bai T, Yao YL, Zhang DQ, Wu YL, et al. (2016) Upregulation of SIRT1-AMPK by thymoquinone in hepatic stellate cells ameliorates liver injury. Toxicol Lett 262: 80-91.

45. Velagapudi R, El-Bakoush A, Lepiarz I, Ogunrinade F, Olajide OA (2017) AMPK and SIRT1 activation contribute to inhibition of neuroinflammation by thymoquinone in BV2 microglia. Mol Cell Biochem 435: 149-162.
46. Karandrea S, Yin H, Liang X, Slitt AL, Heart EA (2017) Thymoquinone ameliorates diabetic phenotype in Diet-Induced Obesity mice via activation of SIRT-1-dependent pathways. PLoS One 12: e0185374.
47. Lu Y, Feng Y, Liu D, Zhang Z, Gao K, et al. (2018) Thymoquinone Attenuates Myocardial Ischemia/Reperfusion Injury Through Activation of SIRT1 Signaling. Cell Physiol Biochem 47: 1193-1206.
48. Salam SA, Mostafa F, Alnamshan MM, Elshewemi SS, Sorour JM (2021) Thymoquinone ameliorates age-related hearing loss in C57BL/6J mice by modulating Sirt1 activity and Bak1 expression. Biomed Pharmacother 143: 112149.
49. Tesarova H, Svobodova B, Kokoska L, Marsik P, Pribylova M, et al. (2011) Determination of oxygen radical absorbance capacity of black cumin (Nigella sativa) seed quinone compounds. Nat Prod Commun 6: 213-216.
50. Zerrouki M, Benkaci-Ali F (2018) DFT study of the mechanisms of nonenzymatic DNA repair by phytophenolic antioxidants. J Mol Model 24: 78.
51. Sutton KM, Doucette CD, Hoskin DW (2012) NADPH quinone oxidoreductase 1 mediates breast cancer cell resistance to thymoquinone-induced apoptosis. Biochem Biophys Res Commun 426: 421-426.
52. Al-Ali A, Alkhawajah AA, Randhawa MA, Shaikh NA (2008) Oral and intraperitoneal LD50 of thymoquinone, an active principle of Nigella sativa, in mice and rats. J Ayub Med Coll Abbottabad 20: 25-27.
53. Talebi M, Talebi M, Farkhondeh T, Samarghandian S (2021) Biological and therapeutic activities of thymoquinone: Focus on the Nrf2 signaling pathway. Phytother Res 35:1739-1753.
54. Velagapudi R, Kumar A, Bhatia HS, Lepiarz-Raba I, Fiebich B, et al. (2017) Inhibition of neuroinflammation by thymoquinone requires activation of Nrf2/ARE signalling. Int Immunopharmacol 48:17-29.
55. Kundu J, Kim DH, Kundu JK, Chun KS (2014) Thymoquinone induces heme oxygenase-1 expression in HaCaT cells via Nrf2/ARE activation: Akt and AMPKÎ± as upstream targets. Food Chem Toxicol 65: 18-26.
56. Dong J, Zhang X, Wang S, Xu C, Gao M, et al. (2020) Thymoquinone Prevents Dopaminergic Neurodegeneration by Attenuating Oxidative Stress Via the Nrf2/ARE Pathway. Front Pharmacol 11: 615598.
57. Amin N, Du X, Chen S, Ren Q,Hussien AB, et al. (2021) Therapeutic impact of thymoquninone to alleviate ischemic brain injury via Nrf2/HO-1 pathway. Expert Opin Ther Targets 25: 597-612.
58. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, et al. (2002) Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc Natl Acad Sci U S A 99:11908-11913.
59. Zhang DD, Hannink M. (2003) Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol Cell Biol 23: 8137-8151.
60. Nemoto S, Fergusson MM, Finkel T (2005) SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1{alpha}. J Biol Chem 280:16456-1660.
61. Kim HN, Han L, Iyer S, de Cabo R, Zhao H, et al. (2015) Sirtuin1 Suppresses Osteoclastogenesis by Deacetylating FoxOs. Mol Endocrinol 29:1498-509.
62. Li Y, Shen G, Yu C, Li G, Shen J, et al. (2014) Angiotensin II induces mitochondrial oxidative stress and mtDNA damage in osteoblasts by inhibiting SIRT1–FoxO3a–MnSOD pathway. Biochem Biophys Res Commun 455: 113-118.
63. Morris BJ (2005) A forkhead in the road to longevity: the molecular basis of lifespan becomes clearer. J Hypertens 23: 1285-1309.
64. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, et al. (2004) Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 23: 2369-2380.
65. CristÃ²fol R, Porquet D, Corpas R, Coto-Montes A, Serret J, et al. (2012) Neurons from senescence-accelerated SAMP8 mice are protected against frailty by the sirtuin 1 promoting agents melatonin and resveratrol. J Pineal Res 52: 271-281.
66. Yu L, Sun Y, Cheng L, Jin Z, Yang Y, et al. (2014) Melatonin receptor-mediated protection against myocardial ischemia/reperfusion injury: role of SIRT1. J Pineal Res 57: 228-238.
67. Yang Y, Jiang S, Dong Y, Fan C, Zhao L, et al. (2015) Melatonin prevents cell death and mitochondrial dysfunction via a SIRT1-dependent mechanism during ischemic-stroke in mice. J Pineal Res 58: 61-70.
68. Fang J, Yan Y, Teng X, Wen X, Li N, et al. (2018) Melatonin prevents senescence of canine adipose-derived mesenchymal stem cells through activating NRF2 and inhibiting ER stress. Aging (Albany NY) 10: 2954-29572.
69. Wang Z, Ma C, Meng CJ, Zhu GQ, Sun XB, et al. (2012) Melatonin activates the Nrf2-ARE pathway when it protects against early brain injury in a subarachnoid hemorrhage model. J Pineal Res 53: 129-137.
70. Zhou B, Zhang Y, Zhang F, Xia Y, Liu J, et al. (2014) CLOCK/BMAL1 regulates circadian change of mouse hepatic insulin sensitivity by SIRT1. Hepatology 59: 2196-2206.
71. Liu J, Zhou B, Yan M, Huang R, Wang Y, et al. (2016) CLOCK and BMAL1 Regulate Muscle Insulin Sensitivity via SIRT1 in Male Mice. Endocrinology 157: 2259-2269.
72. Early JO, Menon D, Wyse CA, Cervantes-Silva MP, Zaslona Z, et al. (2018) Circadian clock protein BMAL1 regulates IL-1Î² in macrophages via NRF2. Proc Natl Acad Sci USA 115: E8460-E8468.
73. Chhunchha B, Kubo E, Singh DP (2020) Clock Protein Bmal1 and Nrf2 Cooperatively Control Aging or Oxidative

Response and Redox Homeostasis by Regulating Rhythmic Expression of Prdx6 Cells 9: 1861.

74. Sun Q, Zeng C, Du L, Dong C (2021) Mechanism of circadian regulation of the NRF2/ARE pathway in renal ischemia-reperfusion. Exp Ther Med 21: 190.

75. Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P (2009) Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science 324: 654-657.

76. Jilg A, Moek J, Weaver DR, Korf HW, Stehle JH, et al. (2005) Rhythms in clock proteins in the mouse pars tuberalis depend on MT1 melatonin receptor signalling. Eur J Neurosci 2845-2854.

77. Fang J, Yan Y, Teng X, Wen X, Li N, et al. (2018) Melatonin prevents senescence of canine adipose-derived mesenchymal stem cells through activating NRF2 and inhibiting ER stress. Aging (Albany NY) 10: 2954-2972.

78. Fu S, Kuwahara M, Uchida Y, Koudo S, Hayashi D, et al. (2019) Circadian production of melatonin in cartilage modifies rhythmic gene expression. J Endocrinol JOE-19-0022.R2. doi: 10.1530/JOE-19-0022.

79. Capellini I, Venditti C, Barton RA (2010) Phylogeny and metabolic scaling in mammals. Ecology 91: 2783-93.

80. Najmi A, Nasiruddin M, Khan RA, Haque SF (2008) Effect of Nigella sativa oil on various clinical and biochemical parameters of insulin resistance syndrome. Int J Diabetes Dev Ctries 28: 11-14.

81. Fallah HH, Amini M, Mohtashami R, Sadeqhi Z, Kianbakht S, et al. (2013) Blood pressure lowering effect of Nigella sativa L. seed oil in healthy volunteers: a randomized, double-blind, placebo-controlled clinical trial. Phytother Res 27: 1849-1853.

82. Mahdavi R, Namazi N, Alizadeh M, Farajnia S (2016) Nigella sativa oil with a calorie-restricted diet can improve biomarkers of systemic inflammation in obese women: A randomized double-blind, placebo-controlled clinical trial. J Clin Lipido l10: 1203-1211.

83. Namazi N, Mahdavi R, Alizadeh M, Farajnia S (2015) Oxidative Stress Responses to Nigella sativa Oil Concurrent with a Low-Calorie Diet in Obese Women: A Randomized, Double-Blind Controlled Clinical Trial. Phytother Res 29: 1722-1728.

84. Lim S, Park S, Koyanagi A, Yang JW, Jacob L, et al. (2022) Effects of exogenous melatonin supplementation on health outcomes: An umbrella review of meta-analyses based on randomized controlled trials. Pharmacol Res 176:106052. doi: 10.1016/j.phrs.2021.106052

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