

A Critique on Mechanism of *Nigella Sativa* as an Anti-Diabetic Drug: Focus on the Therapeutic Dose Based on Assorted Explications

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Abstract:

Background: Globally, Diabetic mellitus is a rapidly progressing metabolic disorder and is becoming a worldwide concern with several complications and deaths every year. Despite conventional anti-diabetic drugs numerous kinds of research are going on to get the best cost-effective therapeutic agents with the least adverse effects for the management of diabetes and its complications. *Nigella sativa* is a spice with multi-effects on various disorders like anti-diabetic, anti-cancer, immune modulator, anti-microbial, anti-inflammatory, anti-spasmodic, relieves pain, bronchodilator, hepato and renal protective, gastro-protective, anti-oxidant properties. Amongst all effects, the anti-diabetic properties remained a cornerstone and was explored. Anti-diabetic effect of *N. sativa* was due to the presence of Thymoquinone, a major constituent responsible for its effect. Since long ago, studies revealed that the active constituent thymoquinone had a significant reduction in fasting and post-prandial blood glucose levels (glycemic control), probably affecting the pancreatic β -cells, on insulin production and secretion; moreover, lipid profile was shown to be improved in both clinical and preclinical trials. However, there are not many studies on the exact dose to be administered for the therapeutic effect clinically. **Conclusion:** The current review investigated and emphasized the molecular mechanism of *N. sativa* based on the pre-clinical, clinical and toxicological evaluations. This aimed for the estimation of effective dose of *N. sativa* therapeutically for healthier out-turn.

Key words: *Nigella sativa*, Diabetes mellitus, Mechanism of action, dose, side effects

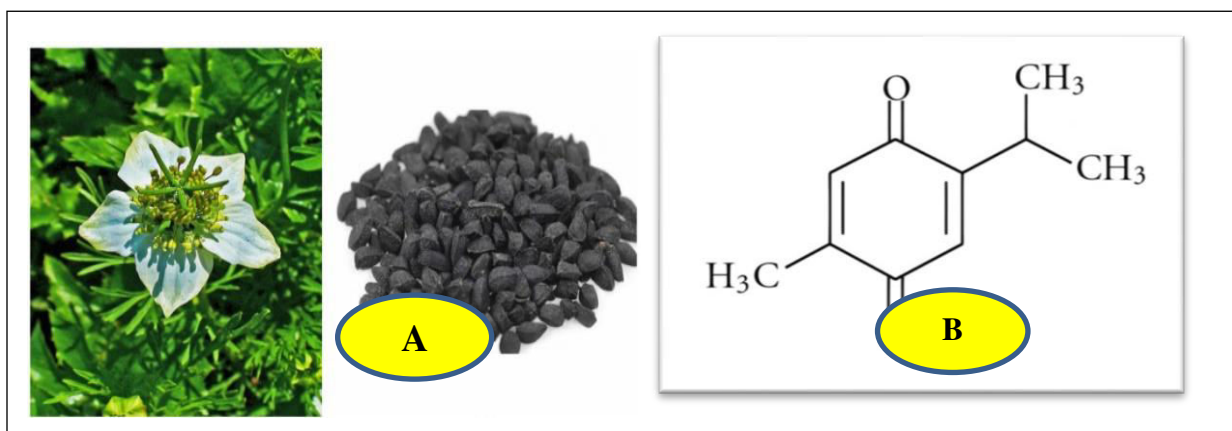
Introduction

Diabetes mellitus is a chronic progressive metabolic disorder characterized by increased blood glucose levels (hyperglycemia), polyuria, insulin resistance, and pancreatic dysfunction^[1]. Diabetes mellitus is associated with many complications which include hypertriglyceridemia, high levels of low-density lipoproteins (LDL), and, low levels of high-density lipoproteins. Because diabetes raises the risk of heart disease and stroke, 50% of diabetic individuals die from cardiovascular diseases^[2]. Most of these deaths are noticed in developing countries with lower and middle-income,^[3] considering the developing economies, India is at its peak and it was estimated that the number of people affected with diabetes will reach 300 million by the year 2025^[4] and around 700 million by 2045. As a consequence of this epidemiological data analyzed, International Diabetes Federation (IDF, 2021) continued studies on pathogenesis and treatment for diabetes mellitus^[5, 6]. All are looking for affordable medications to manage diabetes with the fewest side effects. In the current review, exploration has been made on herbal / Unani drug which has long been in the subject of research for their effects on various chronic illnesses.

Generally, as diabetes is considered as a chronic life style disorder which exists for whole life time, there can be every chance of being incessantly high blood glucose levels that subsequently can generate free radicals and further lead to the genesis of oxidative stress in different parts of the body^[7]. This will additionally may create many complications on different organs of the body like heart, brain, kidneys, eyes and blood vessels. Hence there is a mandatory requirement of such a drug which targets on these free radicals and act as scavengers and anti-oxidants^[8]. Hence choosing drugs that has an action at molecular level is always preferred as they combat the root cause of a disease and have sufficient therapeutic effect.

Since ancient times, medicinal plants have been used by mankind as traditional treatments for a wide range of acute and chronic ailments. According to the World Health Organization (WHO), more than three-fourths of the populations in resource-constrained nations rely on medicinal plants. This might be because of the inaccessibility and cost of allopathic medicines^[3, 9].

Among the plethora of medicinal plants, *Nigella sativa* (NS) is considered an excellent ancient herb with miraculous therapeutic effects which belongs to the family Ranunculaceae. It is commonly called as black cumin (or) kalonji^[10, 11], and has been used in the ancient system of medicine for its potential benefits^[12]. These therapeutic effects of *N. sativa* are due to the presence of the compound, Thymoquinone. Thymoquinone (2-Isopropyl-5-methyl benzo-1,4-quinone) is an active ingredient present in the seeds of *Nigella sativa*^[13].



**Fig – 1: A) *Nigella sativa*, flower and seeds [14]
B) The chemical structure of Thymoquinone [14]**

Nigella sativa is considered a Prophetic medicine in Islam and has a wide variety of therapeutic uses in various illnesses because of which it is known as ‘Habbah Sawda’ or ‘Habbat el Baraka’ (in Arabic, which means ‘Seeds of blessing’), ‘Panacea’ (in Latin, translated as ‘cure all’), ‘kalo jeera’ (in Bangladesh), ‘hei zhong cao’ (in China), ‘kalonji’ (in India). It is a very popular spice widely distributed in Asian countries like India, Pakistan, Bangladesh, Afghanistan and Sri Lanka^[15, 16]. Both, the seeds and oil from *Nigella sativa* can be used for medicinal purposes, and a few of them include anti-cancer, anti-diabetic, anti-hypertensive, anti-inflammatory, anti-microbial, analgesic, immunomodulatory, spasmolytic, gastro-protective, hepato-protective, renal-protective, bronchodilatory and anti-oxidant activities^[17,18,19,20]. Among all the activities it showed spectacular hypoglycemic effect by various mechanisms, which is very essential for the management of Diabetes mellitus in the affected population^[21], and also it is imperative to know about the side effects of the drug being used for therapy. The current study explored the beneficial effects and emphasized mechanistic values of *N. sativa* on glycemic control based on the pre-clinical and clinical effects.

Methodology

We retrieved the data from various known databases like Pubmed, Scopus, Google scholar and SciFinder. To gather the information key words such as ‘thymoquinone’, ‘black cumini’, ‘*Nigella*

sativa compound', pharmacological activity of *Nigella sativa*, therapeutic potential of *Nigella sativa*, 'anti-diabetic effect of *Nigella sativa*' 'effect of *Nigella sativa* on blood glucose levels', '*Nigella sativa* in diabetes', 'toxic effects of *Nigella sativa*'. The search language of publications and articles was done in the English.

Inclusion criteria Articles conveying information about anti diabetic effect of *Nigella sativa* have been included in the study.

Investigatory aspects in Pre-clinical and Clinical studies

***Nigella sativa* as an Anti-diabetic drug on pre-clinical platform:**

Many studies had been carried out on the potential effects of *Nigella sativa* on diabetes mellitus and were found that it reduced blood glucose levels, and NO (Nitrous oxide), HbA_{1c} and altered lipid profile^[22]. Studies were conducted on rats and mice using streptozotocin-induced diabetic model and explored the mechanisms as described below^[23]. A partial regeneration of pancreatic beta cells with the secretion of insulin was also observed with the *N. sativa*^[24]. Previous studies revealed that presence of thymoquinone compound in *Nigella sativa* is responsible for its anti-diabetic effect, also *N. sativa* acts on AMPK (AMP activated protein kinase) thereby inhibits gluconeogenesis in both the liver and muscles, aids in decreased absorption of glucose from the intestine^[25, 26]. The duration of studies, doses and routes of administrations of along with the standard drug and the test drugs were depicted in Table 1.

Table - 1. Effect of *Nigella sativa* on experimental diabetes in animal models

S.No	Models for induction of Diabetes Mellitus in animals	Dose and Duration	Effect of <i>N. sativa</i> /Thymoquinone on blood glucose	References
1	STZ induced diabetes (60 mg/kg, ip) in rats	<i>N. sativa</i> extract (200 and 400 mg/kg oral route) 6 weeks	Serum glucose levels decreases significantly	[27]
2	STZ induced diabetes (90 mg/kg, i.p) in rats.	Methanolic extract of <i>N. sativa</i> (25, 50, 100 and 200 µg/ml in situ intestinal perfusion	Enhanced glucose utilization and decrease glucose absorption from GIT.	[28]

		technique) 3 months	Improved insulin release from beta cells in rats (thereby effective in lowering serum glucose levels)	
3	STZ induced diabeted (30 mg /kg body weight,i.p) in rats	<i>N.sativa</i> 0.5 ml, 1 ml, 1.5 ml per rat was administered orally. 40 days	Significant anti-diabetic effect with three doses due to the regeneration of beta cells of pancreas.	[23]
4	Nicotinamide (110 mg/kg) and Streptozotocin (65 mg/kg, i.p) induced in rats	Thymoquinone (20, 40, 80 mg/kg,p.o.) 21 days	Decreases fasting blood sugar levels effectively. Decreases gluconeogenesis in liver. Increases utilization of glucose by increased sensitivity to the release of insulin from pancreas.	[29,30]
5	STZ induced in rats (65 mg/kg body weight)	Thymoquinone (at a dose of 50 mg/kg body weight) by gastric lavage 4 weeks	Significant decrease in the levels of HbA _{1c} , lipid peroxidase and NO (Nitric Oxide)	[22]
6	STZ induced in rats (30 mg/kg bodyweight)	<i>Nigella sativa</i> seed extract (0,24,48,72 mg/kg body weight) orally 4 weeks	Prevents polyphagia, weight loss and improves blood glucose levels in type diabetic rats.	[31]
7	STZ induced in rats (40 mg/kg body weight,i.p.)	Thymoquinone (10,20 mg/kg, orally) 14 weeks	Significant reduction in blood glucose levels, additionally lipid profile and PPAR γ levels were improved.	[22]
8	STZ (150mg/kg,i.p.) induced	Metformin + Thymoquinone	Showed distinct hypoglycemic effects along with metformin.	[33]

	In mice	(200 mg/kg +50 mg/kg) orally 21 days		
9	Alloxan induced (150mg/kg) in rabbits	<i>N.sativa</i> oil 2.5ml/kg body weight orally 24 days	Found to be effective in reducing blood glucose levels	[34]
10	STZ (50mg/kg body weight,i.p.) induced in rats	Thymoquinone (20 mg/kg/day by gavage) 5 weeks	Serum glucose levels decreases	[18, 35]
11	In vitro biochemical assay	<i>N.sativa</i> silver nano particles	Inhibits alpha amylase activity	[36]

Thymoquinone and *Nigella sativa* extract was found to produce synergistic action on blood glucose levels with standard anti diabetic drugs like metformin^[37] and glibenclimide^[38].

Clinical aspects of *N.sativa*:

With the administration of *N.sativa* clinically, few reports revealed that this plant was effective against hyperglycemia and hyperlipidemia^[39]. Patients given with *N.sativa* seeds, extracts and oil was found to have reduction in fasting blood glucose levels (FBG), post prandial blood glucose (PPBG) levels with improvement in glycated hemoglobin (HbA1c) levels, decreased triglycerides and increased high density lipoproteins (HDL)^[40, 41]. The investigation included a maximum of 113 patients to evaluate the anti-diabetic clinical trials^[42]. In almost all investigations, *N.sativa* was evaluated with the co-administration of any conventional drug, thus might be helpful in analyzing the synergistic effect, with no adverse effects^[43].

Table - 2. Clinical aspects of *Nigella sativa*

S.No	Study design	Drug dose and Duration	Sample size (n)	Effects	References
1	Randomized placebo-control	30 ml <i>Nigella sativa</i> oil for 80 days	41	Fall in fasting blood glucose (FBG) levels and increase in insulin levels.	[44] [45]
2	Perspective study	500 mg NS seeds for 17 months	80	Significant decrease in fasting and post prandial blood glucose and improved HbA1c levels.	[40] [46]
3	Randomized clinical trial	2.5 ml of NS seed oil for 3 months	70	Improved HbA1c, decreased FBG and Post-prandial blood glucose (PPBG)	[41]
4	Randomized single blind control trial	1.5- and 3-ml NS seed oil per day for 20 days	99	Significant decrease in HbA1c levels	[47]
5	Randomized double blind control trial	3g/day NS oil soft capsules for 12 weeks	72	FBG, HbA1c, Triglycerides (TGs) and Basal metabolic index (BMI) changes.	[48]
6	Randomized control trial	500 mg/kg NS seeds per day for 12 months	113	Improved blood glucose levels and enhanced anti-oxidant system.	[42]
7	Randomized, double blind and placebo control trial	1 gm of NS oil per day for 8 weeks	44	Improved HDL levels decreased FBG, liver enzymes and inflammatory mediators. in Non-Alcoholic Fatty Liver Disease (NAFLD) patients	[49] [50] [51]

8	Perspective, open label randomized clinical trial	450 mg NS oil capsule 3 times a day for 12 weeks	44	Significant decrease in serum levels of FBG.	[52]
9	Randomized clinical trial	50 mg of TQ with 1000 mg of metformin for 90 days	60	Great reduction in FBG and PPBG was observed and improved HbA _{1c} .	[31]
10.	Randomized trial	2 g of NS seeds crushed per day for 8 weeks	40	Marked effects on serum glucose levels and insulin were seen.	[53] [54]

Probable mechanism of action of *N.sativa*

Nigella sativa acts as an anti-hyperglycemic agent by the following expected mechanisms from the mentioned data,

- Binds to the insulin receptors in pancreas
- Increases glucose uptake by the cells
- Activates voltage sensitive calcium channels
- Binds to Peroxisome Proliferator Activated Receptor gamma (PPAR-γ) in the nucleus

Conventional hypoglycemic drugs either bind to receptors or only acts on glucose metabolism to exert their mechanism whereas NS has multiple hypoglycemic mechanisms to reduce blood sugar levels.

NS inhibits gluconeogenesis in liver by releasing insulin from secretagogues, it activates insulin receptors which enhances the production of cAMP and also cause calcium dependent depolarization of cell concomitantly blocking ATP-sensitive k⁺ channels.

NS effectuates glucose metabolism through GLUT-2 transporter which will result in decrease number of glycated hemoglobin (HbA_{1c}) (Fig 2).

NS also consequence in genetic transcription by binding to PPAR-γ in nucleus, this mechanism equally plays a crucial role in inhibiting gluconeogenesis.

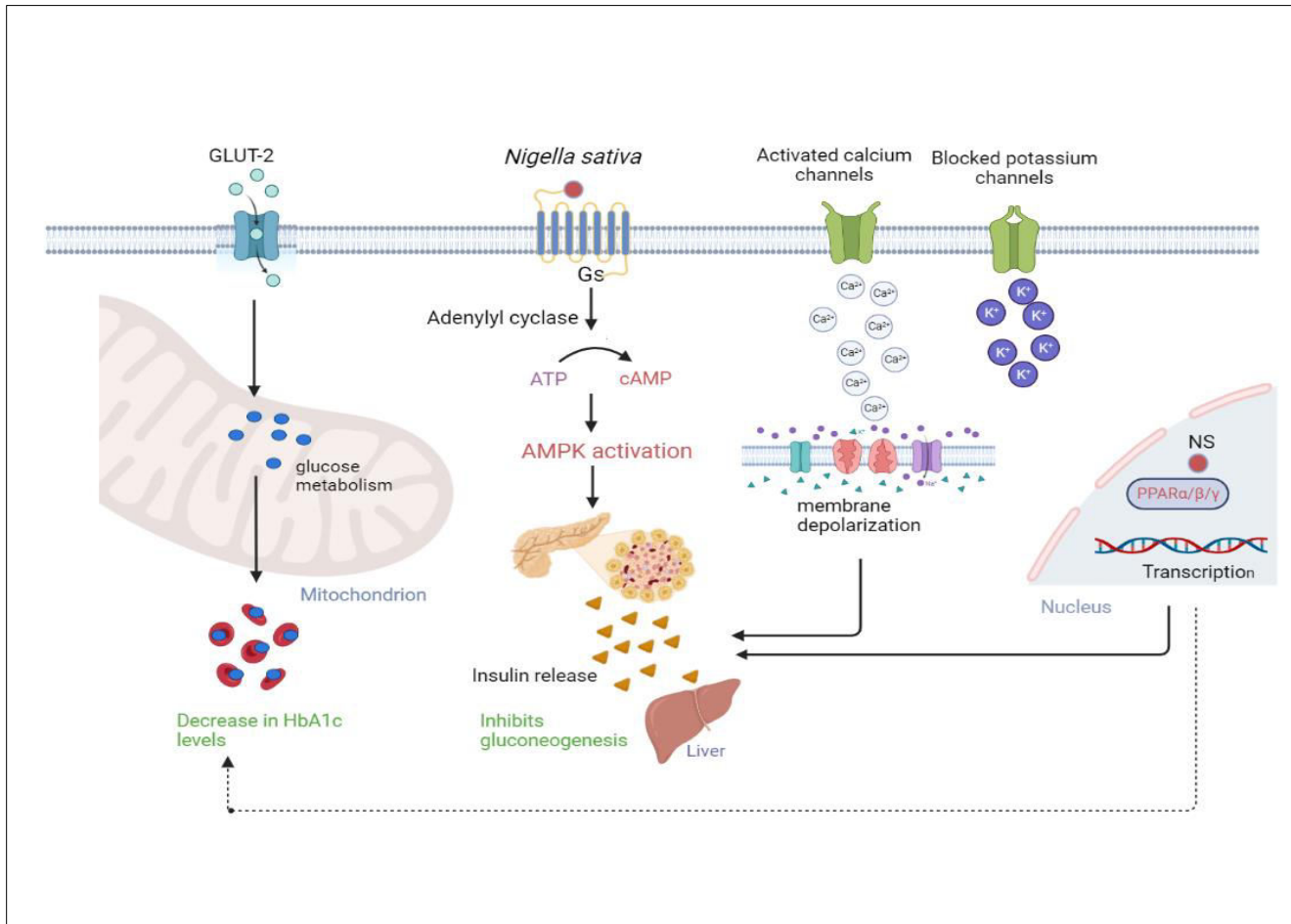


Figure 2. Exploration of probable mechanistic insights of *N.sativa* at molecular basis

Adverse effects/Toxicological investigations of *N.sativa*

Based on the adverse effects of *N.sativa*, toxicological investigations were done by Abukhader et al on possible adverse effects of Thymoquinone in Wistar rats [55, 56]. Various adverse effects with different routes of administration, acute pancreatitis were observed in rats with i.p injection and some short-term toxic effects were seen with oral administration in rats [57, 58]. Deaths were observed at 500mg/kg dose [59]. Few studies have reported adverse effects like abnormal vision, dizziness and drowsiness [60], decreased BP and tachycardia [61], caused nausea, vomiting, stomachache, flatulence [62] and hypersensitivity reactions [63]. The exact clinical and preclinical doses which produce these effects were unknown [64]. Few effects of Thymoquinone were depicted on various organs in Table 3.

Table 3. Side effects of *N.sativa* on experimental animals

S.No	Organs	NS side effects	References
1.	Central Nervous System	Dizziness, drowsiness, fatigue, abnormal vision	59
2.	Cardiovascular System	Tachycardia and hypotension	60,65
3.	Gastrointestinal System	Nausea, vomiting, flatulence, epigastric pain, abdominal cramps, diarrhea or constipation	61,17
4.	Renal system	Reversible nephritis, Crystalluria	66
5.	Joints and muscles	Chondrotoxicity	67
6.	others	Hypersensitivity reactions	63

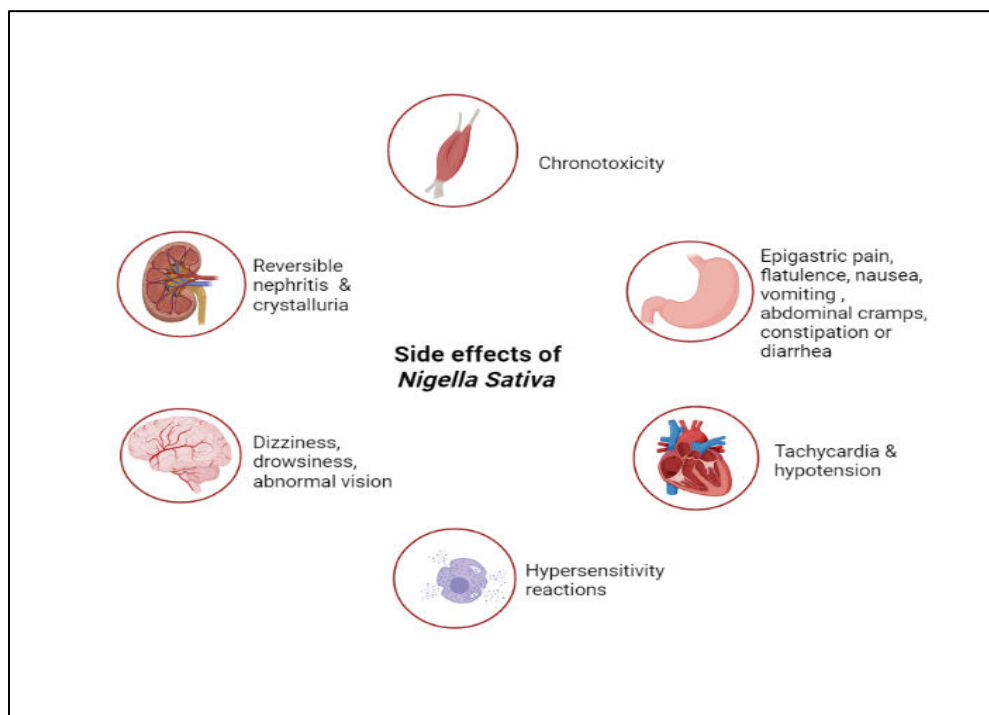


Figure 3. Exploration of Side effects of *Nigella sativa*

Conclusion

It can be concluded that *Nigella sativa* exhibited anti-hyperglycemic effect due to the presence of its chemical constituent, Thymoquinone. The mechanism was derived keeping in view of the above studies, however until present, in the clinical trials reported, the exact dose was in chaos, might be considered as one of the causes for side effects. Thus, a mandate investigation on a large population becomes crucial for fixing the dose so that the side/adverse effects can be closely monitored and resolved simultaneously. Detecting a therapeutic dose, there by inclines to favorable outcome along with patient compliance. In the present review, though the mechanism was acquired, still a significant, satisfactory and more detailed pharmacodynamics is possible with further studies. Additionally, as consumption of the conventional drug with the herbal medicines as a combination has been recommended in the present era, there is an option for a possible synergistic effect to be identified if exists. Hence, in future, experimental investigations on determination of therapeutic dose of *N.sativais* essential to elucidate the exact mechanism at molecular level which in turn may be helpful to conquer the side effects.

Conflicts of Interest

The authors declared no conflicts of interest.

Acknowledgement : Not applicable

Authors Contributions

SBhas written the manuscript, guided and edited by SR, SAM has contributed in preparation of diagrammatic illustration in the manuscript, RKV and SB collected the information required for writing Manuscript, DVRNB has guided and given final approval for submission.

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