THE THERAPEUTIC POTENTIAL OF *NIGELLA SATIVA* IN THE INHIBITION OF ADVANCED GLYCATION END PRODUCT (AGE) FORMATION: A SYSTEMATIC REVIEW

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ABSTRACT

Introduction: The purpose of this paper is to systematically review the therapeutic potential of *Nigella sativa* in the inhibition of AGE formation and its properties that regulate AGE level. **Methods:** The selected studies consist of interventions towards in vitro samples from blood origin and compared AGE inhibition between treated and untreated control groups. Six databases were searched for related articles using keywords derived using PICO format. The PRISMA guidelines were used for the purpose of standardizing systematic review writing. **Results:** Six articles were accepted as meeting the inclusion criterion and were reviewed for the effectiveness of *Nigella sativa* in AGE inhibition. All studies showed positive results. These studies demonstrated significant and dose dependent activity of *Nigella sativa* in inhibiting AGE formation, presenting significant results even at lower concentrations. Thymoquinone was consistently identified as the component associated with the antiglycating effect of *Nigella sativa*. **Conclusion:** This review provides evidence for the decreased formation of AGEs in the presence of Nigella sativa proposing its use as a natural antiglycating drug and its effectiveness in the treatment of diabetes, its complications and other AGE-related diseases.

KEYWORDS: *Nigella sativa*, Thymoquinone, Black seed, Advanced glycation end products, Glycation and Glycation inhibition

INTRODUCTION:

Obesity that is typically caused by overeating and physical inactivity has been long known to be one of the major causes of developing chronic diseases that can lead to death. Recent findings have suggested that advanced glycation end products may also be a contributing factor affecting metabolic health (Uribarri et al., 2015). Advanced glycation end products (AGEs) are formed when a reducing sugar binds to a free amino acid group of proteins, lipids, or nucleic acid (Uribarri et al., 2010). AGEs are

harmful compounds also called glycotoxins which accumulate in the body endogenously and exogenously as age increase. Recent studies have given attention to AGE as a factor in aging as well as its pathogenic effect on metabolic health (Chaudhuri et al., 2018). High accumulation of AGE in cells may contribute to the development of diseases and induce different complications in diseases such as diabetes, cardiovascular disease and neurodegenerative disease. Conditions such as hyperglycemia and oxidative stress increase the production of endogenous AGEs which further builds up in the body (Abate et al., 2015).

Many compounds of both synthetic and natural have been researched and tested in efforts of finding inhibitors to prevent AGE accumulation. Synthetic or pharmacological AGE inhibitors have been found to give adverse side effects (Chetan et al., 2015). *Nigella sativa* is a widely used therapeutic herb throughout the world. It is more commonly known as black seed and is rich with historical and religious background for its curative potential, particularly in Islamic literature. The seeds and oils of *Nigella sativa* have been used in the treatment of various diseases and ailments. It is also regarded as the top ranked evidence based herbal medicine (Aftab et al., 2013). Among the pharmalogical actions of *Nigella sativa* include antidiabetic, anticancer, antimicrobial, anti-inflammatory, hepato-protective, renal protective, gastro-protective and antioxidant properties (Aftab et al., 2013). According to Rashmi, Dinesh and Ahmad (2018), *Nigella sativ*a possesses inhibitory roles in the process of glycation of proteins and DNA, and has shown to significantly decrease AGE production *in vitro*. Thus, there is a need for expanding research to identify natural inhibitors such as *Nigella sativa* capable of suppressing AGE formation as effective as and if not more effective than synthetic inhibitors. This review aims to address the research question does treatment with *Nigella sativa in vitro* effectively inhibit AGE formation compared to negative controls (no treatment)? Therefore, information regarding the therapeutic potential of *Nigella sativa* on inhibition of AGE formation and the properties of *Nigella sativa* that contributes in AGE level regulation is reviewed and discussed.

METHODS:

Systematic review process

The systematic review methodology follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009). There are four main components in the PRISMA guidelines that include identification of the sources of the journal articles, screening of the article based on title, eligibility based on the inclusion criterion and screening to identify studies for further analysis.

Search strategy

Six online databases were used to obtain peer reviewed articles namely Science Direct, Scopus, Pubmed, SAGE Journals, Cochrane Library, and Google Scholar. Additional sources were also obtained from manual searches through reference list of obtained articles. The search strategy used in this study to obtain as many relevant studies pertaining to the topic is based on the PICO elements, Population (P), Intervention (I), Comparator (C), and Outcome (O). Individual key search terms were entered for each database combining them with Boolean operators as relevant. The combination of keywords used were "glycation" AND "*Nigella sativa*" OR "thymoquinone" OR "black seed", "advanced glycation end product" AND "*Nigella sativa*" OR "thymoquinone" OR "black seed" and "glycation inhibition" AND "*Nigella sativa*" OR "thymoquinone" OR "black seed".

Inclusion criteria

Studies were selected if they targeted interventions towards *in vitro* samples from blood origin, and compared AGE inhibition between treated and untreated control groups. To be included, studies have to be peer reviewed articles published in English starting January 2010 until December 2018. Studies were excluded if they did not evaluate *Nigella sativa* on any AGE formation viewpoint.

Methods of the review

Titles and abstracts were screened to identify articles potentially meeting the inclusion criteria. For those articles, full text versions were retrieved and screened to determine whether they met inclusion criteria and answer the research question. Screening process was repeated three times.

Collating, summarizing, and reporting results

An evidence table was used to collect data of relevant study information for articles meeting inclusion criteria. Information such as the author's name, type of sample, type of study design, publication year and summarized results were presented in table 1 as adopted from Guidelines for systematic reviews by The American Occupational Therapy Association (Richards & Polk, 2017)

Assessment of level of evidence

The strength of the findings in relation to study design is determined using the level of evidence of each study. Although there can be study limitations at all levels, results from a Level I, II or III study will provide stronger evidence than results from Levels IV or V. Levels of evidence for the included studies were determined using descriptions set by Sackett, Rosenberg, Gray, Haynes & Richardson (1996).

Risk of bias across studies

The risk of bias was assessed using the Cochranes risk of bias assessment tool (Higgins & Green, 2011). In addition, the National Toxicology Program was also used as a reference to guide risk of bias assessment for *in vitro* studies (Rooney, 2015). Based on the reference, the risk of bias approach was extended from experimental animal to address *in vitro* studies. In total, there are seven domains for risk of bias assessment: (1) Random sequence generation; (2) Allocation concealment; (3) Blinding of participants and personnel; (4) Blinding of outcome assessment; (5) Incomplete outcome data; (6) Selective reporting and (7) Other bias. Cochrane risk of bias tool was used to define each domain and to assess for judgement (high, low or unclear) for individual elements of each domain.

RESULTS

Database screening and article selection

The article selection process is outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram (Fig. 1). The initial search identified a total of 1231 references. After removing duplicates using EndNote X8 software, 630 articles were identified. After scanning the titles and reviewing complete abstracts of these studies, 42 articles were accepted for further screening including 1 article identified when screening bibliographies of relevant published articles in the field. After examination of full text articles, 6 intervention studies were included in the review. Common reasons for exclusion were that the studies measured blood glucose level as an indicator of AGE inhibition by *Nigella sativa* and the effectiveness of *Nigella sativa* on AGE inhibition was not evaluated.

Of the 42 full text articles examined, there were 9 purely irrelevant articles which were not significant to this review. There was 1 thesis and 5 review papers excluded because they were also not relevant to this review topic. Meanwhile, 17 articles were rejected because they did not evaluate *Nigella sativa* on any AGE formation viewpoint. In addition, 2 studies compared the effectiveness of *Nigella sativa* with other therapeutic herbs such as *Molinga olifera* and a group of other natural extracts, which did not meet the inclusion criteria. Hence, it did not answer the research question of this review. One article which did not evaluate the effectiveness of *Nigella sativa* in AGE formation inhibition was rejected. The paper discussed the therapeutic effects of *Nigella sativa* on superoxide dismutase (SOD) activity influenced by AGE formation, therefore the direct effects of *Nigella sativa* as an intervention in AGE formation inhibition was not determined.

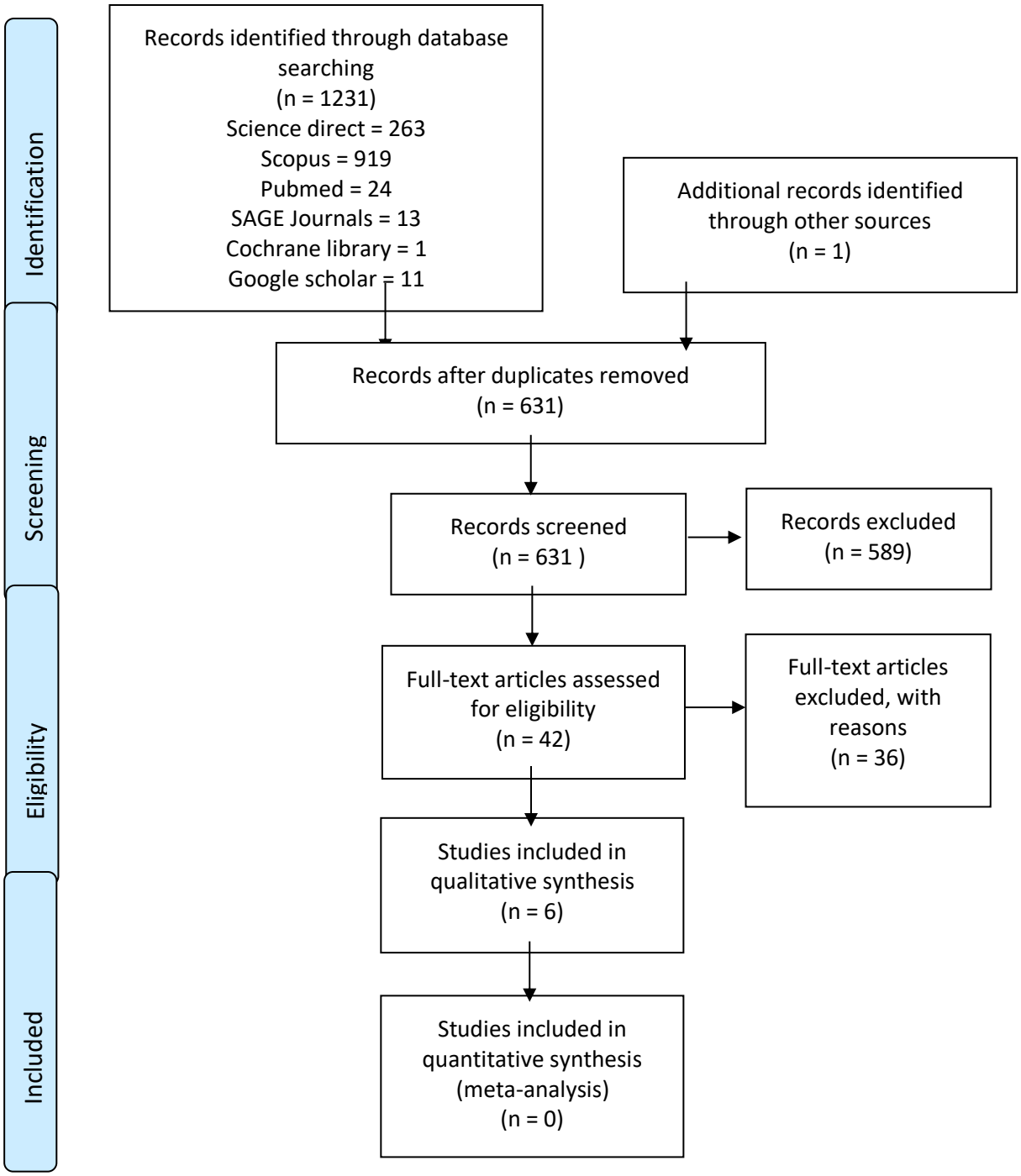

Figure 1: PRISMA Flow Diagram

Description of included studies

Six studies on the efficacy of *Nigella sativa* were reviewed. The details of included studies that investigated AGE inhibiting effects of *Nigella sativa* are summarized in Table 1. Studies were experimented on samples from blood origin to identify the therapeutic potential of *Nigella sativa* on AGE formation. Among the 6 studies, 1 study (1) targeted bovine erythrocytes, 2 studies (2,5) tested on bovine serum albumin, 1 study (4) tested on human serum albumin, 1 study (6) focused on human plasma and 1 study (3) experimented on human plasma, human serum albumin and bovine serum albumin. All 6 studies were experimental designs with presence of different concentrations of *Nigella sativa* as interventions and absence of *Nigella sativa* as controls. All studies used different experimental assays to evaluate AGE formation inhibition except for 2 studies (1,4) which used AGE-specific-fluorescence studies to measure AGE formation. In addition, 3 (1,2,3) of 6 studies utilized thymoquinone as an intervention in AGE inhibition while another 3 studies (4,5,6) evaluated *Nigella sativa* seed extract as the intervention in the study.

Table 1: Summary of findings on *Nigella sativa* as intervention in AGE inhibition

Author/ Year	Level of Evidence/Study Design/Sample	Intervention and Control Groups	Results
STUDY 1 **Anwar, Khan, Sadaf, & Younus (2014)** **10.1016/j.ijbiomac.2014.06.003**	Level II Experimental Design Bovine erythrocyte	*Intervention* SOD incubated with 0.5 M glucose or 10mM methylgloxal (MG) or combination of 0.5 M glucose & 10mM MG in presence of 0,10,20,50 mM TQ. *Control* Absence of TQ	**AGE fluorescence studies** A progressive decrease in AGE-specific fluorescence (formation of AGEs) at 450 nm with increasing TQ concentration was observed in all three cases. The control exhibited insignificant decrease in fluorescence at 450 nm. Therefore, TQ protected the enzyme to some extent against formation of AGEs induced by glycation with glucose, MG or a combination of both, and the protection increased with increasing concentration of TQ ($p < 0.05$). *Properties of Nigella sativa responsible for AGE inhibition*: TQ as antiglycating agent.
STUDY 2 **Benvidi, Rezaeinasab, Gharaghani, & Abbasi (2018)** **10.1016/j.ijbiomac.2017.10.135**	Level II Experimental Design Bovine serum albumin (BSA)	*Intervention* BSA (10 mg/ml) was simultaneously incubated with 100 mg/ml of glucose containing 0.02% NaN3 in presence of TQ at concentration of 0.0, 1.0, 5.0, and 10.0 mg mL^{-1W}. *Control* Solutions including BSA	**DPV experiments** When the concentration of TQ was increased, formation of AGE products was extremely decreased and minor increase in the current peaks was seen in the case of TQ 10 mg mL^{-1}($p < 0.05$). **UV-Vis experiments** Dose-dependent inhibition of BSA glycation ($p < 0.05$). *Properties of Nigella sativa responsible for AGE inhibition*: Thymoquinone

Author/ Year	Level of Evidence/Study Design/Sample	Intervention and Control Groups	Results
		and BSA with glucose without test molecules (TQ).	
STUDY 3 **Losso, Bawadi, & Chintalapati (2011)** **10.1016/j.foodchem.2011.02.076**	Level II Experimental Design Human blood samples Human serum albumin (HSA) Bovine serum albumin (BSA)	**Hemoglobin-δ-glucanolactone** *Intervention* 400 µl of blood mixed with 80 µl of 0.2 M PBS containing 50mM of δ-Glu and 0-20 µM of TQ. *Control* Absence of TQ.	**Hemoglobin-δ-glucanolactone** The mixtures containing Hemoglobin-δ-glu-thymoquinone showed dose-dependent inhibition of AGE products formation. At a concentration of 20 µM of thymoquinone strongly inhibited and reduced the formation of early AGE products formation by about 39% ($p < 0.01$).
		HSA glucose assay *Intervention* HSA (50 mg/ml) was simultaneously incubated with 144 mg/ml of glucose in 50mM PBS (pH 7.4) containing 0.02% NaN3 in the presence of 0-20 µM thymoquinone. *Control* HSA and the test compound but without glucose.	**HSA glucose assay** When thymoquinone was incubated with HSA before addition of glucose, thymoquinone inhibited 82% of post-Amadori glycation and AGE product formation ($p < 0.01$).

Author/ Year	Level of Evidence/Study Design/Sample	Intervention and Control Groups	Results
		Interaction of TQ and MGO *Intervention* HSA incubated with 100mM MGO in presence of 0-20 µM TQ in 0.1M phosphate buffer. *Control* Absence of TQ. **GK peptide-ribose assay** *Intervention* GK peptide (80mg/ml) in 0.1 ml of sodium phosphate buffer containing 0.02% NaN3 at pH 7.4 mixed with 0.1 ml of 120 mg/ml ribose in 0.5 M sodium phosphate buffer and TQ at 10- 20 µM. *Control* Mixture of GK and ribose.	**Interaction of TQ and MGO** Thymoquinone at 10µM or 20µM significantly inhibited 59% and 68% of MGO-catalyzed HSA glycation, respectively ($p < 0.01$). **GK peptide-ribose assay** Thymoquinone at 10µM and 20µM inhibited 66% and 78% of late glycation end products respectively ($p < 0.01$). *Properties of Nigella sativa responsible for AGE inhibition*: TQ reduce absorption of potent carbonyl species present in food derived AGEs.

Author/ Year	Level of Evidence/Study Design/Sample	Intervention and Control Groups	Results
STUDY 4 **Mahmood, Moin, Faizy, Naseem, & Aman (2013)** **10.15373/22778179**	Level II Experimental Design Human serum albumin (HSA)	*Intervention* HSA in concentration of 1 mg/ml, glucose in concentration of 50mM and the ethanolic extract of *Nigella sativa* seeds in concentrations of 0.25, 0.5, 0.75 and 1 mg/ml was dissolved in PBS *Control* Absence of *Nigella sativa* extract	**AGE-specific fluorescence** A concentration dependent decrease in AGE fluorescence was observed in HSA samples that were incubated with 4 concentrations (0.25, 0.5, 0.75, 1 mg/ml) of ethanolic extract of *Nigella sativa* seeds ($p< 0.05$). **SDS-PAGE of HSA** Electrophoretic pattern of glycated HSA treated with ethanolic extract of *Nigella sativa* at concentrations of 0.25,0.5,0.75,1.0 showed a reduction in the intensity and broadening of bands showing a decrease in glycation and hence cross linkage and aggregate formation. This inhibitory effect was seen in a dose dependent manner ($p< 0.05$). *Properties of Nigella sativa responsible for AGE inhibition*: Antioxidant activity of polyphenols, tocopherols and thymoquinone.
STUDY 5 **Rashmi, Dinesh, & Ahmad (2018)**	Level II Experimental Design Bovine serum albumin (BSA)	*Intervention* Aqueous solution of BSA (10mg/ml) was incubated with fructose (100mg/ml) and with *Nigella sativa* seed extracts (1mg/ml) in 0.1M phosphate buffer.	**Effect of *Nigella sativa* on browning:** Both the extracts K1 and K2 caused significant decrease in the browning of BSA by fructose ($p< 0.05$). However, K2 caused more inhibition (47.96%) of glycation as compared to K1 (34.94%). **Effect of *Nigella sativa* seed extracts on fructosamine:** K2 (39.42%) was more

Author/ Year	Level of Evidence/Study Design/Sample	Intervention and Control Groups	Results
		K1- methanol as solvent K2-water as solvent *Control* Without seed extract	potent in preventing the formation of fructosamines as compared to K1 (14.59%) with respect to control. **Effect of *Nigella sativa* seed extracts on the carbonyl content:** Carbonyl content was significantly reduced by 58.28% in K1 and 57.06% in K2 as compared to glycated BSA ($p< 0.05$). **Effect of *Nigella sativa* seed extracts on Protein Aggregation Index:** A very significant reduction of amyloid cross-ß structure in presence of K1 and K2 ($p< 0.05$). *Properties of Nigella sativa responsible for AGE inhibition*: Not mentioned
STUDY 6 **Hira, Fatma, Shoaib & Riffat (2013)**	Level II Experimental Design Human plasma	*Intervention* Plasma samples were incubated with glucose (5.5, 25 and 50mM) and *Nigella sativa* extracts (50, 250, 500 mg/ml) in phosphate buffer saline (PBS). *Control* Normal plasma incubated with 5.5, 25 and 50mM glucose.	**Glycation analysis** NEG was significantly reduced when 500 mg/ml *Nigella sativa* concentration was used compared to controls. ($p< 0.05$) Decline in NEG remained significant with diluted inhibitor concentrations. ($p< 0.05$) *Properties of Nigella sativa responsible for AGE inhibition*: Due to different phenolic components.

Note. [TQ = Thymoquinone; SOD = Superoxide dismutase; AGE = Advanced glycation end product; DPV = Differential pulse voltammetry; UV-Vis = UV-Vis spectrophotometry; PBS = Phosphate buffer saline; MG = Methylglyoxal; δ-Glu = Hemoglobin- δ-gluconolactone; G-K peptide = N-acetyl-glycyl-lysine methyl ester; SDS-PAGE = Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis; NEG = Non enzymatic glycation]

Results extracted

AGE in plasma or serum albumin decreased in different clinical circumstances with *Nigella sativa* in all of the studies. A consistent result demonstrating statistically significant and dose-dependent activity of *Nigella sativa* towards AGE formation inhibition was reported in 5 articles (1,2,3,4,6). A significant but not dose-dependent result was observed by Rashmi, Dinesh and Ahmad (2018) due to samples only investigated at a single concentration. Furthermore, 3 (1,2,3) studies that utilized thymoquinone as the intervention recognized thymoquinone, the most abundant phytochemical found in *Nigella sativa*, as a contributor to the inhibitory activity of *Nigella sativa*. The other 3 (4,5,6) articles commended *Nigella sativa* seed extract for their positive results. From the three articles, 2 (4,6) had also suggested thymoquinone as the potential component responsible for the antiglycating effects of *Nigella sativa*. The remaining study (5) did not propose any components relating to this. The mechanism of AGE inhibiting *Nigella sativa* was also identified in 2 studies (2,4) proposing thymoquinone's ligand like and antioxidant property as the causal factor to its therapeutic effect. Moreover, all of the studies acknowledge the use of *Nigella sativa* in reducing AGE formation as a promising property in the management and prevention of diabetic complications.

DISCUSSION

The aim of the review is to identify the therapeutic potential of *Nigella sativa* in the inhibition of AGE formation and investigate its properties that regulate AGE level. The review found two prominent findings that were directed to answer the research question of this review. These findings were the reduction of AGE in the presence of *Nigella sativa* and thymoquinone as *Nigella sativa*'s component responsible for this role. The review also found four general findings from all the six included papers. Firstly, the review found that AGE formation in samples treated with *Nigella sativa* was significantly lower than untreated samples and a dose-dependent activity was observed. Higher concentrations display extremely decreased AGE formation, nevertheless more diluted concentrations showed significant effective inhibitory potential. The non-enzymatic reaction to form AGE is subdivided into three main stages. The early, mid and late stage involves formation of unstable Schiff base which rearranges into amadori product, degradation of amadori product to a variety of reactive dicarbonyl compounds and the formation of irreversible AGEs respectively (Singh, Bali, Singh & Jaggi, 2014). Two (3,5) of 6 studies experimented on *Nigella sativa's* inhibitory potential at different stages of AGE production. Losso, Bawadi and Chintalapati (2011) and Rashmi, Dinesh and Ahmad (2018), recorded thymoquinone and *Nigella sativa* seed extract respectively, to significantly reduce early and late AGE formation. Moreover, 1 study (3) also demonstrated strong inhibition of mid stage advanced glycation end product formation. Therefore, the therapeutic effect of *Nigella sativa* targets all stage of AGE production formation.

The second finding recognizes thymoquinone as the ingredient that regulates AGE level. Three studies (1,2,3) investigated *Nigella sativa*'s inhibitory potential by using thymoquinone as their intervention, while 3 (4,5,6) other articles experimented on *Nigella sativa* seed extract in their studies. According to Losso, Bawadi and Chintalapati (2011), thymoquinone may reduce the absorption of potent carbonyl species found in food-derived AGEs. These toxic compounds can induce oxidative stress and promote inflammatory diseases. Mahmood, Moin, Faizy, Naseem and Aman (2013) and Hira, Fatma, Shoaib and Riffat (2013) suggested the antioxidant activity of thymoquinone and the presence of different phenolic compounds in *Nigella sativa* respectively, as contributors to the AGE limiting property of *Nigella sativa*. Furthermore, Rashmi, Dinesh and Ahmad (2018) identified that aqueous extracts of *Nigella sativa* exhibited better AGE inhibiting potential compared to the methanolic extract.

The third finding reports the mechanism responsible for the antiglycating effects of *Nigella sativa*. There were only 2 (2,4) studies that described the possible mechanism. Benvidi, Rezaeinasab, Gharaghani and Abbasi (2018) suggested the attachment of ligand like thymoquinone to amino groups of BSA to promote inhibition of glycation and formation of Schiff base and AGEs. On the other hand, Mahmood, Moin, Faizy, Naseem and Aman (2013) explained the antiglycating activity of *Nigella sativa* in relation to its antioxidant activity. *Nigella sativa* prevents autoxidation of glucose which would ultimately inhibit formation of ketoamines involved in AGE formation, hence preventing AGE production early on in the process. On the other hand, among the 6 studies, 4 (1,3,5,6) did not discuss or mention the mechanism related to antiglycating properties of *Nigella sativa*. The mechanism involved in AGE inhibition by *Nigella sativa* is still not fully understood and in fact requires more in-depth research to discover its comprehensive mechanism.

The forth finding presented the therapeutic use of *Nigella sativa* in disease management. In the 6 experimental studies, blood samples were incubated with sugar molecules to induce glycation. Five (1,2,3,4,6) of 6 studies used glucose as their glycating agent, while the remaining 1 (5) used fructose to produce glycated samples. This is for the reason that, hyperglycemic conditions have shown to increase AGE production abundantly. After obtaining positive results, all 6 studies firmly proposed the use of

glycation inhibiting *Nigella sativa* in the treatment of diabetes and its complications. According to Losso, Bawadi and Chintalapati (2011), AGEs in tissues and serum accumulate at an accelerated rate in diabetic patients and play an important role in diabetic complications, age-related cardiovascular disease and osteoarthritis. Hence, the identification of *Nigella sativa* as a natural AGE inhibitor can be developed as a natural drug in controlling diabetes and preventing other diseases induced or affected by AGE formation.

STRENGTH AND LIMITATION

The strength of this review includes obtaining of related articles from 6 selected databases which increases the possibility of a comprehensive review. This review also provides current and up to date knowledge as it only includes studies since January 2010 up until December 2018. The main limitation of this review is different use of experimental designs between the 6 studies analyzed which limits comparability. The review findings infer results from all studies with regard to positive or negative significant outcomes in spite of their experimental design. The small number of studies and large heterogeneity in study design also means that the conclusions about the effectiveness of *Nigella sativa* as an intervention should be interpreted with caution.

CONCLUSION

In conclusion, this systematic review identifies the therapeutic use of *Nigella sativa* as an AGE inhibitor with the potential to treat diabetes, its complications and other glycation complications. Lastly, this review suggests the role of *Nigella sativa* as a potent antiglycating agent which requires further in-depth research such as the mechanism involved in AGE inhibition and precise dose required to ensure effective treatment before the discovery can be applied in clinical settings.

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