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Anti-diabetic effect of a combination of black seed (*Nigella sativa*) and cumin (*Cuminum cyminum*), a two-step study from bench to bed

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ABSTRACT

Background: Type 2 Diabetes mellitus (T2DM) is a prevalent chronic condition causing one-fifth million deaths globally. Standard treatments for diabetes are not sufficiently effective, highlighting the need to find adjunctive treatments to improve glycemic control. *Nigella sativa* (NS) and *Cuminum cyminum* (CC) are two herbal medicines known for their antioxidant, anti-inflammatory, and antidiabetic properties.

Objective: This study aims to assess the impact of supplementation with a combination of NS and CC on glycemic regulation among individuals with T2DM. The research was conducted in two phases, pre-clinical and clinical.

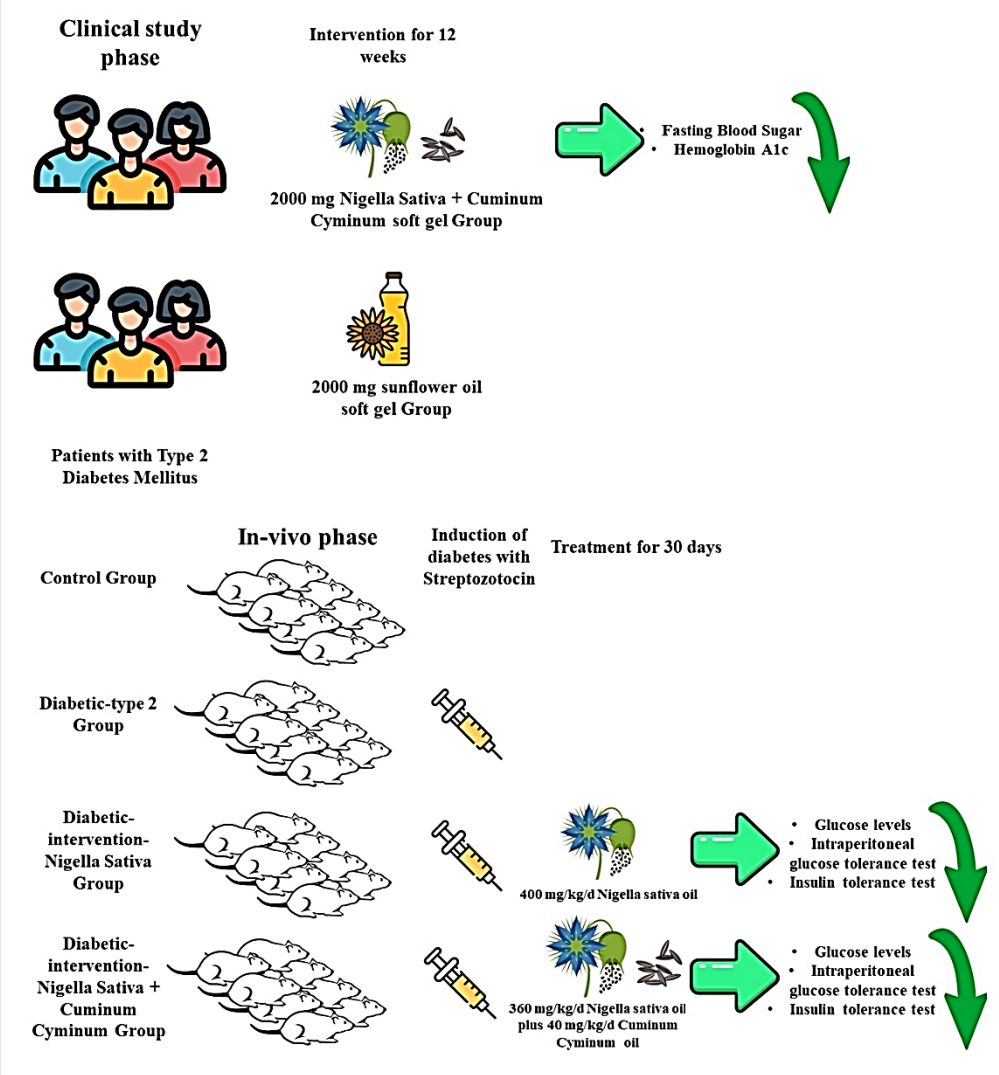
Methods: In the in-vivo phase, thirty-two male mice were allocated into four groups, each comprising eight mice. These groups were categorized as follows: control, diabetic-type 2, diabetic-intervention-NS, and diabetic-intervention-NS+CC. Following the induction of diabetes using Streptozotocin, the mice in NS and NS-CC groups received 400 mg/kg/d or 360 mg/kg/d NS oil plus 40 mg/kg/d CC oil respectively for 30 days. An insulin tolerance test (ITT), an intraperitoneal glucose tolerance test (IPGTT), and blood glucose were conducted subsequently. In the clinical study, eighty patients who have been clinically diagnosed with type 2 diabetes were randomly assigned to two distinct groups to receive a daily dosage

of 2000 mg NS-CC soft gel or sunflower oil soft gel for 12 weeks. The examination of fasting blood glucose (FBG) and hemoglobin A1C (HbA1C) was performed both before and after the completion of the intervention.

Results: Preclinical findings exhibited a notable decrease in glucose levels in NS (decrease from 271.1±21.71 to 167.6±8.14 mg/dl) and NS-CC (decrease from 275.4±25.57 to 136±13.07 mg/dl). Also, ITT and IPGTT levels showed significantly more reduction in NS and NS-CC compared to the non-treated diabetic group. Treating with NS-CC, but not NS alone decreased ITT to a normal level with no significant difference with the non-diabetic group. In phase 2, supplementation with NS-CC led to a significantly greater reduction of the mean of fasting blood sugar (FBS) (P=0.001) concentration and HbA1C (P<0.001) compared to placebo.

Conclusions: NS-CC oil may reduce insulin resistance and blood glucose levels in murine models and individuals with T2DM.

Keywords: *Nigella sativa*, *Cuminum cyminum*, Type 2 diabetes mellitus, Glycemic control, Diabetic mice



INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by high blood sugar levels, increased lipid levels, and reduced insulin levels. This condition results in a decline in both insulin secretion and insulin activity [1]. The prevalence of DM is escalating globally, posing a significant health challenge. According to estimates from the International Diabetes Federation, there are currently 382 million people with diabetes worldwide, a number projected to increase to six hundred million by 2035 [2]. In the USA, the prevalence of prediabetes and diabetes among persons over the age of eighteen is 34.5 and 13.0%, respectively [3]. According to studies, people with diabetes are more likely to die, have a stroke, develop atherosclerosis, and experience cardiovascular events [4-5]. Numerous academic studies have demonstrated how diabetes lowers people's quality of life by increasing their risk of serious consequences like stroke, amputation, renal failure, and blindness, which increase morbidity and early death [6]. Recognizing a lack of comprehensive research on the efficacy and safety of botanicals with potential therapeutic effects, the World Health Organization (WHO) has highlighted the need for more investigation. This concern stems from the potential cardiovascular risks associated with certain anti-diabetic medications and the widespread use of medicinal herbs among individuals seeking alternative treatments [7]. The first line of diabetes treatment is blood glucose management which includes insulin therapy, exercise, and the use of hypoglycemic medicine [8]. A study conducted in England found that after using hypoglycemic medications for ten years, patients must begin insulin therapy [9]. Recently, there has been an emerging fascination with the application of plant-derived commodities for chronic disease prevention via appropriate nourishment within a novel realm of nutritional science known as Functional Food Science. The Functional Food Committee (FFC) has outlined a definition for functional foods (FFs) as “Natural or processed foods containing biologically active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms'' [10]. Collaborating with the Academic Society of Functional Foods and Bioactive Compounds (ASFFBC), as well as regulatory bodies such as the FDA and other governmental agencies, the FFC has made efforts to establish precise definitions and classifications for Functional Foods [11]. Recently, they proposed a 16-step process for categorizing foods as functional [12]. Bioactive compounds present in plant, yeast, insect, single-cell, and animal-based food products, although in small amounts, play a vital role in promoting optimal human health. It is crucial to define the effective and safe dosages of these compounds that can yield positive responses in individuals, thereby enhancing their well-being. The impact of bioactive compounds on the body stems from their interactions with cellular functions and metabolic processes [13].

Nigella sativa (NS) Linn, a member of the Ranunculaceae botanical family, is commonly distributed across Western Asia, the Middle East, and Europe. Recognized as 'black seed' or 'kalonji', this herb has been widely incorporated into culinary practices as a spice and condiment. [14]. Traditionally, NS has been utilized as a medicinal plant in Arabian countries, and the Indian sub-continent to address various ailments including high blood pressure, bronchial asthma, gastrointestinal issues, diabetes, cancer, headaches, dysentery, back pain, infections, and inflammation [15]. The functional components of NS include thymoquinone (TQ) (30%-48%), p-cymene (7%-15%), carvacrol (6%-12%), sesquiterpene longifolene (1%-8%), 4-terpineol (2%-7%), tanethol (1%-4%), thymol, β-pinene, α-pinene, and γ-terpinene, α-thujene. Among these components, TQ has the most efficacy by reducing oxidative stress [16]. NS oil

has been observed to enhance glucose uptake by HepG2 cells through the activation of AMP-activated protein kinase (AMPK), facilitating healing processes [17]. Administration of NS has demonstrated significant effects on laboratory parameters associated with hyperglycemia and diabetes management, including reductions in fasting blood glucose (FBG) levels, postprandial blood glucose levels, and glycated hemoglobin levels, alongside increases in insulin secretion and insulin sensitivity [18]. Black seed oil has succeeded in improving endocrine pancreatic activity and raising insulin levels in diabetes patients' serum [17].

Cumin, scientifically known as *Cuminum cyminum* (CC), is a member of the Apiaceae family and is indigenous to India, Iran, the Mediterranean, and Egypt. Famed for its antioxidant properties, this plant has a rich history in traditional medicine, where it has been used as a stimulant, carminative, and coagulant [19]. Animal studies have provided evidence supporting the anti-diabetic effects of CC's essential oil [20]. The active components of this plant include Cumin aldehyde (approximately 49.4%), α-terpinen-7-al (6.8%), β-pinene (6.3%), g-Terpinene (6.1%), γ- terpinene (6.1%), p-cymen-7-ol (4.6%) and thymol (2.8%) [21]. Among these components, Cumin aldehyde, also known as 4-isopropyl Benzaldehyde, exhibits the most potent activity. It acts as an inhibitor of enzymes involved in the carbohydrate metabolism pathway, specifically, glycosidase and aldose reductase. The presence of these enzyme inhibitors may explain the anti-diabetic effects of cumin [22]. Therefore, insulin sensitivity can be increased by limiting the inflammatory pathways in the peripheral tissues and using substances with anti-inflammatory properties [23]. While previous investigations into the effects of CC on T2DM have involved laboratory animals, one study has involved a sample of twenty human subjects with T2DM. The findings showed that green cumin positively and significantly impacted serum glucose levels and lipid profile [24]. Both cumin and NS oil help to control blood sugar by reducing the amount of TNF alpha, NF-kb, IL-1, and IL-6 and increasing the level of adiponectin. Interestingly, it has been reported that owing to the overall mandatory metabolic control, such male patients have a lower incidence of prostate cancer [18-19].

Despite the various beneficial effects of NS and CC, further improvement is required in terms of evidence regarding the combined effect of NS and CC. Consequently, the present research attempts to assess the potential of combining NS and CC oils to enhance insulin sensitivity in diabetic mice induced by a high-fat diet and streptozotocin. Subsequently, the impact of this combined treatment on glycemic control in patients with T2DM will be investigated.

MATERIALS AND METHODS

Part 1 (In vitro study): Male C57BL/6 mice, aged 6 weeks, were sourced from the Animal Study Department of Royan Institute when they were five weeks old. They were allowed to adapt to their environment in a temperature-controlled animal facility with a 12-hour light and 12-hour dark cycle for one week. Throughout this period, the mice had access to their respective diets and water ad libitum, unless stated otherwise.

Following the acclimatization period, the experimental animals were randomly divided into two dietary groups: one group was fed a standard chow diet comprising 7% fat, 20% protein, and 73% carbohydrates (wt./wt.), while the other group received a high-fat diet containing 60% fat, 20% protein, and 20% carbohydrates (wt./wt.). Weekly monitoring of body weight and FBG was conducted, adhering to all ethical guidelines for the use of experimental animals, which were approved and specified.

Eventually, after ten weeks of feeding mice with an assigned diet, animals were allocated into four groups (n=8 per group): control, diabetic-type2, diabetic-intervention-NS, and diabetic-intervention-NS+CC.

Induction of DM2: The experimental rodents were fed HFD for 2.5 months which brought about glucose intolerance and insulin resistance in periphery cells and tissues, followed by two injections of Streptozotocin (STZ) (100mg/kg) 15 minutes after injection of Nicotinamide (240mg/kg) every other day (experimental day one and experimental day 3) resulted in moderate pancreas destruction and reduction in its mass; a reliable model resembling the pathology of human T2DM [25]. Animals exhibiting blood glucose levels exceeding 180 mg/dl were chosen for inclusion in this study [26]. While STZ causes necrosis of insulin-secreting cells, Nicotinamide affords partial protection of beta cells against STZ, resulting in a moderate degradation of B cells rather than complete inhibition of insulin secretion [25].

Rodents were monitored daily for characteristic signs of diabetes expressed in all injected mice: hyperglycemia, polydipsia, polyuria, hypoinsulinemia in type 1, and insulin function deficiency in type 2 models.

Upon the onset of symptoms, diabetic mice were administered NS oil alone or in combination with CC oil via gavage for 30 days. Mice in the diabetic-intervention-type I group received a dosage of 400 mg/kg/d NS oil, while those in the diabetic-intervention-type II group were administered 360 mg/kg/d NS oil along with 40 mg/kg/d CC oil. Subsequently, all experimental rodents underwent intraperitoneal glucose tolerance tests (IPGTT) and insulin tolerance tests (ITT) to assess and characterize their metabolic profiles, glucose tolerance, and whole-body insulin sensitivity across all groups. GTT and ITT are powerful tools to investigate metabolism-associated diseases and assess the general metabolic phenotype of the body and alterations in glucose metabolism [27]. Moreover, blood glucose was checked weekly during the study, and insulin level was measured before mice were tested using blood samples from the tail.

Part 2 (Human study): This study followed a randomized, double-blind, controlled clinical trial design. A total of eighty patients with T2DM, aged between 40 and 65 years, who had been clinically diagnosed with T2DM for a duration of 1-10 years, along with a body mass index (BMI) ranging from 18.5 to 30 kg/m2, and were currently receiving Metformin (500-2500 mg/d), were recruited from Fayyaz Bakhsh Hospital in Tehran. The individuals with any liver or renal diseases, inflammatory diseases, or lung diseases; those being on glucocorticoid drugs or non-steroidal anti-inflammatory drugs (NSAIDs) or insulin; who were taking any herbal supplements three months before the study were excluded from our study. Patients who experienced changes in their standard medications during the 12-week study period, those who were pregnant or lactating, and individuals with uncontrolled diabetes (Hb A1C ≥ 8.5%) were excluded as well. Ethical approval for this study was acquired from the Research and Ethical Committee of Shahid Beheshti University of Medical Sciences (code: IR.SBMU.RETECH.REC.1400.1132).

Intervention: All patients received detailed explanations regarding the study's objectives and procedures, and informed consent was acquired from all patients. Subsequently, patients were categorized based on their age and sex. Through a double-blind randomization method, patients were randomly allocated to either the intervention group or the placebo. Randomization was conducted using a table of random numbers generated on-site. The allocation process was carried out by an investigator not involved in administering the intervention or gathering data.

Patients in the intervention group received 2000 mg CC -NS soft gel with a ratio of 1 to 10 for CC L to NS (Mehr et al. Company, Iran) daily for 12 weeks (1000 mg soft gel, once every 12 hours). Subjects in the placebo group received 2000 mg of soft gel containing sunflower oil daily (1000 mg soft gel, once every 12 hours). Tehran

Darou Company prepared "CC," "NS," and placebo soft gels.

A questionnaire including demographic information, clinical history, and drug history was obtained from each participant at the baseline. To evaluate the regular dietary intake of the participants, a registered dietitian conducted interviews and filled out three days of dietary records (2 weekdays and 1 weekend day) before and after the intervention.

Anthropometric Parameters and Blood Pressure: Furthermore, blood pressure measurements were taken while the participants were in a seated position, using the right hand, and the individual's arm was positioned at heart level. Five minutes before the measurement, the person was resting completely, and the sleeves of the clothes should be wide enough to prevent loss of blood flow. It should be noted that if the blood pressure (systolic or diastolic) is greater than or equal to 140/90, the patient has hypertension, and the measurement should be repeated at least twice to confirm the diagnosis. The time interval between the two measurements should be 12 minutes. Weight was measured with a precision of 100 grams, with the participants wearing lightweight clothing, utilizing a Seca scale. Height was measured without footwear, with a precision of 0.1 cm, using a measuring tape.

Blood Samples and Analytic Methods: After a fasting period of 12-14 hours, a 5 ml sample of venous blood was collected from each patient at the beginning and end of the study. Hemoglobin A1c (HbA1c) levels were determined in the whole blood sample using Pars Azmoon kits (Pars Azmoon Co., Tehran, Iran) on the day the samples were taken. The chromatography method was employed to determine Hb A1C levels. The blood serum was separated through centrifugation at room temperature (20-25 °C), at a speed of 3000 rpm, for 10 minutes. The resulting serums were then aliquoted into micro-tubes and frozen at a temperature of -80 °C until biochemical analysis could be performed. Enzyme-linked immunosorbent assay (ELISA) kits (Pars Azmoon Co., Tehran, Iran) were utilized to determine fasting blood sugar (FBS) levels.

Statistical Analysis: According to the previous studies and considering the 20% difference in wound healing rate for the two supplement and placebo groups with a 5% probability of error and a test power of 80%, the sample size was defined as thirty-six subjects per group. Accounting for a 10% potential dropout rate, this number was increased to forty individuals per group.

Statistical analyses were carried out using SPSS software (version 16.0; SPSS et al.). All quantitative parameters demonstrated a normal distribution based on the Shapiro-Wilk or Kolmogorov-Smirnov test. Within-group and between-group comparisons were conducted using t-tests and paired t-tests, respectively. The findings are presented in terms of mean ± standard deviation (SD), with a P-value≤0.05 being deemed statistically significant for all analyses.

RESULTS

Part 1 (In vitro study): Changes in glucose levels were measured for four weeks in the control group, T2DM groups, and the treatment group. We detected a notable decrease in blood glucose levels among the T2DM groups following treatment with either NS or NS-CC. As presented in Figure 1, the blood sugar level of the diabetic NS group decreased from 271.1±21.71 mg/dl on day 0 to 167.6±8.14 mg/dl on day 30 ($P<0.0001$). More improvement was observed in the NS-CC group (changing from 275.4±25.57 to 136±13.07, $P<0.0001$). Compared to NS, NS-CC treatment resulted in more reduction of blood glucose. However, the difference was not significant. We noted no significant disparity in blood sugar levels between non-diabetic controls and NS-CC 30 days post-treatment. After injecting insulin into T2DM, control, and treatment groups, the insulin sensitivity of the untreated group and the disruption of insulin receptors can be detected due to the high blood sugar of the mice.

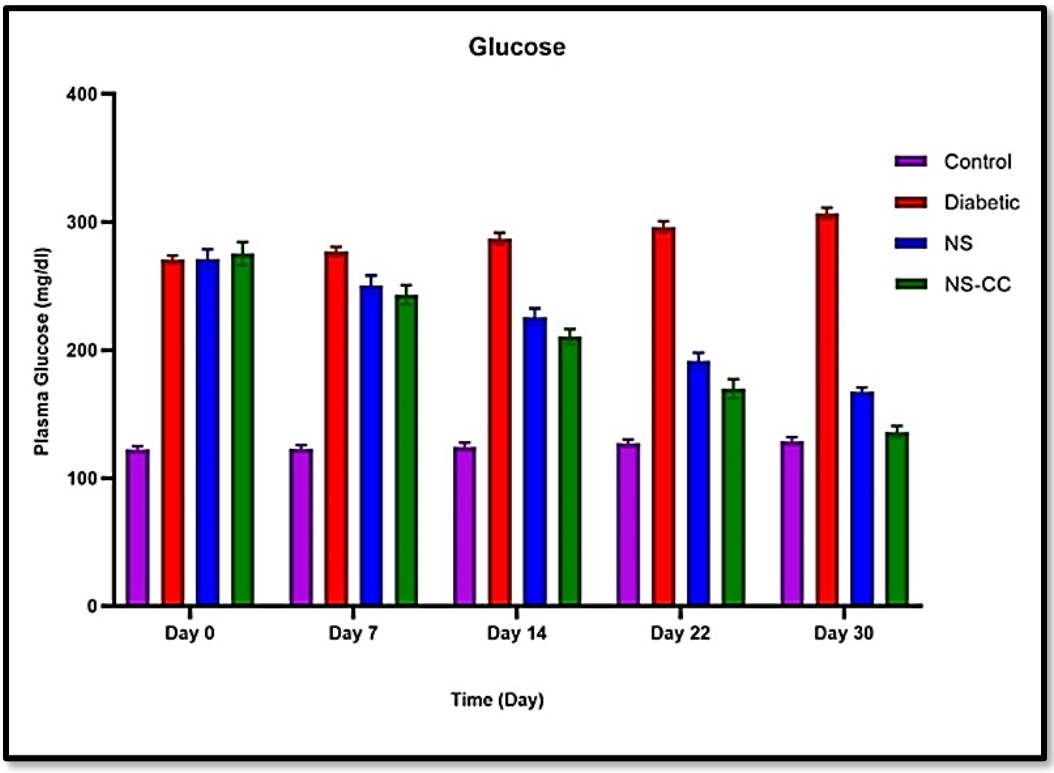


Figure 1. Weekly review of DM2 glucose changes. The changes in blood sugar of each group were investigated from the beginning of the mice becoming diabetic (pre) and then up to four weeks later (all data are plotted as Mean+/-SEM.).

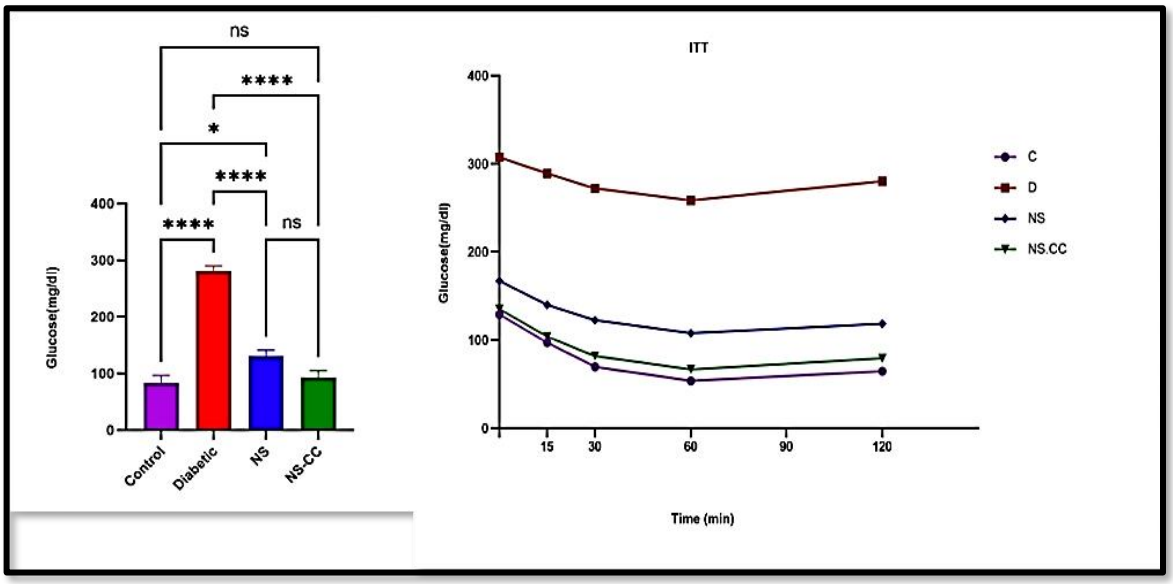


Figure 2. Insulin challenge measurement of DM2 mice. All data are plotted as Mean+/-SEM.

Conversely, the treatment group exhibited a marked reduction in blood sugar and improved insulin activity (Figure 2). ITT values were 82.72±30.52, 281.4±18.4, 131.1±23.09, and 93.25±26.99 in the control, diabetes-induced, NS, and NS-CC groups, respectively. ITT of NS and NS-CC groups were significantly lower than ITT of diabetic mice.

Interestingly, ITT values are comparable with a control group with no significant difference after treatment with NS or NS-CC. The difference was

insignificant, although the ITT level was lower in the NS-CC group.

Comparing the graphs in the glucose challenge test also confirmed the imbalance of blood sugar metabolism in the type 2 diabetic group ($p<0.0001$). However, four weeks of treatment has resulted in a significant balance of plasma glucose (Figure 3). GTT 120 was 146.4±24.22, 532.7±36.15, 257.0±54.27, and 194.1±54.71 in the control, diabetes-induced, NS, and NS-CC groups, respectively. GTT 120 was significantly reduced in both the NS and NS-CC groups compared to the diabetic group. Furthermore, there was no noteworthy distinction between either the NS or NS-CC-treated mice and the placebo group. Although the GTT 120 of the NS-CC group was lower than that of NS alone, the difference was insignificant.

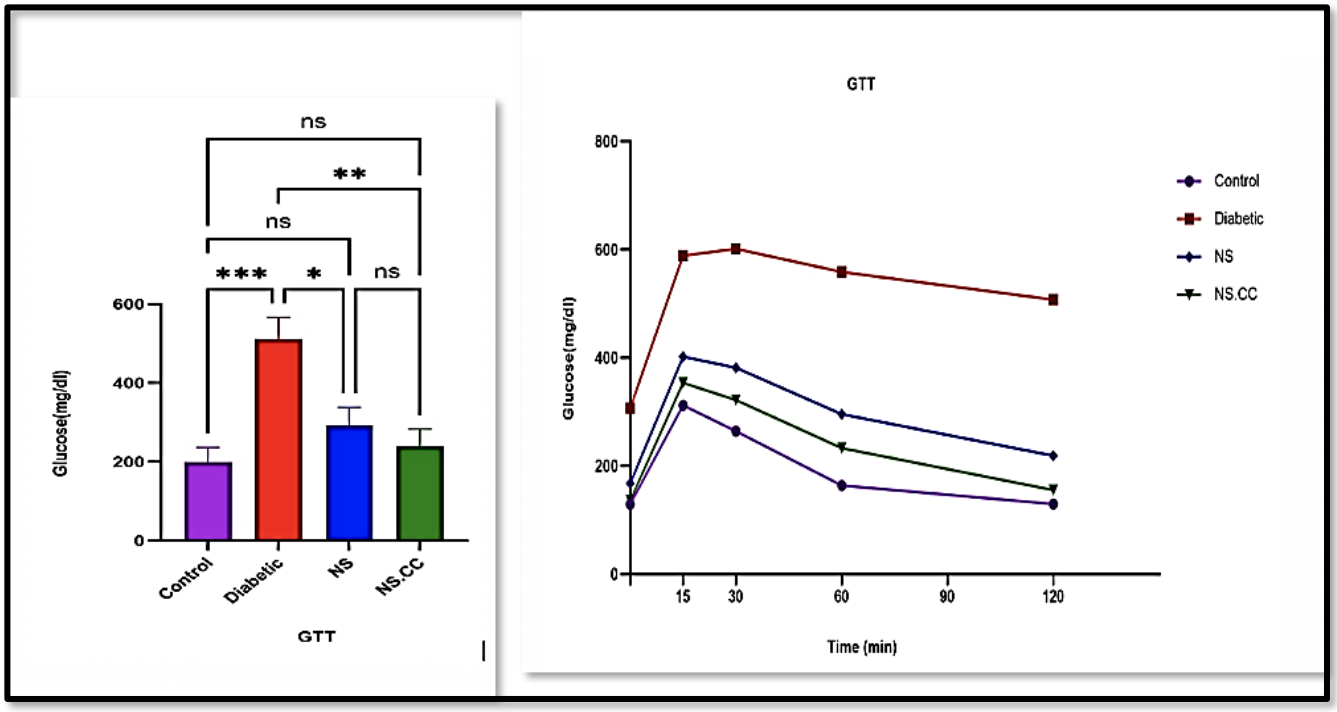


Figure 3. Measurement of glucose challenge in DM2 mice. A) 120-minute blood glucose level, B) Glucose Tolerance Test (GTT). All data are plotted as Mean+/-SEM

Part 2 (Human study): From the murine phase of the study, we concluded that the NS-CC treatment is more effective than the NS treatment. In phase 2, 79 subjects (34 males and forty-five females) completed the study. One participant in the intervention group withdrew from the study due to a lack of willingness to continue. The average age of patients was 57.59 ± 10.4 and 58.55 ± 10.57 years in the intervention and control groups, respectively. The patient's BMI was 27.44 ± 2.67 and 27.43 ± 3.44 kg/m2 in the intervention and control groups, respectively. The general characteristics of study groups are presented in Table 1. There were no notable differences in age and gender distribution between the two groups (P= 0.862, 0.551, respectively). At the baseline, there were no significant discrepancies in weight, BMI, and the percentage of hypertension between the two groups (P=0.28).

Table 1. Participants' characteristics in intervention and placebo groups.

Variable		Intervention (n=39)	Placebo (n=40)	P-value
Sex	Male	17 (43.6%)	17 (42.5%)	0.551
	Female	22 (56.4%)	23 (57.5%)	
Age (year)	Male	57.59±10.4	58.55±10.57	0.862
	Female	57.63±9.93	58.21±9.7	
Weight (Kg)		77.53±9.29	77.86±11.17	0.901
BMI (Kg/m2)		27.44±2.67	27.43±3.44	0.944
Hypertension		16 (41.02%)	22 (55.0%)	0.105

*Data for Sex and Hypertension are expressed as numbers (percentage), while Age, Weight, and Body Mass Index (BMI) are presented as Mean ± SD.

Serum glucose and HbA1C levels were measured as indicators of short-term and 3-month control of diabetes, respectively, at the beginning and end of the study. The changes were then compared between the two groups.

Table 2 illustrates the results of comparisons made within and between the intervention and placebo groups regarding serum concentrations of FBS and HbA1C at baseline and after 12 weeks.

Table 2. Serum concentrations of fasting blood sugar (FBS), and Hb A1 in the intervention and placebo group

Serum parameters	Intervention (n=39)	Placebo (n=40)	P-value*	P-value***
FBS (mg/dl)				
Baseline	194.36±73.59	211.82±71.09	0.287	0.468
Week twelve	160.18±55.55	201.77±64.88	0.003	0.001
Changes	-34.18 (35.14)	-10.05 (43.24)	0.008	0.001
P-value**	<0.001	0.150		
Hb A1C (%)				
Baseline	8.07±1.60	8.27±1.69	0.581	0.603
Week twelve	7.38±1.34	8.11±1.5	0.025	<0.001
Changes	-0.69 (0.74)	-0.16 (0.76)	0.003	<0.001
P-value**	<0.001	0.185		

All variables are reported as mean (standard deviation). *Using Independent Samples T-test; **Using Paired Samples T-test; ***Using analysis of covariance (ANCOVA), adjusted based on body mass index at the beginning of the study and baseline values, P-value < 0.05 is statistically significant, FBS: fasting blood sugar, Hb A1C: hemoglobin A1C

At baseline, there was no statistically significant difference in the mean FBS between the two groups before and after adjusting for BMI. The FBS concentration decreased during the intervention (from

194.36±73.59 to 160.18±55.55, P<0.001), and the placebo group (from 211.82±71.09 to 201.77±64.88, P=0.150).

The HbA1C also fell both during the intervention (from 8.07±1.60 to 7.38±1.34, P<0.001) and the control group (from 8.27±1.69 to 8.11±1.5, P<0.185).

These changes were only significant in the intervention group before and after adjustment for BMI and baseline values. Therefore, supplementation with NS and CC reduced the mean of FBS concentration greater than placebo (P=0.008) and led to a significant reduction in Hb A1C compared with placebo. (P=0.003).

DISCUSSION

Our results showed the lowering of glucose blood levels after treatment with NS or a mixture of NS and CC in type 2 diabetic C57BL6 mice, as well as improving ITT and GTT. The weekly examination of these mice's metabolic activities shows that the effect of oils is time dependent. We can expect more treatment for glucose and insulin disorders in a more extended period. As the highest effect was observed from the combination of NS (90%) oil and CC (10%) oil, in the second phase, we continued the study with 1000 mg NS (900 mg) and CC (100 mg) soft gel twice daily. After three months of treatment, this combination resulted in a significant decrease in both blood glucose and HbA1C levels.

The observed decrease in glucose levels, insulin resistance, and enhanced GTT among diabetic mice utilized in this experiment corroborate previous findings indicating improvements in pancreatic cell function and increased insulin production. In a study involving Female rats induced with diabetes, the administration of 400 mg/kg NS oil for 21 days led to reductions in blood glucose levels, alongside improvements in Zenker's necrosis, myositis, and hyaline degeneration [26]. In two studies by Fararh et al., male Syrian hamsters were induced with 65 mg/kg STZ and 230 mg/kg nicotinamide in the first study and 65 mg/kg STZ in the second. They received 400 mg/kg NS oil over the course of four weeks. The first study reported an increase in pancreatic insulin immunoreactivity, a surge in serum insulin levels, and a reduction in blood sugar [27]. In the second study, reductions in HbA1c and blood glucose levels were observed, along with decreased glucose production in isolated hepatocytes when exposed to gluconeogenic precursors (glycerol, lactate, and alanine). Furthermore, the phagocytic index of peritoneal macrophages and phagocytic activity, as well as the lymphocyte count in peripheral blood were enhanced [28]. Additionally, our results agree with an in-vitro study on fractions of CC. Patil and colleagues reported that the insulinotropic properties of Cuminaldehyde and cuminol (25 µg/ml) showed a 3·34- and 3·85-fold increase in insulin secretion, respectively, compared to the control. They also demonstrated that the insulinotropic characteristics of both compounds were dependent on glucose and were caused by the closure of the ATP-sensitive K (K+-ATP) channel and the elevation of intracellular Ca2+ concentration [20].

Several human studies have shown NS oil to have anti-diabetic effects [29-32]. Adam et al. gathered the data from clinical trials on NS in a systematic review [31]. Thirteen trials were enrolled. The supplementation of T2DM patients on glucose-lowering oral agents or a diet with NS oil for eight weeks improved their glycemic profile by decreasing FBG and HbA1c levels and enhancing insulin secretion [31]. The glucose-lowering effect of CC was studied less. However, like our results in a randomized clinical trial (RCT) by Jafari et al., the effect of eight weeks of treatment with 100 and 50 mg/d CC was compared with a placebo. Both doses of 100 mg/d and 50 mg/d of CC exhibited significant reductions in FBG and HbA1c levels while also increasing insulin sensitivity, as determined by the homeostatic model assessment of insulin resistance (HOMA-IR) [17].

Although previous studies have demonstrated the therapeutic potential of NS or CC as complementary or adjunctive treatments for managing DM and its

complications, this study is, to the best of our knowledge, the first to investigate the combined effects of NS and CC oil. Like the drug Metformin, NS has been reported to promote uptake of glucose, enhance insulin sensitivity, and stimulate the AMPK pathway [33]. Furthermore, it decreases intestinal glucose absorption [34]. NS also has the potential to modulate the carbohydrate-digesting enzymes to inhibit and manage the fluctuation of postprandial blood glucose levels, the same as acarbose, voglibose, and miglitol. In addition to its direct effects, NS may enhance the effectiveness of anti-diabetic drugs and exert a synergistic effect, thereby improving blood glucose management. Furthermore, the combination of NS with effective herbal drugs may reduce the required dosage and potential toxicity, leading to improved overall health outcomes for patients [31].

According to the evidence obtained from clinical trials, the impact of CC and its bioactive constituents on producing favorable effects can be evaluated by assessing several diabetes biomarkers. The impact of aldehyde compounds of CC on glycemic status is primarily attributable. The presence of these compounds can inhibit alpha-glucosidase and aldose reductase, resulting in decreased reactive oxygen species (ROS) production and glucose uptake [25-35]. Furthermore, CC can stimulate insulin secretion by increasing calcium influx and influencing nesfatin-1 [36]. In addition, other bioactive components found in CC serve to maintain the integrity of pancreatic β-cells, thereby improving the secretion of insulin and reducing insulin resistance in hepatocytes [20]. Similarly to thiazolidinedione, CC sterols can activate PPAR-γ and enhance the activity and quantity of glucose transporter-4 [37].

However, despite the positive findings of our study on the beneficial effects of NS combined with CC in the management of T2DM, there are still several limitations that need to be addressed before clinical use. The methodology employed for the gathering and examination of data lacks adequacy in its robustness. Like other herbal resources, the bioavailability of these herbs varies based on farming place and technique. We did not assess the amount of CC and NS bioactive compounds in the current study. Modulation of the dose is needed before general recommendation. We studied the effective dose in the preclinical setting before the human phase. However, the dose is selected based on available evidence, and we should have extensively checked the doses as this expanding field of research is now demanding [38]. Aging of gastrointestinal mucosa as well as the age-related cellular susceptibility to diabetes-associated oxidative stress [39-40] are also variables worth future investigation. All studied populations were receiving metformin. The drug-herb synergistic effect must be studied for other glucose-lowering oral agents and injections.

Future studies need to address further risk of unwanted pharmacodynamic and pharmacokinetic interactions. Our study has several strengths. Many herbal compounds have synergistic effects that can multiply their individual effects. In this investigation, we evaluated the impact of NS and CC on lowering glucose levels. Both NS and CC are widely available and cheap herbs throughout the world. Our study had preclinical and clinical phases to increase the success potentiation and reduce the failure rate.

CONCLUSION

In conclusion, the combination of NS-CC oil has demonstrated the ability to reduce blood glucose levels and insulin resistance in C57BL6 mice and has shown a glucose-lowering effect in T2DM patients. Further research is required to validate our findings and address any gaps identified in our study.

List of Abbreviations: NS: *Nigella sativa*, CC: *Cuminum cyminum*, IPGTT: Intraperitoneal glucose tolerance test, ITT: Insulin tolerance test, FBS: Fasting blood sugar, Hb A_1C: Hemoglobin A_1C, DM: Diabetes mellitus, WHO: World Health Organization, FFs: Functional foods, FFC:

Functional Food Center, FDA: Food and Drug Administration, ASFFBC: The Academic Society of Functional Foods and Bioactive Compounds, TQ: Thymoquinone, AMPK: AMP-activated protein kinase, FBG: Fasting blood glucose, HFD: High fat diet, STZ: Streptozotocin, BMI: Body mass index, NSAIDs: Non-steroidal anti-inflammatory drugs, ELISA: Enzyme-linked immune sorbent assay, SD: Standard deviation, T2DM: Type 2 diabetes mellitus, RCT: Randomized clinical trial, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, ROS: Reactive oxygen species.

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