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# Anti-hypercholesterolemic and anti-hyperglycaemic effects of conventional and supercritical extracts of black cumin (*Nigella sativa*)

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ABSTRACT

**Objective:** To explore the hypoglycaemic and hypocholesterolemic potential of conventional and supercritical extracts of black cumin.

**Methods:** Purposely, rat modelling was carried out for 2 months by designing three studies *i.e.* study I (normal rats), study II (hyperglycaemic rats) and study III (hypercholesterolemic rats). Each study was further divided into three groups based on diet *i.e.* control, functional diet (contained extract of black cumin prepared by using conventional solvent) and nutraceutical diet (contained extract of black cumin prepared by supercritical fluid extraction system).

**Results:** During whole trial, an abating trend was observed in the level of serum cholesterol with maximum reduction (12.8%) in nutraceutical group of study III. Low density lipoprotein and triglyceride level was also lowered maximum in study III as 17.1% and 11.6%, respectively. Whereas, highest decline in glucose level was in nutraceutical group of study II as 11.2%.

**Conclusions:** Inclusion of black cumin extracts in diet significantly lowers the occurrence of hyperglycaemia and hypercholesterolaemia. Furthermore, hypoglycaemic and hypocholesterolemic potential of nutraceutical diet is more prominent as compared to functional diet.

## 1. Introduction

Poor dietary habits, changing lifestyles and escalating consumption of processed food have paved the way towards various physiological dysfunctions. Metabolic disorders like hyperglycaemia and hypercholesterolaemia have become a great threat for sustaining healthy human life. Prevention of these malfunctions has become a major public health concern worldwide especially in developing nations. Thus, researchers are converging their attention for the identification of natural remedies to handle metabolic syndromes. For the purpose, numerous phytochemicals have been isolated from food items especially herbs and spices. These phytochemicals are widely used in the intervention and prevention of numerous disorders owing to their therapeutic potential and high pharmacological safety [1].

Diet based therapy with special reference to polyphenols has been invigorated worldwide and people are using natural food materials as an intervention against various maladies. In the past few decades, several attempts have been carried out to explore the pharmacological characteristics of a delicate and attractive spicy herb known by scientific name *Nigella sativa* (*N. sativa*) Linnaeus which belongs to the family Ranunculaceae [2]. It is also known as black cumin or black seed [3]. For thousands of

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years, black cumin has been used as spice and preservative in numerous foods like bread, pickles and other products [4]. Black cumin is rich in oil, proteins, minerals and carbohydrates [5]. Fixed and essential oils of black cumin are rich in active ingredients having health promoting potential [6]. Furthermore, studies showed that most of the fat contents are in the form of Ω-3 and Ω-6 fatty acids [7]. The oil of black cumin is enriched with unsaturated fatty acids, terpenoids and various kinds of quinones like thymoquinone, along with some alkaloids in lesser quantities. Collectively these all are good for enhancing memory [8]. Along with balanced fatty acid composition, it also contains various bioactive components and tocopherols. Most of the biological activity has been attributed to the major constitutes of the essential oil: thymoquinone (24.5%–57.0%), ρ-cymene (10.7%–40.3%) and α-thujen (1.9%–8.2%) [9,10].

Black cumin seeds contain considerable amounts of alkaloids like nigellicine, nigellidine and nigellimine, which are reported as cholesterol lowering agents [11]. Thymoquinone is effective against cancer, oxidative stress, diabetic complications and immune dysfunction as explored by several pharmacological investigations. Moreover, it has a major role in the regulation and maintenance of body homeostasis and hypocholesterolemic effect [8,12].

In recent years, black cumin is in limelight as an anti-diabetic drug owing to its ability to maintain integrity of β-cells. Diabetes mellitus is one of the leading causes of mortality all over the globe and if uncontrolled, it can target at multi-organ systems. It was observed that most diabetic cases are type II, while type I diabetes occur in childhood. According to the estimates, 376 million people worldwide in 2030 will be affected with diabetes [13]. Furthermore, studies have shown that dyslipidaemia is a major cause for cardiovascular diseases which ultimately results in high rates of morbidity and mortality among people all over the world [14]. It has been explored in various research investigations that maintained level of plasma cholesterol is important for protection from cardiovascular disease, as hypercholesterolaemia plays a vital role in the occurring of atherosclerosis [15]. Protection from cardiovascular disease is also important in a community when people are already suffering from other chronic diseases like diabetic mellitus and cancer [16].

The vegetable oil is conventionally extracted by mechanical cold-pressing process or using a solvent. Conventional methods such as solvent extraction and soxhlet, although effective for extraction, can lead to degradation of heat sensitive compounds as well as leave traces of toxic solvents in the solute [17]. This is a concern for food and pharmacy industry, because of the increasing regulation of harmful solvents used [18]. On the other hand, supercritical fluid extraction is one of the most promising techniques that attract the interest of process engineers. With supercritical fluid extraction, higher yields and better-quality products can be achieved. Although this process requires high pressures, there is no risk of fire or toxicity; solvent removal is simple, efficient and storage capability of extract can be extended. Moreover, process residue has certain nutritional value and can be used to feed cattle [19].

The objective of this research was to explore and compare the hypoglycaemic as well as hypocholesterolemic potential of conventional and supercritical fluid extracts of black cumin. This would help in more efficient recovery of bioactive moieties from black cumin along with carving the way for the development of functional foods.

## 2. Materials and methods

### 2.1. Procure of materials and preparation of powder

The present study was conducted in the Functional and Nutraceutical Food Research Section, National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Black cumin of indigenous variety was procured from local supermarket considering the quality attributes like uniformity in shape, colour and size followed by cleaning. For bioevaluation, Sprague Dawley rats were housed in the animal room of National Institute of Food Science and Technology. Diagnostic kits were purchased from Sigma–Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia). Cleaned black cumin was ground to a fine powder using grinding. Resultant black cumin powder was stored in air tight plastic bags for extraction of bioactive moieties and bioefficacy trial.

### 2.2. Preparation of black cumin extracts

#### 2.2.1. Conventional solvent extraction

The conventional solvent extract was prepared by using aqueous methanol (50% v/v) selected based on preliminary trials. For the purpose, powdered black cumin was taken in a conical flask and solvent was added in ratio of 1:6 (black cumin powder: solvent). Afterwards, the mixture was shaked in orbital shaker at 180 rpm at 50 °C temperature for 50 min. Finally, extract was filtered and subjected to rotary evaporator (Eyela, Japan) for removal of surplus solvent [20].

#### 2.2.2. Supercritical fluid extraction

Supercritical black cumin extract was obtained by using supercritical fluid extractor (SC-$CO_2$), model SFT-150 (supercritical fluid extractor incorporation USA) at 7500 psi pressure and 40 °C temperature for 180 min [21]. The solvent and extracting conditions in both conventional and supercritical fluid extractions were selected based on preliminary trials and antioxidant profiling of different solvents.

### 2.3. Ethical approval

Ethics approval was given by the head of the National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan; by reviewing the suggestions of Animal Experimentation Ethics Committee, University of Agriculture Faisalabad. Animal experiments were conducted accordance with the instructions for the care and use provided by the committee and instructed by the university.

### 2.4. Animal feed modelling

Ninety Sprague Dawley rats were housed in animal room of National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. Initially, some rats were sacrificed to establish baseline trend. The study was carried out in three categories separately. Study I comprised of rats fed on normal diet, whereas in study II and III, high sucrose and high cholesterol diets (Table 1) were given to induce hyperglycaemia and hypercholesterolaemia in them, respectively.

Efficacy study was further divided into three segments *i.e.* normal, hyperglycaemic and hypercholesterolemic rats. Three

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