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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

types of diet were given to three groups *i.e.* control, functional and nutraceutical diets.

During entire experimental period of two months, the animal room was maintained at a temperature and relative humidity of (23 ± 2) °C and 55% ± 5% respectively, with 12:12 h light: dark cycle (NIH Publications No. 8023, revised 1978). The rodent diet prepared by the incorporation of conventional extracts and supercritical extracts was accordingly labelled as functional and nutraceutical diet. During trial, normal, functional and nutraceutical diets were given to the respective groups under each study to evaluate the effects of individual treatment on the selected parameters including serum lipid profile, glucose and insulin levels.

2.5. Indexes observation

2.5.1. Feed, drink intakes and body weight gain

Average feed and water intake of each group were measured on daily basis during the whole study period [22]. Increase in body weight of rats from all experimental groups was measured weekly throughout the study period.

2.5.2. Serum lipid profile

The blood was collected by direct cardiac puncture and sera were obtained by centrifugation at 5000 rpm for 5 min at 4 °C. Serum lipid profile of rats was measured by following their respective protocols. Serum cholesterol level of rats was measured using CHOD–PAP method following the protocol of Kim *et al.* [23]. High density lipoprotein (HDL) and low density lipoproteins (LDL) in serum samples were calculated by method as mentioned by Suleria *et al.* [24]. Triglycerides in serum sample were estimated by liquid triglycerides method as illustrated by Buriro and Tayyab [25].

2.5.3. Serum glucose and insulin levels

For each study, the collected sera were evaluated for glucose concentration by GOD–PAP method as described by Kim *et al.* [22], whereas insulin level was assessed by following the method of Ahn *et al.* [26].

2.6. Statistical analysis

Data obtained were statistically analysed by employing ANOVA to check the level of significance followed by mean comparisons by Tukey's honest significant difference test [27].

3. Results

3.1. Feed intake, drink intake and body weight

It was noticed that treatments imparted non-significant effect on feed intake in all three studies. However, time interval effected significantly the feed intake of all studies. During two-month trial, feed intake in control group, functional and nutraceutical groups has increased from (13.5 ± 0.4) to (19.4 ± 0.9) g/rat/day, (13.3 ± 0.4) to (19.1 ± 0.8) g/rat/day and (12.7 ± 0.3) to (18.9 ± 0.8) g/rat/day, respectively, in study I. Similarly, in study II (hyperglycaemic rats) maximum feed intake was increased in control group from 1st to 8th week [(14.4 ± 0.6)-(21.2 ± 1.0) g/day/rat] followed by functional and nutraceutical group, [(14.1 ± 0.6)-(21.0 ± 1.1) g/day/rat and (13.9 ± 0.4)-(20.7 ± 0.9)

Table 1
Composition of diets given to trial animals.

Ingredients (%)	Normal diet	High sucrose diet	High cholesterol diet
Corn oil	10	10	10.0
Corn starch	66	26	64.5
Casein	10	10	10.0
Cellulose	10	10	10.0
Salt mixture	3	3	3.0
Vitamins	1	1	1.0
Cholesterol	–	–	1.5
Sucrose	–	40	–

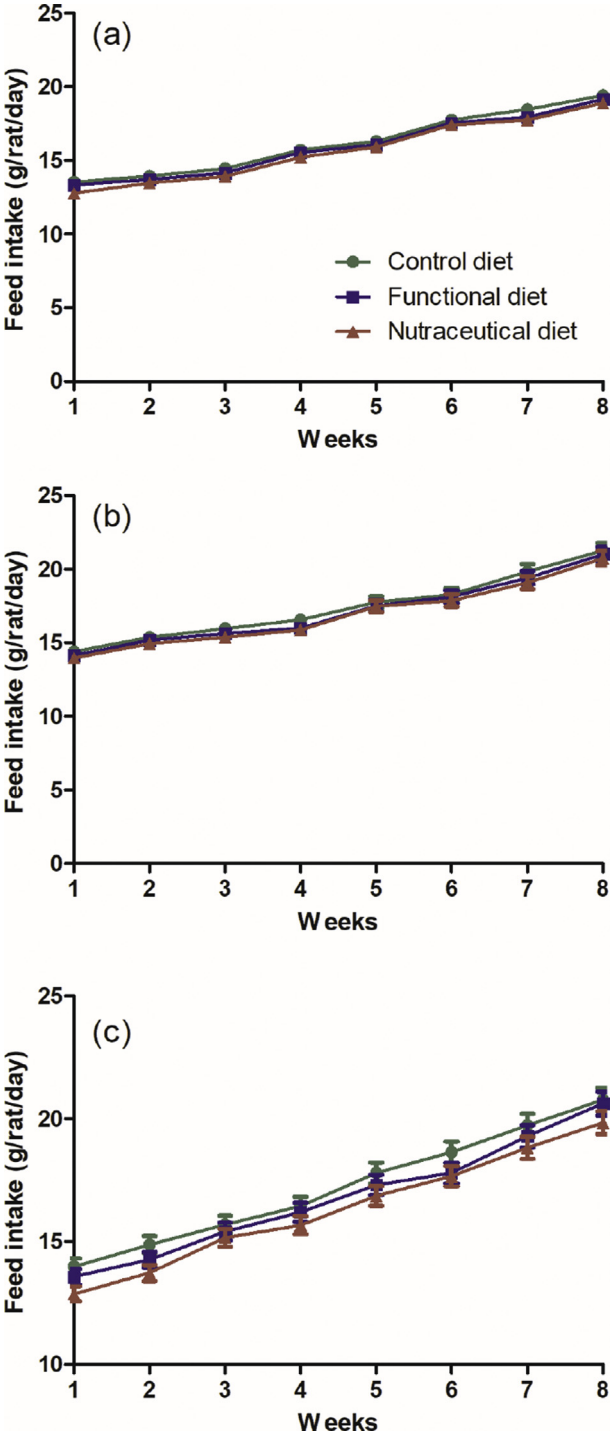

Figure 1. Feed intake of rats increased in all studies from 1st week to 8th week.
a) normal rats, b) hyperglycaemic rats, c) hypercholesterolemic rats.

g/day/rat], respectively. Likewise, in study III (hypercholesterolemic rats) maximum increase in feed intake was observed in control group from (13.9 ± 0.5) to (20.7 ± 0.9) g/rat/day, while, functional and nutraceutical group also showed increase in feed intake from (13.5 ± 0.7) to (20.6 ± 0.9) g/rat/day and (12.8 ± 0.3) to (19.8 ± 0.8) g/rat/day, respectively, throughout the study period (Figure 1).

In case of water intake, it was noticed that treatment had non-substantial effect while intervals had momentous effect on water intake in all study groups. In study I, water intake in control group, functional and nutraceutical groups have increased from (19.6 ± 0.8) to (24.0 ± 1.2) mL/rat/day, (18.5 ± 0.7) to (23.7 ± 1.1) mL/rat/day and (18.3 ± 0.9) to (23.1 ± 1.0) mL/rat/day, correspondingly, during whole trial. Furthermore, in study II the water intake increased throughout the study tenure in control, functional and nutraceutical groups from (20.0 ± 0.9) to (25.1 ± 1.2) mL/rat/day, (19.9 ± 0.9) to (24.3 ± 1.2) mL/rat/day and (18.9 ± 0.9) to (23.9 ± 1.1) mL/rat/day, respectively. Likewise, in hypercholesterolemic rats the maximum increase was observed in nutraceutical group followed by functional and control group as (18.5 ± 0.8) to (23.5 ± 1.2) mL/rat/day,

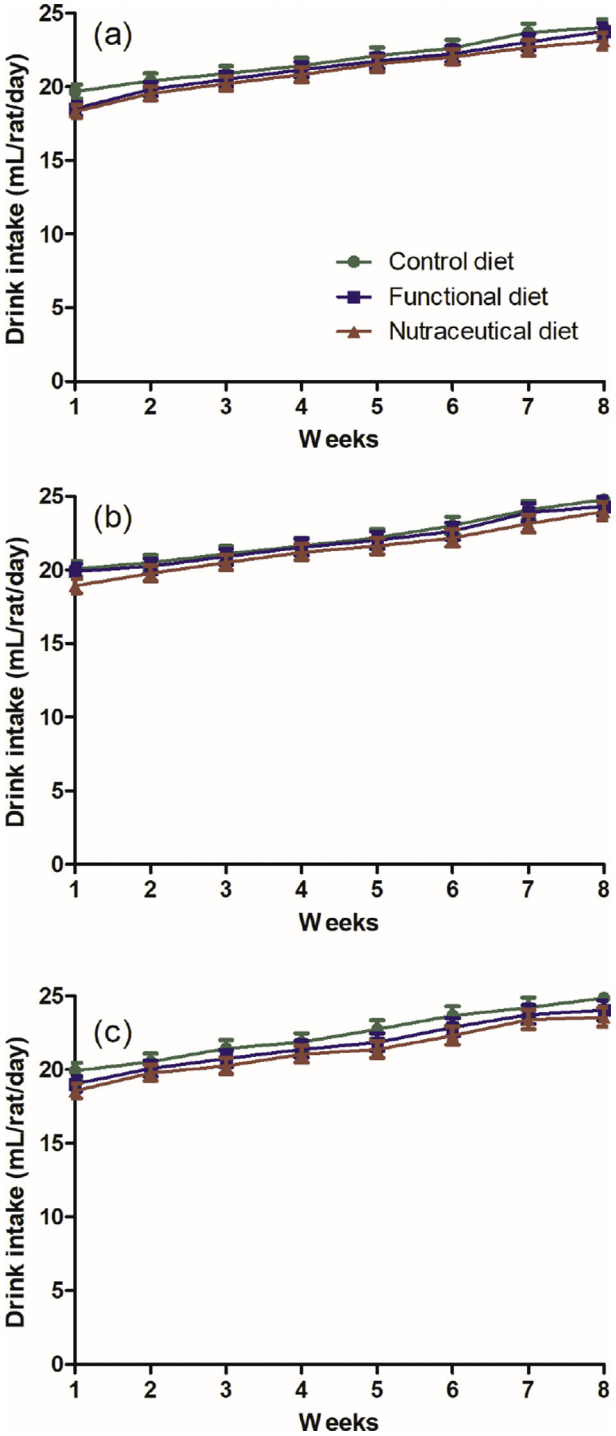

Figure 2. Drink intake of rats increased in all studies from 1st week to 8th week.
a) normal rats, b) hyperglycaemic rats, c) hypercholesterolemic rats.

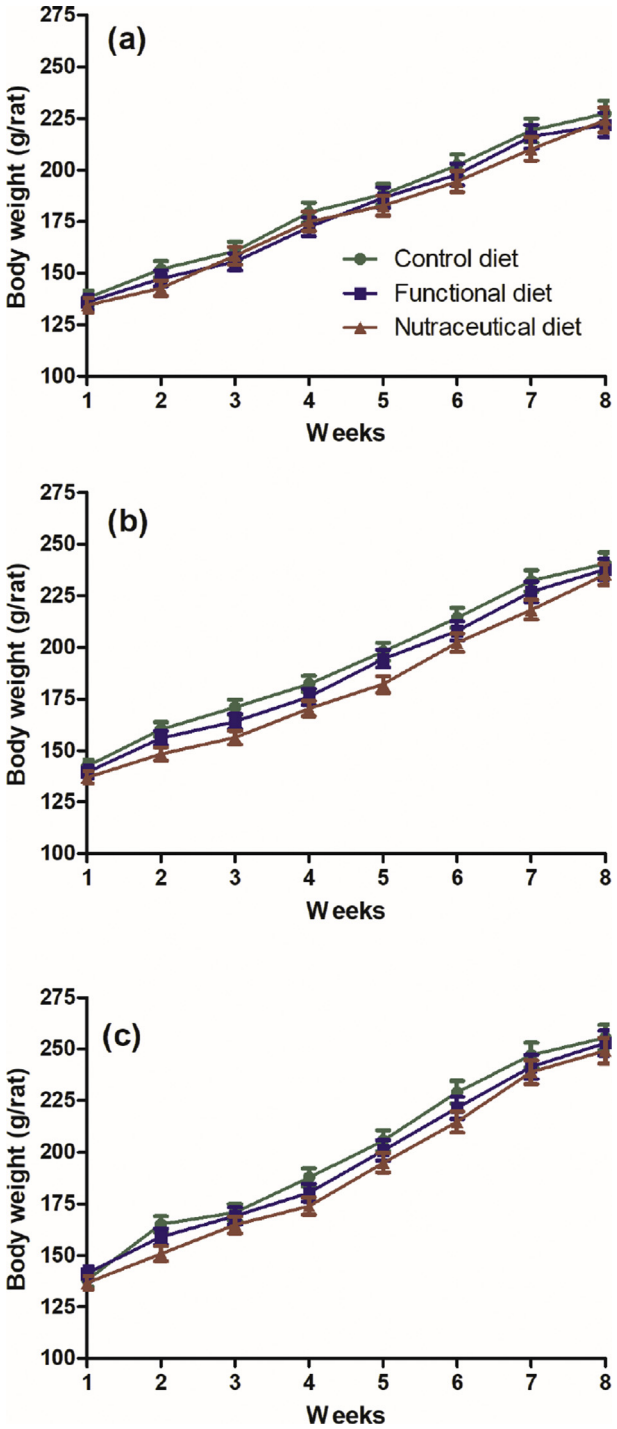

Figure 3. Body weight of rats increased in all studies over the period of 8 weeks.
a) normal rats, b) hyperglycaemic rats, c) hypercholesterolemic rats.

(19.0 ± 0.6) to (23.0 ± 1.1) mL/rat/day and (19.9 ± 0.8) to (24.8 ± 1.3) mL/rat/day, respectively (Figure 2).

In case of body weight treatments and interval imparts significant effect on body weight in all studies. In normal group of rats (study I), maximum body weight was observed in control diet group [(183.3 ± 7.6) g/rat/week] followed by functional [(179.2 ± 6.2) g/rat/week] and nutraceutical [(177.7 ± 6.5) g/rat/week] groups. The body weight for control, functional and nutraceutical groups was increased from (137.8 ± 5.2) to (227.4 ± 8.2) g/rat/week, (135.9 ± 5.5) to (221.8 ± 8.1) g/rat/week and (134.4 ± 6.2) to (224.2 ± 9.5) g/rat/week, respectively, throughout the study trial. Likewise, in hyperglycaemic group of rats (study II) the highest body weight was observed in control group [(192.6 ± 6.6) g/rat/week] followed by functional [(187.8 ± 7.9) g/rat/week] and nutraceutical [(181.2 ± 8.9) g/rat/week] group. Similarly, in case of hypercholesterolemic rats (study III) highest body weight was noticed in control group [(199.8 ± 8.6) g/rat/week]; however, minimum in nutraceutical [(190.4 ± 8.8) g/rat/week] group. In both study II and III the body weight of all groups showed a significant weight gain during study period (Figure 3).

3.2. Hypocholesterolemic potential

3.2.1. Cholesterol

In study I, mean serum cholesterol level was affected significantly by treatments and maximum level was observed in control [(83.0 ± 4.0) mg/dL] followed by functional [(77.8 ± 3.9) mg/dL] and nutraceutical [(75.9 ± 3.4) mg/dL] groups at 60 d (Table 2). In studyI, reduction in serum cholesterol level was 4.4% and 5.3% in rat groups fed on functional and nutraceutical, respectively. Likewise, in hyperglycaemic study group (study II), maximum reduction in serum cholesterol level was observed in nutraceutical group (9.1%) followed by functional group (7.5%). The serum cholesterol level in control group has increased while a decreasing trend observed in functional and nutraceutical groups during the trial period.

In study III (hypercholesterolemic rats), maximum mean serum cholesterol level was observed in control group trailed by functional and nutraceutical group. During the sixty days trial maximum reduction was assessed in nutraceutical group from (145.7 ± 6.3) to (127.0 ± 5.0) mg/dL followed by functional (146.2 ± 6.5) to (130.5 ± 6.2) mg/dL. Maximum decline in serum cholesterol level was in groups fed on nutraceutical (12.8%) followed by functional (10.7%).

3.2.2. HDL

In study I, the group of rats fed on nutraceutical diet exhibits maximum mean HDL level which non-significantly varied in functional and control group. Similar trend was observed in study II that HDL increased non-momentously (2.7%) in nutraceutical diet group followed by functional (2.5%) groups as compared to control group. Likewise, in study III a non-significant decrease in HDL level was observed in control while it elevated in functional and nutraceutical diet *i.e.* 2.7% and 3.1%, respectively (Table 3).

3.2.3. LDL

In study I, maximum mean LDL level was observed in control group that substantially diminished in nutraceutical and functional diet, respectively (Table 4). A respective decrease of 5.5% and 6.6% was measured in functional and nutraceutical diet. Similarly, in study II control group exhibited momentously higher mean LDL level as compared to nutraceutical and functional diet groups. Over the period, maximum decline was observed in nutraceutical group (12.2%) followed by functional group (10.1%) in study II. Likewise, in study III, maximum

Table 3
HDL (mg/dL) of rats in different studies.

Studies	Diet groups	0 d	30 d	60 d
Study I	Control	35.2 ± 1.5	35.3 ± 1.4	35.5 ± 1.0
	Functional	35.8 ± 1.5	36.2 ± 1.9	36.5 ± 1.1
	Nutraceutical	36.6 ± 1.7	37.0 ± 1.8	37.4 ± 1.3
Study II	Control	39.4 ± 1.4	38.9 ± 1.5	38.6 ± 1.6
	Functional	39.6 ± 1.4	40.1 ± 1.6	40.6 ± 1.7
	Nutraceutical	40.2 ± 1.5	40.8 ± 1.6	41.3 ± 1.8
Study III	Control	55.6 ± 2.0	54.8 ± 2.0	54.3 ± 2.1
	Functional	53.7 ± 2.0	54.6 ± 2.1	55.2 ± 2.0
	Nutraceutical	54.5 ± 2.4	55.5 ± 2.3	56.2 ± 1.8

Study I (normal rats), study II (hyperglycaemic rats), study III (hypercholesterolemic rats).

Table 2
Cholesterol (mg/dL) of rats in different studies.

Studies	Diet groups	0 d	30 d	60 d
Study I	Control	81.9 ± 4.3	82.6 ± 4.6	83.0 ± 4.0
	Functional	81.4 ± 4.0	79.3 ± 4.0	77.8 ± 3.9*
	Nutraceutical	80.2 ± 4.1	77.8 ± 3.5	75.9 ± 3.4*
Study II	Control	98.0 ± 4.5	99.9 ± 4.3	101.2 ± 4.8
	Functional	97.6 ± 4.9	93.4 ± 4.1	90.2 ± 4.1*
	Nutraceutical	97.3 ± 4.3	92.2 ± 3.9	88.4 ± 4.0**
Study III	Control	144.4 ± 6.4	151.7 ± 6.9	157.1 ± 7.2*
	Functional	146.2 ± 6.5	136.8 ± 5.7	130.5 ± 6.2**
	Nutraceutical	145.7 ± 6.3	135.1 ± 6.8	127.0 ± 5.0***

Study I (normal rats), study II (hyperglycaemic rats), study III (hypercholesterolemic rats). Asterisks indicate that the differences (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$) between the means of different diets (control, functional and nutraceutical) are statistically significant as determined by one-way ANOVA with posthoc Tukey's honestly significant difference test.

Table 4
LDL (mg/dL) of rats in different studies.

Studies	Diet groups	0 d	30 d	60 d
Study I	Control	31.1 ± 1.4	31.3 ± 1.3	31.5 ± 1.0
	Functional	30.7 ± 1.4	29.7 ± 1.1	29.0 ± 1.2
	Nutraceutical	30.3 ± 1.2	29.2 ± 1.0	28.3 ± 1.3
Study II	Control	47.0 ± 2.1	47.9 ± 2.3	48.5 ± 1.8
	Functional	46.5 ± 2.2	43.8 ± 2.2	41.8 ± 1.7**
	Nutraceutical	45.9 ± 2.3	42.7 ± 2.1	40.3 ± 1.9**
Study III	Control	60.8 ± 2.5	64.0 ± 2.0	66.3 ± 2.4*
	Functional	61.6 ± 2.4	55.7 ± 2.3	51.7 ± 2.3**
	Nutraceutical	61.3 ± 2.3	55.4 ± 2.4	50.8 ± 2.5**

Study I (normal rats), study II (hyperglycaemic rats), study III (hypercholesterolemic rats). Asterisks indicate that the differences (*: $P < 0.05$; **: $P < 0.01$) between the means of different diets (control, functional and nutraceutical) are statistically significant as determined by one-way ANOVA with posthoc Tukey's honestly significant difference test.

decline of 17.12% was observed in rats fed on nutraceutical diet trailed by functional diet group (16.00%).

3.2.4. Triglycerides

Treatments impart significant effects on triglycerides level in all the three studies; whilst, days interval imparted non-momentous effect in study I while significant effect in case of study II and III. Mean triglycerides in study I was at highest level in control followed by functional and nutraceutical groups. In functional group a reduction of 3.4% was observed, whilst, in nutraceutical group 4.4% decline in triglycerides level was noticed from 0 to 60th day. Similarly, in study II, maximum reduction was observed in nutraceutical group followed by functional group as 8.2% and 6.6%, respectively. Likewise, in study III nutraceutical diets showed greater suppression for this attribute as compared functional and control group. Nutraceutical diet showed maximum reduction (11.6%) trailed by functional group (10.0%) (Table 5).

3.3. Hypoglycaemic potential of black cumin extracts

3.3.1. Glucose

Mean serum glucose level in study I was maximum in control group trailed significantly by functional and nutraceutical groups. Functional and nutraceutical diets cause 4.1% and 5.2% reduction in the level of glucose from 0 to 60th day. Whereas, in study II nutraceutical group illustrated maximum reduction in mean glucose level (11.3%) trailed by functional group (10.2%). Likewise, in high cholesterol fed rats (study III) maximum alleviation of glucose was noticed in nutraceutical diet group followed by functional group as 9.1% and 7.4%, correspondingly (Table 6).

3.3.2. Insulin

Insulin level in study I was (8.8 ± 0.2), (8.6 ± 0.2) and (8.5 ± 0.3) μU/mL in control, functional and nutraceutical diets, respectively at 60th day. It was observed that in control, functional and nutraceutical groups of normal rats the level of insulin raised non-significantly with time interval. Nonetheless in study II, maximum increase in insulin level was recorded in nutraceutical group followed by functional diet group as 8.5% and 6.6%, correspondingly. Similarly, in study III, control group showed a decrease in mean insulin value from (10.5 ± 0.4) to (10.4 ± 0.4) μU/mL while increasing trend was observed in both functional (4.0%) and nutraceutical (6.2%) diet groups (Table 7).

Table 5
Triglycerides (mg/dL) of rats in different studies.

Studies	Diet groups	0 d	30 d	60 d
Study I	Control	67.4 ± 2.5	67.7 ± 2.1	67.9 ± 2.2
	Functional	66.8 ± 2.1	65.4 ± 2.3	64.5 ± 2.3*
	Nutraceutical	65.2 ± 2.3	63.5 ± 2.1	62.3 ± 2.4*
Study II	Control	77.7 ± 2.8	79.1 ± 2.7	80.1 ± 2.9
	Functional	76.3 ± 2.6	73.4 ± 2.5	71.2 ± 2.8*
	Nutraceutical	75.4 ± 2.5	71.8 ± 2.3	69.2 ± 2.7*
Study III	Control	95.5 ± 4.1	99.9 ± 4.0	103.1 ± 5.0*
	Functional	96.7 ± 3.9	90.9 ± 4.1	87.0 ± 4.8**
	Nutraceutical	95.9 ± 3.2	89.5 ± 3.9	84.7 ± 4.4**

Study I (normal rats), study II (hyperglycaemic rats), study III (hypercholesterolemic rats). Asterisks indicate that the differences (*: $P < 0.05$; **: $P < 0.01$) between the means of different diets (control, functional and nutraceutical) are statistically significant as determined by one-way ANOVA with posthoc Tukey's honestly significant difference test.

Table 6
Glucose (mg/dL) of rats in different studies.

Studies	Diet groups	0 d	30 d	60 d
Study I	Control	91.2 ± 4.2	92.0 ± 4.3	92.5 ± 4.1
	Functional	90.3 ± 3.8	88.1 ± 4.1	86.6 ± 3.9*
	Nutraceutical	89.7 ± 3.9	87.1 ± 3.9	85.0 ± 4.7*
Study II	Control	137.2 ± 6.1	145.2 ± 6.5	150.5 ± 6.7
	Functional	136.7 ± 6.2	128.7 ± 6.2	122.7 ± 6.3**
	Nutraceutical	135.5 ± 6.0	126.6 ± 6.1	120.2 ± 6.4***
Study III	Control	100.0 ± 4.0	102.5 ± 4.9	104.3 ± 4.7
	Functional	99.5 ± 4.1	95.1 ± 4.3	92.1 ± 4.1*
	Nutraceutical	99.3 ± 3.9	94.2 ± 4.6	90.3 ± 4.8**

Study I (normal rats), study II (hyperglycaemic rats), study III (hypercholesterolemic rats). Asterisks indicate that the differences (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$) between the means of different diets (control, functional and nutraceutical) are statistically significant as determined by one-way ANOVA with posthoc Tukey's honestly significant difference test.

Table 7
Insulin (μU/mL) of rats in different studies.

Studies	Diet groups	0 d	30 d	60 d
Study I	Control	8.7 ± 0.3	8.7 ± 0.3	8.8 ± 0.2
	Functional	8.3 ± 0.4	8.5 ± 0.3	8.6 ± 0.2
	Nutraceutical	8.2 ± 0.6	8.4 ± 0.4	8.5 ± 0.3*
Study II	Control	12.3 ± 0.5	12.2 ± 0.5	12.0 ± 0.5
	Functional	12.1 ± 0.5	12.5 ± 0.3	12.9 ± 0.3*
	Nutraceutical	11.7 ± 0.4	12.3 ± 0.4	12.7 ± 0.4**
Study III	Control	10.5 ± 0.4	10.5 ± 0.4	10.4 ± 0.4
	Functional	9.9 ± 0.3	10.1 ± 0.4	10.3 ± 0.4
	Nutraceutical	9.6 ± 0.3	9.9 ± 0.3	10.2 ± 0.4*

Study I (normal rats), study II (hyperglycaemic rats), study III (hypercholesterolemic rats). Asterisks indicate that the differences (*: $P < 0.05$; **: $P < 0.01$;) between the means of different diets (control, functional and nutraceutical) are statistically significant as determined by one-way ANOVA with posthoc Tukey's honestly significant difference test.

4. Discussion

Globally, nutraceuticals and functional foods are in limelight owing to their health enhancing characteristics. It has been noticed that functional foods have established their therapeutic worth by alleviating numerous lifestyle related disorders like inflammations, hyperglycaemia and hypercholesterolaemia [28]. The present observations agree with the findings of Meral *et al.* [29] and Kanter *et al.* [30]. They concluded that feed intake and weight gain were higher in control group as compared to black cumin enriched treatments. Furthermore, it was reported that feed intake and weight gain increased with high-fat diets while supplementation of antioxidants can control the deposition of fat in the body [31]. Reduction in feed and drink intakes may be owing to black cumin slight anorexic effects [31]. Later, Sultan *et al.* [32] observed that control group has maximum feed intake while groups fed on diets rich in black cumin fixed oil showed least intake *i.e.* (21.26 ± 2.13) and (20.17 ± 1.22) mg/rat/day, respectively. They also noted elevated water intake with the passage of study period. It has also been observed that weight gain of groups fed on black cumin fixed and essential oils is 13.9% and 11.4% lower than

control group. Moreover, Parhizkar *et al.* [31] observed that during treatment period, the body weight of control and supercritical extract fed group of rats was increased at the initiation of study afterwards it maintained at a certain point. This influence of extracts on body weight might be related to the black cumin action on metabolism of lipid.

In present study the serum cholesterol level of rats decreased in groups fed on nutraceutical diets as well as functional diets, whilst, it shows an elevated trend in control group. The findings are in accordance with the outcomes of Le *et al.* [33], which was reported that ether extracts of black cumin significantly reduced the cholesterol level of rats. Previously, it has also been depicted that volatile oil of black cumin is very effective against hyperlipidaemia [34]. Cholesterol lowering property of black cumin may be owing to reduction in hepatocytic synthesis of serum cholesterol or by lowering reabsorption in small intestine [24]. It was conjectured that activation of peroxisome proliferator-activated receptor is responsible for cholesterol reducing mechanism [35].

Results regarding the serum LDL level are in accordance with previous literature as Salem [36] reported the reduction of non- HDL cholesterol fractions by induction of black cumin extracts in diet. Afterwards, Buriro and Tayyab [25] elucidated that low fat diet supplemented with black cumin and 3% sunflower oil cause notable lowering of LDL cholesterol of blood serum. Likewise, it was reported that 30 mg administration of black cumin per kg body weight of rats for 20 weeks study interval significantly lowered the LDL level of blood from (35.73 ± 3.56) to (24.35 ± 2.40) mg/dL. Besides, Sultan *et al.* [37] expounded that at dose 1% and 2%, black cumin powder reduced serum low density lipoprotein level 24.7% and 24.3%, respectively. One of their peers, Al-Naqeep *et al.* [38] while working on atherosclerosis gave conclusions that feeding of rabbits by black cumin enriched diet significantly decrements the LDL level of serum. Frequent changes in the plasma lipid concentration are observed in diabetic patients that certainly contribute to the development of vascular diseases. The free radical generation associated with LDL modification can further lead to atherosclerosis [39]. The present findings agree with the findings of Kanter *et al.* [30]. It was hypothesized the cholesterol deposition was due to increased activities of cholesterol synthesizing enzyme and reduced activities of antioxidants. Considering the negative association of serum cholesterol with tocopherols, it can be hypothesized that supplementation of diets with rich sources of tocopherols can reduce the elevated cholesterol level [40]. Recently, it has been reported that supplementation of *N. sativa* momentously lowered plasma levels of cholesterol, LDL and triglycerides to about 15.65, 14.10 and 20.64 mg/dL, respectively [41].

Current findings regarding the serum triglyceride level are in line with the findings of Le *et al.* [33], which proved that the *N. sativa* significantly decreased the triglycerides level of blood as compared to control group. Likewise, Kökdil *et al.* [42] gave orally 1 mL of black cumin fixed oil/kg body weight to the diabetic rats for 4 weeks and suggested that the triglycerides levels significantly decreased during the research work. Besides, Murugesan *et al.* [43] testified that when the black cumin was given at 150 mg/kg body weight for 15 d, it significantly declined the triglyceride level of blood that was (132.02 ± 0.69) mg/dL at the first day and (119.06 ± 0.44) mg/dL at the termination of study.

It was observed that the serum HDL level increased non-significantly in all three studies. The findings are in harmony with explorations of El-Dakhakhny [44] and they reported an increase in HDL level by oral feeding of black cumin oil to rats as compared to control group. According to a report, dose rate of 30 mg/kg body weight of albino rats significantly increased the level of HDL in serum.

The results obtained regarding the effects of black cumin on serum glucose level in current research are similar with the findings of Mohtashami *et al.* [45], in which they used volunteer humans in the research and gave them 2.5 mL of black cumin fixed oil twice a day for two weeks. At the beginning of the study the glycaemic index of that group was (102.4 ± 20.8) mg/dL that was decreased to (91.5 ± 12.5) mg/dL. In that research, it was noticed that the fixed oil dose of 5 mL per day has significantly reduced the blood glucose level of people and did not cause any side effect. Similarly, Kökdil *et al.* [42] suggested that the blood glucose level momentously decreased when the rats were supplemented with 1 mL of black cumin fixed oil per day for four weeks. Meanwhile, in another exploration a hypothesis was developed that elevated glucose level in diabetic rats may in turn trigger the enhanced insulin release in serum due to gluconeogenesis in liver is altered [46].

Afterwards, Kanter [47] concluded that notable reduction in glucose and elevation in serum insulin level in diabetic rats can be observed by an administrating 400 mg/kg/day and 50 mg/kg/day of black cumin powder and thymoquinone, respectively. Hypoglycaemic activity of black cumin may be due to stimulation of release of insulin and partial proliferation of pancreatic beta-cells, mediated by extra pancreatic actions [30]. Moreover, hyperglycaemia is developed by hepatic production of glucose diabetic patients which tends to decrease due to black cumin extract treatments [48]. It has also been observed that level of various enzymes increased in serum ultimately reduces the permeability of glucose precursors to hepatic system and lowering the process of gluconeogenesis [29]. In case of serum insulin level, the data obtained from the present research was comparable with the findings of Le *et al.* [33], in which they demonstrated that petroleum ether extract of black cumin exerted an *in vivo* insulin-sensitizing action.

In conclusion, this research investigates that black cumin conventional as well as supercritical fluid extracts possess significant hypoglycaemic and hypocholesterolemic potential. Furthermore, supercritical fluid extracts are more efficient as compared to conventional extracts which may be owing to superior recovery of bioactive moieties through supercritical fluid extraction technique as compared to conventional technique. The findings of current investigation would be helpful in designing various functional foods to curtail the metabolic dysfunctions like hyperglycaemia and hypercholesterolaemia.

Conflict of interest statement

The authors showed no conflict of interest.

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