A comprehensive insight into potential roles of *Nigella sativa* on diseases by targeting AMP-activated protein kinase: a review

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Abstract

Objectives *Nigella sativa* (NS) is a known medicinal herb with numerous therapeutic effects such as antidiabetic, anti-proliferative, anti-inflammatory, and anti-cancer activities. It has been indicated that NS can regulate cellular metabolism by adjusting transduction signaling pathways. Adenosine monophosphate-activated protein kinase (AMPK) is one of the main physiological processes, such as energy hemostasis, cellular metabolism, and autophagy regulators. Herb-derived medicines have always been considered as one of the main AMPK activators, and surprisingly recent data has demonstrated that it can be a target for NS and its derivatives. **Evidence acquisition** The literature search was conducted in PubMed, SCOPUS, Embase, ProQuest, and Google Scholar electronic resources. Published articles up to September 2020 were considered, and those of which investigated *Nigella sativa* effects on the AMPK pathway after meeting the inclusion criteria were included.

Results The search was performed on several online databases such as PubMed, Scopus, Embase, ProQuest, and Google Scholar from inception until January 2020. Among the initial search, 245 studies were found. After removing duplicated data and meeting the inclusion criteria, only 14 studies were selected. They included the effects of NS and its bioactive compounds as anti-hyperglycemic ($n = 5$), on liver function ($n = 4$), cancers ($n = 3$), and on Neuroinflammation and Atherosclerosis ($n = 2$). Most of the included studies are animals or in-vitro investigations.

Conclusion In this review, we discuss the latest findings on the molecular mechanism of NS effecting the AMPK signaling pathway. We also focus on the therapeutic effects of NS, including the prevention and treatment of metabolic and pro-inflammatory disease by targeting the AMPK pathway.

Keywords *Nigella sativa* · AMP-activated protein kinase

Introduction

AMP-activated protein kinase (AMPK) plays an essential role in regulating cellular metabolism and energy homeostasis. Low intercellular ATP levels and an increased ratio of AMP/ATP are two main activators of AMPK [1]. In fact, AMPK, by turning on catabolic pathways, mediates higher ATP production [2]. AMPK can be regulated by upstream kinase phosphorylation. The role of AMPK is more obvious in energy metabolism-related diseases such as diabetes, Atherosclerosis, cancer, and obesity, which positive energy balance is a well-known risk factor for all chronic diseases [3–5].

Nigella sativa is a medicinal herb that has been traditionally used to treat various diseases, including asthma, dyslipidemia, diabetes, and many other metabolic disorders in the Middle and Eastern Asia, and Southern Europe for many decades [6]. *Nigella sativa*'s medicinal properties might be due to its

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numerous active components, including Thymoquinone, thymol, and Nigellone [6, 7]. Thymoquinone (TQ) is the most potent bioactive component that has been extracted from *Nigella sativa*'s seeds. There is a hypothesis that *Nigella sativa* (NS), an activator of AMPK, might play a role in alleviating the intensity of metabolic diseases [1, 8, 9]. AMPK phosphorylation, besides decreasing the phosphorylation of mTORC2, a rapamycin-insensitive protein complex molded by serine/threonine kinase mTOR, plays a critical role in the inhibition of malignant tumors progression [10]. Also, the thymoquinone anti-cancer effect may be due to the regulatory effect in p-S6K expression and Liver kinase B1 activity [11]. Although several studies indicated that Thymoquinone might be responsible for some of the antidiabetic, anti-hypertensive, anti-dyslipidemia, and anti-tumor effects of *Nigella sativa*, there are few studies to determine the molecular mechanisms by which *Nigella sativa* exerts its function.

Methods

Search strategy

A systematic search was performed in four databases including PubMed, SCOPUS, Embase, ProQuest, and Google Scholar electronic databases until September 2020 using the following keywords: (“*Nigella sativa*” or “black cumin” or “Thymoquinone” or “black seed”) and (“AMP-activated protein kinase” or “AMPK” or “5’ adenosine monophosphate-activated protein kinase” or “5’ AMP-activated protein kinase”). Also, we conducted a manual search in reference lists of all relevant studies to avoid missing any eligible study. The search was limited to English language articles published up to January 2020.

Eligibility criteria

Eligible criteria for including studies were: (1) all clinical trials, (2) animal studies, and (3) in vitro models. All published articles in non-English language journals and abstract-limited studies were excluded.

Data extraction and quality assessment

At first, after confirming eligibility of studies by two independent investigators (VM and OT), standardized data collection form and research questions were used for screening and extracting data [12]. In the next step, the following information was extracted from each eligible study: first author's name, year of publication, country of origin, type of study, methods or models, quantity and gender of participants, dosage, and duration of the intervention, and main outcomes. If there was a

disagreement between the researchers, accuracy and quality of the included data was evaluated by a third researcher.

Results

Selected articles

A flow diagram of the study selection is summarized in Fig. 1. In total, 245 articles were retrieved, of which 67 were duplicated, resulting in 178 non-duplicated publications. Of these 178 publications, 161 articles did not meet the inclusion criteria and were excluded. Following that, three articles were excluded because of not meeting the eligibility criteria. Finally, 14 articles met the selection criteria and were included in the review. Table 1 shows a summary of studies on the effect of *Nigella sativa* in various experimental models by targeting AMPK signaling.

Characteristics of the included studies

Most of the included studies were animal or in vitro studies. Study duration and dosage of NS or TQ were different in the considered investigations. Among all of the studies, five studies investigated the anti-hyperglycemic effect of NS and its bioactive compounds on diabetic rats or different cell lines [13–17]. Also, four studies were carried out with the aim of evaluating the possible mechanisms of NS on the liver function by investigating its cellular regulating metabolism [18], anti-inflammatory [19], and hepatoprotective effects [20, 21]. Cancer studies were conducted on different human renal and colon cell lines [3, 11, 22]. However, only one study has assessed the impact of NS on neuroinflammation and Atherosclerosis. These studies were administered on aortic vascular smooth muscle cells and BV2 mice microglia [23, 24].

Constituents of *Nigella sativa*

So far, several chemical components have been described based on different samples of *Nigella sativa*. The black seed of NS contains protein (26.7%), carbohydrates (24.9%), fiber (8.4%), and fats (28.5%) [25]. The seed is also rich in various vitamins (A, B1, B2, B3, B9, and C) and minerals (iron, copper, zinc, phosphorus, calcium) [26]. The main triacylglycerols (TAGs) in NS are tri-linoleoyl, oleoyl-di-linoleoyl, palmitoyl-di-linoleoyl, palmitoyl-oleoyl-linoleoyl, and dioleoyl-linoleoyl [27, 28]. Some other fatty acids and sterols such as myristic acid, palmitoleic acid, linoleic acid, linolenic acid, arachidonic acid, cholesterol, campesterol, β -sitosterol, Δ 5-avenasterol, Δ 7-stigmasterol, and Δ 7-avenasterol are also reported in NS [29]. However, the proportion of saturated to unsaturated fatty acids in NS oil varies in different regions, and its composition can be affected by the extraction method and the seed originality. The

Fig. 1 Flow diagram of the literature search and study selection process

phytochemical analysis has shown NS seeds contain alkaloids such as isoquinoline, terpenes including α -adherin, and saponins. Evidence indicates that Thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone, 30–48%), thymohydroquinone, dithymoquinone, p-cymene (7–15%), carvacrol (6–12%), 4-terpineol (2–7%), t-anethol (1–4%), sesquiterpene longifolene (1–8%), α -pinene and thymol, are the most important active ingredients in NS [30].

Pharmacological and biological properties of *Nigella sativa*

Accumulated studies have reported a wide range of NS and its bioactive components health benefits. Its anti-bacterial effects

have been reported by acting against gram-positive (*Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria as well as synergic effects in combination with gentamycin and spectinomycin [31]. Also, the black seed NS may enhance T-helper cell (T4), CD4 and increase natural killer (NK) cell activity in humans, which justify the anti-viral effects of NS [31].

Findings from different animal and human models suggest a potent anti-inflammatory property for *Nigella sativa* oil and its active component, Thymoquinone. This activity is due to pro-inflammatory mediators such as IL-1 β , IL-6, TNF- α , IFN- γ , and PGE2 down-regulation along with NO, cyclooxygenase-1 (COX-1) and COX-2, and histone deacetylase (HDAC) production [32]. In addition,

Table 1 Summary of *Nigellasativa* effects on AMPK pathway signaling

References	Disease	Experimental model	Effect	Mechanism of action
Cui et al./2019 [20]	Liver injury	Human Chang liver cells	Hepatoprotective	TQ inhibited JNK, ERK, and P38 phosphorylation induced by acetaminophen
Zhang et al./2018 [11]	Renal cell cancer	Human renal carcinoma cell lines ACHN and 786-O	Anti-metastatic properties, including inhibiting migration, invasion, and EMT was mainly dependent on TQ-induced autophagy	TQ inhibited the metastasis of RCC cells by inducing autophagy via the AMPK/mTOR signaling pathway.
Kou et al./2018 [22]	Renal cell carcinoma	Human renal tubular epithelial cell line, the human RCC cell lines 769-P and 786-O	NS suppress the metastatic phenotype and reversed the epithelial-mesenchymal transition	<i>Nigella sativa</i> (NS) up-regulated phosphorylation levels of LKB1 and AMPK in RCC 769-P and 786-O cell lines. NS mediated high expression of E-cadherin and low expression of Snail, which could be further enhanced by overexpression of LKB1, indicating a critical role of LKB1 in TQ-inhibited EMT of RCC.
Chen et al./2018 [3]	Colon cancer	Human cancer cell lines HepG2, SKOV-3, and SW620	Anti-cancer function	NS inhibited cell growth and induced vacuolization, mild apoptosis, autophagy by mTOR inhibition, promoted autophagy flux and inhibited p-mTOR by AMPK activation in SW620 cells
Velagapudi et al./2017 [23]	Neuroinflammation	BV2 mouse microglia cell line ICLC ATL03001	Anti-inflammatory activity in microglia	Thymoquinone inhibits NOX-mediated ROS generation and activates both AMPK and SIRT1 in BV2 microglia
Yang et al./2016 [21]	Liver injury	LX-2 cells are immortalized human hepatic stellate cell line	Hepatoprotective effect	Hepatoprotective effect of TQ is associated with SIRT1-AMPK signaling targeting HSCs
Pei X et al./2016 [24]	Atherosclerosis	Rat aortic VSMCs	NS prevented atherosclerosis and diabetes complication	TQ inhibited Ang II-induced VSMC proliferation and migration through the AMPK/PPAR- γ /PGC-1 pathway
Acevedo et al./2015 [13]	Diabetes	Diabetic rats	Antidiabetic effect	Activation of AMPK resulted in translocation of glucose transporter 4 (GLUT4) to the plasma membrane which mobilized glucose into the cell
Yuan et al./2014 [14]	Diabetes	HepG2 cells	Anti-hyperglycemic effect	NS increased glucose consumption by liver hepatocytes (HepG2 cells) through activation of AMP-activated protein kinase
Ting Bai et al./2014 [19]	Hepatic fibrosis	Mouse liver tissue	Anti-inflammation and hepatoprotective	NS inhibited TLR4 signaling pathway and activated the LKB1–AMPK signaling pathway.
Haas, M. J. et al./2014 [18]	Hepatic cell	HepG2 hepatoma cells	Regulating cellular metabolism	FOXO proteins are phosphorylated by kinases such as (AKT) and 5'-AMP-activated protein kinase (AMPK) on threonine
Benhaddou-Andaloussi et al./2011 [15]	Diabetes	Diabetes rats	Antidiabetic activity	Activation of the AMPK pathway in skeletal muscle and liver and increased content of Glut4 in skeletal muscle
Benhaddou-Andaloussi et al./2010 [16]	Diabetes	C2C12 myotubes, 3 T3-L1 adipocytes, and H4IIE hepatocytes	Antidiabetic effect	NSE was observed to stimulate PPAR- γ activity
Ali et al./2008 [17]	Diabetes	C2C12 myotubes, hepatocytes H4IIE and 3 T3-L1 adipocytes	Antidiabetic effect	The antidiabetic effect of NS, therefore, could be attributed to an increased sensitivity to insulin and enhancement of the insulin-independent AMPK pathway. That could be related to the inhibitory effects of NS on the mitochondrial respiratory chain.

Abbreviations: **AMPK**; AMP-activated protein kinase, **EMT**; epithelial-mesenchymal transition, **RCC**; Renal carcinoma cells, **NS**; *Nigella sativa*, **LKB1**; Liver kinase B1, **ROS**; Reactive oxygen species, **SIRT1**; Sirtuin1, **TQ**; Thymoquinone, **HSCs**; Hepatic stellate cells, **VSMCs**; Vascular Smooth Muscle Cell, **PGC-1**; Proliferator-Activated Receptor-Gamma Coactivator-1, **GLUT-4**; Glucose transporter type 4, **TLR4**; Toll-like receptor 4, **FOXO**; Forkhead box protein O1, **PPAR**; Peroxisome Proliferator-Activated Receptor

Thymoquinone has shown potential radical scavenging and antioxidant effects by improving the oxidative status and boosting immune system enzymes including, catalase (CAT), lipid peroxidase (LPO), glutathione (GSH) and nitric oxide (NO) [33, 34].

Toxicological properties of *Nigella sativa*

There is no evidence of any change in liver enzymes function or toxicity effects on the liver after 28 days supplementation with 1 g/kg *Nigella sativa* [35]. Also, the toxicity of NS fixed oil has been assessed in mice and rats by determining LD50 values and the possibility of hematological, histopathological, and chemical changes. Obtained results have demonstrated no change in vital hepatic enzymes, including alanine transaminase (ALT), Aspartate transaminase (AST), and histopathological modification (kidney, liver, pancreas, heart) in treated animals [36]. However, in contrast with a significant increase in hematocrit and hemoglobin, there was a substantial decrease in the leukocyte and platelets counts. Overall, none of the human studies have reported any side effects or toxicity of *Nigella sativa* and its constituents; however, a fall in leukocytes and platelets must be taken into consideration while supplementing large doses of NS and its constituents [25, 26].

Discussion

AMPK is an energy-sensing enzyme that plays a vital role in the regulation of several metabolic and cellular processes. Accumulating data proposes that targeting the AMPK pathway could exhibit useful benefits in the treatment of diseases such as type 2 diabetes, cancer, hypertension, and Atherosclerosis.

Cancer

The effects of NS and its component, Thymoquinone (TQ), have been investigated in various cancers [37, 38]. It has been suggested that *Nigella sativa* plays an inhibitory role in some signaling pathways and molecular mechanisms that involve in initiation and progression of different types of cancers. Among known mechanisms leading to autophagy, the AMPK/mTOR consider as a critical pathway [39–41], which positively regulates AMPK phosphorylation and inhibits mTOR phosphorylation. mTOR consists of two different subtypes, including mTORC1 and mTORC2, which of these mTORC1, but not mTORC2, demonstrates an essential role in boosting autophagy [40, 42, 43]. In this regard, Zhang et al. in an in-vivo study on rats, showed anti-metastatic properties of TQ in renal carcinoma cells (RCC). In fact, the possible mechanism of autophagy induction by TQ could be

through up-regulating AMPK expression while reducing mTORC1 and p-S6K expression, which leads to the potential RCC metastasis suppression [11].

Liver kinase B1 (LKB1), a regulatory protein in adjusting cells energy balance, in combination with AMPK known as LKB1/AMPK signaling pathway, is a malignant tumor progression pathway which phosphorylates APMK at the Thr172 site. Furthermore, LKB1 can act as a significant tumor suppressor by inducing cell cycle arrest, autophagy, and apoptosis. A study by Bo Kou et al. (2018) confirmed that TQ mediates malignant cells apoptosis as well as inhibiting tumor invasiveness in RCC through increasing LKB1 and AMPK phosphorylation without changing their concentrations [22].

Diabetes

Nigella sativa is known to have antidiabetic effects [6]; therefore, its effects on AMPK pathway in diabetes has been investigated in several studies [44]. Benhaddou-Andaloussi et al. indicated the activating the role of *Nigella sativa* Essential oil (NSE) on the AMPK pathway in an in-vivo study, which in turn leads to gluconeogenesis and Acetyl-CoA carboxylase (ACC) activities reduction in the liver. This reduction, particularly in ACC, would be along with fatty acid oxidation elevation and higher insulin sensitivity. Also, Meddah et al. were the first who indicated that NS could inhibit glucose intestinal absorption in-vitro [45].

Additionally, NSE exerted an insulin-sensitizing action by increasing translocation and synthesis of Glut4, which plays a vital role in controlling hyperglycemia in skeletal muscles [15]. In another study, Benhaddou-Andaloussi [16] investigated the role of NSE on three different cell lines, including C2C12 muscle, 3 T3-L1 adipocytes, and H4IIE hepatocytes cells. Adipocyte cells did not show any significant increase neither in Akt nor ERK1/2. However, C2C12 cells treated with NS showed an increase in P-Akt and ERK1/2 concentration, while H4IIE cells displayed an enhancement in Akt phosphorylation but not the ERK1/2. The content of AMPK and its substrate ACC increased when treated with NS in muscle and hepatocyte cells, but not in adipocytes.

Moreover, it has been observed that NSE acts like an agonist for Peroxisome Proliferator-Activated Receptor PPAR- γ and improves its activity by 50% in Cell line [16]. In an in-vivo study Acevedo et al. [13] investigated the involved molecular mechanism in glucose transporter regulation. Rats were fed with NS and Thymoquinone (TQ) fortified diet for four months, which resulted in a significant increase in AMPK phosphorylation and Glut4 protein content. However, they did not report any evidence of Akt phosphorylation activation neither in NS nor the TQ group [13].

Hepatic disorders

Numerous studies have suggested the crucial role of AMPK on glucose and lipid metabolism regulation in hepatic cells [46–48]. Besides, researchers have investigated *Nigella saliv*'s effect on AMPK in the liver. For instance, Ting Bai et al. study (2014) demonstrated that the anti-fibrotic effect of TQ is due to phosphorylation of LKB1 which mediates AMPK expression [19].

Furthermore, mTOR acts as a regulator of cell growth and protein synthesis. It has been suggested that AMPK may phosphorylate the mTOR subgroup, which leads to their inactivation. Therefore, NS may lower liver fibrosis progression by inhibiting mTOR activity while inducing the expression of the AMPK gene. In another study, Yong Yang et al. (2016), in an in-vitro study in hepatic stellate cells (HSC), induced alcoholic liver disease (ALD) by using ethanol, which resulted in AMPK inhibition and as a consequence ACC stimulation and lipid accumulation [21]. Interestingly, an improvement was observed in LKB1 suppression and AMPK activation, after treating HSCs with TQ for Besides, TQ significantly increase Sirtuin1 (SIRT1) and inhibit TGF-B signaling. Any

disruption in the SIRT1/AMPK axis may lead to fat accumulation and inflammatory response.

Moreover, this finding showed that PPAR- γ activation by the LKB1-AMPK pathway might demonstrate another hepatoprotective effect of TQ [21]. Also, after the induction of liver injury by acetaminophen (APAP), JNK phosphorylation raised and AMPK phosphorylation diminished. As expected, TQ treatment showed a hepatoprotective effect by a considerable reduction in JNK phosphorylation and activation of the AMPK pathway [20].

Atherosclerosis

The role of NS in Atherosclerosis has been investigated for many years. Pei et al. conducted a study to understand the mechanism of NS on Atherosclerosis [24]. Many cancer studies indicated that AMPK and PPAR- γ could have anti-proliferative and anti-tumor effects [49–51]. Consistent with this study's results, TQ can increase AMPK and PPAR- γ activation through the cell cycle progression, leading to lower VSMCs proliferation and inhibition of the progression of Atherosclerosis [24].

Fig. 2 The possible mechanism for the effect of *Nigella sativa* in preventing disease by targeting the AMPK pathway. **Abbreviations:** ACC; Acetyl-CoA carboxylase, PPAR; Peroxisome Proliferator-Activated Receptor, SIRT1; Sirtuin1, NF- κ B; nuclear factor kappa-light-chain-enhancer of activated B, AMPK; AMP-activated protein

kinase, mTOR; mammalian target of rapamycin, PGC-1; Proliferator-Activated Receptor-Gamma Coactivator-1, ROS; Reactive oxygen species, VSMCs; Vascular Smooth Muscle Cell, AKT; protein kinase B, GLUT-4; Glucose transporter type 4, NS; *Nigella sativa*

Neuro-inflammation

Inflammation is a normal physiological response to stimulators, which activate the immune cells to destroy pathogens and repair the damage [52]. However, a wide range of disorders like cardiovascular diseases and multiple sclerosis could be initiated in case of an intense or uncontrolled chronic inflammation [53]. Several studies have indicated that AMPK is involved in regulating inflammation in different types of cells [40, 54]. Moreover, evidence supports the effect of *Nigella sativa* on reducing inflammatory cytokines and its participation in modulating inflammatory pathways [7, 55]. Velagapudiet et al. (2017) examined possible neuroprotective effects of *Nigella sativa* on the molecular level in BV2 microglial cells [23]. This study showed significant accumulation of SIRT1 in treated cells by TQ, which may result in the activation of AMPK. Moreover, it has been indicated that TQ can block LPS-induced nuclear factor kappa-light-chain-enhancer of activated B (NF- κ B) activation through the SIRT1/AMPK pathway [23].

The potential effects of *Nigella sativa* on AMPK activation in diseases are summarized in Fig. 2.

Future directions

Activating the AMPK pathway may lead to increased cell growth and proliferation, which is thought to be a paradox in cancer, since treatment with AMPK activators may result in tumoral cells proliferation [56]. Therefore, more studies are needed on the evaluation of *Nigella sativa* effects on the AMPK pathway in cancer. Additionally, AMPK can induce phosphorylation of tumor suppressor p53 [40]. Thus, examining the effect of *Nigella sativa* on p53 phosphorylation may be considered as a novel approach for future studies.

Previous studies have suggested the possibility of AMPK pathway interaction with SIRT1 and (FGF-21), which may lead to an increase in proliferator-activated receptor- γ coactivator-1) PGC-1 α) gene expression and enhancement of mitochondrial function and improve *GLUT4* translocation to the cell membrane [57]. Moreover, the AMPK pathway activation in the liver leads to suppression of Forkhead transcription factor FKHR (Foxo1), an increase of gluconeogenic enzyme gene expressions, and lipogenic enzymes genes down-regulation [58]. Therefore, the evaluation of the impacts of *Nigella sativa* on AMPK signaling pathways is recommended for the future studies.

Conclusion

Nigella sativa and its component, Thymoquinone, have positive effects on cancer, diabetes, Hepatic disorders, Atherosclerosis, and Neuro-inflammation prevention, which may explain by the

AMPK pathway. NS, by increasing the expression/ phosphorylation of AMPK and reversibly regulating expression/ phosphorylation of mTORC2, plays an essential role in apoptosis, inhabitation of tumor progression, and suppress the metastasis, especially in renal carcinoma cells. In collaboration with AMPK, NS and TQ have regulatory effects on p-S6K expression and Liver kinase B1 activity, which are involved in NS anti-cancer effects. The glucose-lowering effect of NS and its derivatives can be explained by the reduction of ACC activity via the AMPK pathway, which increases fatty acid oxidation and insulin sensitivity. The effect of NS on AMPK and PPAR- γ activation can explain its inhibitory effects on the progression of Atherosclerosis and proliferation of tumors. In fact, mTOR activity inhibition induces AMPK gene up-regulation and PPAR- γ activation, which could be associated with the LKB1-AMPK pathway and hepatoprotective effects. The SIRT1/AMPK pathway is an important way in the anti neuro-inflammation effect of TQ by blocking NF- κ B activation.

In general, By this study, we provide detailed information about NS supplementation, improving role on significantly lower lipid accumulation, AMPK phosphorylation, PPAR α up-regulation, and lipogenic genes down-regulation. This suggests that NS may have protective effects against hepatic disorders and diabetes. Also, NS mediates higher autophagy through boosting AMPK phosphorylation and attenuating mTOR phosphorylation resulting in a lower risk of different cancers. AMPK activation also in the liver leads to the stimulation of fatty acid oxidation and inhibition of lipogenesis and glucose production and may play a major role in the regulation of glucose and lipid metabolism through its effects on energy metabolism as a suggestion NS administration leads to AMPK activation in the liver, which in turn decreases hepatic lipid metabolism. Overall, regardless of recent advances in the mechanistic understanding of AMPK activation by NS and its component, numerous critical questions still remained to be answered.

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Compliance with ethical standards

Competing interests All of the authors declared no personal or financial conflicts of interest.

Consent for publication Not applicable.

Abbreviations ACC, Acetyl-CoA carboxylase; AKT, protein kinase B; ALD, alcoholic liver disease; AMPK, AMP-activated protein kinase; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinases; FOXO, Forkhead box protein O1; GLUT-4, Glucose transporter type 4; HSCs, Hepatic stellate cells; LKB1, Liver kinase B1; mTOR, mammalian target of rapamycin; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B; NS, *Nigella sativa*; PGC-1, Proliferator-Activated Receptor-Gamma Coactivator-1; PPAR, Peroxisome Proliferator-Activated Receptor; RCC, Renal carcinoma cells; ROS, Reactive oxygen species; SIRT1, Sirtuin1; TGF- β , Transforming growth factor-beta; TLR4, Toll-like receptor 4; TQ, Thymoquinone; VSMCs, Vascular Smooth Muscle Cell

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