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Original Research Article (Experimental)

# A potent nutraceutical combination of *Cinnamomum cassia & Nigella sativa* for Type 1 diabetes mellitus

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ABSTRACT

*Background:* *Nigella sativa (black cumin)* and *Cinnamomum cassia (Cinnamon)* are an integral part of the Indian diet, and have also been sourced in the ayurveda, the traditional Indian system of medicine, for their medicinal properties. Both the herbs individually have been successfully evaluated for their preliminary antidiabetic potential.

*Aim:* Herein, we dived deeper into antidiabetic properties of these herbs, by investigating the combinatorial effect of both herbs, on parameters of diabetes and further, as an adjunct to metformin therapy, for assessing the pharmacodynamics of herb-drug interaction in diabetes mellitus. The objectives were to screen the combinatorial extract of Nigella sativa & Cinnamomum cassia's (NSCCe) alone and in combination with metformin for its potential in mitigating symptoms of diabetes mellitus-alone, and as an adjunct therapy with metformin.

*Methods:* Diabetes was induced in the animals by a single intraperitoneal injection of streptozotocin. Animals were divided into seven groups with 6 animals each: Vehicle control, Negative control, Positive control (Metformin 50 mg/kg), treatment groups 4 and 5 received NSCCe at the doses of 100 mg/kg and 200 mg/kg, respectively. Groups 6 and 7 received the same doses, in combination with Metformin (50 and 25 mg/kg). Following a 28-day dosing period, plasma glucose levels, lipid profile and renal function profile were evaluated. Histopathological examinations were performed to measure any morphological change in kidney, liver and pancreatic tissue.

*Results:* Combination of *Nigella sativa & Cinnamomum cassia* extracts significantly normalized plasma glucose levels, lipid profile and kidney function parameters, compared to the diabetic control group. Animals treated with the combinatorial extract and metformin showed more prominent effects on these parameters. Significant reversal in the pancreatic cell damage was observed on treatment with NSCCe.

*Conclusions:* This study generates evidence to support Nigella sativa & Cinnamomum cassia as an adjunctive in diabetes treatment protocols.



## 1. Introduction

Plant-based medicaments have served as the most important therapeutic agents to treat various diseases [1]. However, in recent time due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine, there is a renewed and growing interest in the use of plant derived biologically active compounds as potent medicaments [1]. Diabetes mellitus is an endocrine disorder characterized by hyperglycaemia [2] and altered carbohydrate metabolism. In India, the number of people suffering from diabetes is expected to rise from 20 to 57 million in 2025 [2]. Type 1 diabetes mellitus (T1D) is one of the major factors contributing towards diabetes in youth, and children. As evident from the global epidemiological data, $\geq$ 85% of all diabetes cases in youth <20 years are attributed to T1D [3]. The incidence of T1D in adults is marginally lower than in children, however, about one fourth of persons diagnosed with T1D are adults [3]. Scrutinizing the recent Indian epidemiological data from the Registry of People with Diabetes [4] with Young Age at

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Onset (YDR), T1D seemed to be most prevalent cause of diabetes in youth, contributing over 60% of the cases. T1D is the result of autoimmune mediated destruction of β-cells which leads to dependence of the subject on exogenous insulin throughout his life. Current therapies utilized for T1D are very limited, and mostly centred to insulin administration. Insulin treatment is associated with a major drawback of glycaemic instability. Moreover, the repeated dose of parenteral insulin, and lack of efficient and feasible oral medications, make these therapies highly patient non-compliant. This is of prime concern in T1D, since considerable cases of such kind are reported in the pediatric population, and the therapy is life-long. Hence, search for alternative treatment areas, and research showing improvements in the disease parameters as compared to currently established treatments are always welcome. One such less explored but highly promising area is herbal medicine. The introduction of medicinal herbs that lower blood glucose level has brought a revolution in the field of medicine [5] and are considered as the next generation medicines for the management of diabetes [6]. WHO (2001) estimated that 80% of world population rely on medicinal plants for their primary health care needs [7]. The ethnobotanical information reveals that about 800 plants like *Gymnema sylvestre, Brassica juncea, Hibiscus rosa sinesis, Lantana camara, Momordica charantia, Pterocarpus marsupium* etc. possess anti-diabetic potential [8]. *Nigella sativa,* traditionally used as the component of food can reduce glucose absorption in intestine and hepatic gluconeogenesis [9]. Thymoquinone is an active component of *Nigella sativa* that is reported to reduce the blood glucose level as well as body weight [10]. Thus, the plant is a potent nutraceutical for the management of diabetes mellitus. On the other hand, methyl hydroxyl chalcone polymer (MHCP), a biologically active substance of *Cinnamomum cassia* was proposed to be effective insulin mimetic which activates the pathways leading to glucose utilisation in cells [11]. These herbs have been reported to delay carbohydrate digestion by competitively inhibiting α-glucosidase a membrane bound enzyme of the intestinal brush border [12]. *Nigella sativa and C. cassia*, both contain polyphenols which facilitates lipid and carbohydrate metabolism. It also controls hyperglycemia, dyslipidemia, insulin resistance, oxidative stress and stress sensitive signalling pathways and inflammatory pathways [13,14]. Thus, the present study was undertaken to evaluate pharmacodynamic interaction of the combinatorial extract of *Nigella sativa* and *C. cassia*, (NSCCe) in T1D induced rats. The NSCCe was compared with metformin as standard, and was evaluated for α-glucosidase inhibitory activity, biochemical estimation such as glucose, lipids, creatinine, blood urea nitrogen, and histopathology.

## 2. Materials and methods

### 2.1. Chemicals

Streptozotocin (Batch number KL3456A) was purchased from SRL Laboratories, India. Metformin was received as gift sample from USV Ltd, Mumbai, India. Glucose estimation kit, triglyceride estimation kit, total cholesterol estimation kit, low density lipoprotein direct kit and high density lipoprotein direct kit were procured from Transasia Bio-medicals Ltd, Mumbai, India.

### 2.2. Plant material

The seeds of *Nigella sativa* & *C. cassia* were purchased from a local vendor in Vile Parle (East) Mumbai. The specimens of the plants were authenticated from Agharkar Research Institute, Pune & the voucher samples were kept for reference. The voucher no. is S-170 for *Nigella sativa* & S/B 114 for *C. cassia*.

### 2.3. Animals

Male Wistar rats weighing between 150 and 200 gm were procured from the animal house of SPP School of Pharmacy & Technology Management SVKM's (NMIMS). The animals were maintained in well ventilated room with 12 h light and 12 h dark cycle in polypropylene cages. Standard feed and tap water was provided *ad libitum* throughout the experimentation period. The study was designed according to the OECD guidelines, and the protocol of the research work approved by the CPCSEA and the Institutional Animal Ethics Committee (Protocol approval number: 1027/PO/a/07/CPCSEA).

### 2.4. Preparation of plant extracts

*Nigella sativa* (Family-Ranunculaceae) *& C. cassia* (Family Lauracea) were extracted by ethanol 95% v/v. The powdered seeds and bark of *Nigella sativa* and *C. cassia*, respectively were defatted by petroleum ether. Extraction was carried out in Soxhlet apparatus. The Soxhlet apparatus was heated at 65 °C for 6 h to prepare the extract of 100 gm of dried powdered *Nigella sativa* and *C. cassia.* The suspension was filtered and the residues were re-extracted. These different solvent extracts were pooled and concentrated in rotary flash evaporator at temperature 45 °C. Percentage yield of the extract was 4% for *Nigella sativa* and 5.2% for *C. cassia*.

#### 2.4.1. Phytochemical screening of the extracts

Both extracts were screened for major phytochemical ingredients, namely tannins, flavonoids, phenolic compounds and saponins. using the methods of Khandelwal [15]. Analysis yielded the presence of phenolics, flavonoids and tannins.

#### 2.4.2. Preparation of the combinatorial extract NSCCe

The extracts were then combined in 1:1 ratio by preparing a suspension in distilled water using 0.2% of sodium CMC as a suspending agent. The quantity was based on doses, either 200 mg/kg or 100 mg/kg [16,17].

### 2.5. In vitro studies

#### 2.5.1. α-glucosidase inhibitory activity

This test was undertaken to screen postprandial glucose excursion potential through α-glucosidase inhibitory activity of NS and CC extract per se and in combination (NSCCe). α-glucosidase inhibition plays an important role by delaying the carbohydrate metabolism in small intestine and hence decreasing the postprandial blood glucose and insulin levels.

The $IC_{50}$ values for the extracts were calculated by extrapolation method [18].

### 2.6. Animal studies

#### 2.6.1. Acute toxicity study [19].

Acute toxicity was performed using the ethanolic extract of *Nigella sativa & C. cassia* in female albino mice. From the three groups containing three female mice, the extract was administered orally at dose of 2000 mg/kg. Distilled water was administered to control group, orally by gavage. Animals were observed individually once during the first 30 min, periodically during the first 24 h, with special attention during the first 4 h, thereafter, for 14 days. If no mortality was recorded, it was planned to conduct a confirmatory test for another 14 days for validation of the results obtained in step 1. The dose was found to be safe, as confirmed with repetition of the test, and hence further animal studies were conducted.

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