

UTILIZATION OF MICROWAVE ASSISTED BLACK CUMIN SEED EXTRACT AS HYPOCHOLESTEROLEMIC AGENT IN ALBINO RATS

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ABSTRACT

Main aim of this study was to evaluate hypocholesterolemic potential of microwave-assisted black cumin (*Nigella sativa*) extracts (MABCEs) in a rat bioassay. Efficacy trial in this study comprised of 25 male albino rats which were divided into 5 groups having 5 rats each. Out of these 25 rats, 20 were hypercholesterolemic and 5 were normal rats. Hypercholesterolemia was induced by providing high cholesterol diet for 15 days, and after the onset of hypercholesterolemia these rats were administered with different concentrations of the MABCE *i.e.* 150, 300 & 450 mg/kg B.W. for a period of 28 days. The administration of extract displayed significant lowering in the lipid profile of the experimental rats. The 300mg/kg B.W. dose of black cumin MAE provided the optimum results giving cholesterol, triglyceride and LDL-c content lowered by 14.9%, 11.32% and 12% and value of HDL-c elevated by 12.88% compared to the hypercholesterolemic control. Similarly, there was a percent elevation in levels of SOD and CAT by 19.83% and 13.97%. The current study concluded that MABCEs have hypocholesterolemic effect thus can be used for its therapeutic property.

Keywords: Microwave assisted extraction, phenolics, hypercholesterolemia, antioxidant, black cumin

INTRODUCTION

Nutraceuticals have been known throughout the world and are found to be well credited for their use as therapeutic agents. The properties these nutraceuticals possess have great effect inside the body and assists with the structural and functional maintenance also preventing from acquiring various diseases (Espin *et al.*, 2007). There are a number of plants and seed which provide with the different phytochemicals including polyphenols, flavonoids, anthocyanins etc. that we consume on our daily basis (Bech-Larsen and Scholderer, 2007). Different diseases are being treated in current age by the help of these nutraceuticals that are present in the plants consumed in our diet. Such diet-based therapies are the need of the hour as the drug load is increasing by the use of multiple medicines for different disorders (Espin *et al.*, 2007; Bech-Larsen and Scholderer, 2007).

Black cumin also commonly known as Kalonji in Urdu whereas its scientific name is *Nigella sativa* is a vital plant especially the seeds as they have the medicinal history for treating different diseases and is known to be a considerable part of the family named *Ranunculaceae* (Aggarwal and Kunnumakkara, 2009). The plant of black cumin seed has acquired a notable place because of its use as a medicine. Study shows that this seed has been in use since a long time traditionally by consuming the seed and in its extract form as well (Ramadan and Morsel, 2003). It provides with the treatment of different autoimmune diseases also in earlier days it was used for treating headaches, fever, stroke, heart related diseases. It was shown to have reduced the effect of inflammation thus promoting anti-inflammatory property of the seed (Cheikh-Rouhou *et al.*, 2007).

The disturbance in the lipid concentrations in the human body may lead to hyperlipidaemia or hypolipidemia (Bamosa *et al.*, 2002). An increase in the level of lipids including cholesterol, LDL and triglycerides is the cause of

hyperlipidaemia and the incidence of this abnormality has increased hugely in the past years. The common condition that is found in our society is the hypercholesterolemia which is contributes to the development of ischemic heart disease (Ramadan and Morsel, 2003). The World Health Organization (WHO) has reported that the ischemic heart disease has been the major cause of deaths in Pakistan in 2012 that killed around 111.4 thousand individuals (WHO, 2019). The need to cure and prevent this disease from increasing by the hour is very important thus we require therapeutic agents that will do the job. Black cumin seed in earlier studies reported to have hypercholesterolemic properties and was able to prevent from such condition (Khalid *et al.*, 2019).

There are many different procedures used in order to extract the phenolics present in a plant material. Some of the earlier conventional procedures included maceration, decoction and distillation, respectively. However, in this modern age the use of non-conventional procedures is increasing day by day. Some of these procedures include the ultra-sound assisted extraction (UAE), microwave assisted extraction (MAE), pulse-electric field extraction (PEF) and so on. These non-conventional techniques provide a number of benefits as compared to the conventional techniques. Microwave assisted extraction (MAE), one of the non-conventional technique for the extraction of oils and extracts from plants is being used greatly these days. The method specifically involves the microwave radiations which assists the extraction. This technique has been reported as one of the better techniques as it provides with an increased yield of extract, far efficient extraction, reduced use of the solvent and lesser time to achieve the extraction (Liu *et al.*, 2013). Likewise, in our study the phenolics are extracted through the microwave assisted extraction technique from the seed of our interest *i.e.* black cumin seed.

The aim of this study was to extract the essential oils from the seed of black cumin by the method of microwave assisted extraction and then administrating

the extract with higher phenolics to the experimental rats in order to determine the hypercholesterolemic effect of the extract obtained.

MATERIAL AND METHODS

Plant Material

Black cumin seeds (*Nigella sativa*) were procured from a local market in Lahore. The seeds of black were washed, and then air-dried at room temperature to avoid any dirt particles or other impurities. The seeds after drying were crushed in an electric grinder for 1 min until a fine powder was obtained to further proceed with the experiments.

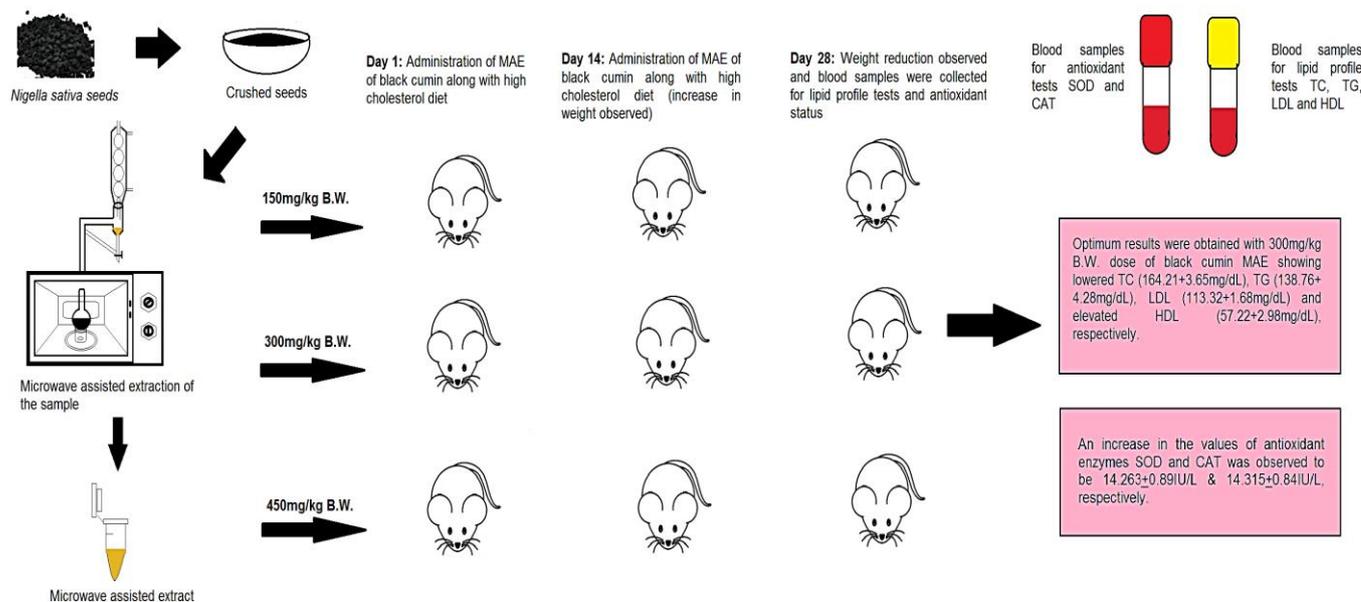


Figure 1 Graphical representation of experimental design. Microwave assisted extraction of *Nigella sativa* seeds and administration of extracts in different doses to the study rats. After the administration analysis of the blood samples to determine the lipid lowering effect of MAE of black cumin seeds

Experimental animals

Male albino rats were purchased from animal house of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, specifically weighing 200g-250g. The rats were then acclimatized on a basal diet for one-week time period. The environmental conditions were controlled through the trial period i.e. temperature (23 ± 2 °C) and relative humidity ($55 \pm 5\%$) along with 12- h light-dark period (Makni et al., 2008).

Induction of Hypercholesterolemia

Experimental hypercholesterolemic diet was designed using corn oil (10%), corn starch (64.5%), cholesterol (1.5%), protein (10%), cellulose (10%), mineral (3%) and vitamins (1%). Groups B₀, B₁, B₂, & B₃ were subjected to high cholesterol diet for first 15 days for the purpose of inducing hypercholesterolemia. The induction was validated by examining their total cholesterol content at 15th day (Imran et al., 2018).

Experimental design

The 25 rats in our study were divided into 5 groups i.e. N₀, B₀, B₁, B₂, and B₃. Group N₀, the normal group was subjected to normal basal diet throughout the study, while group B₀ (hypercholesterolemic control group) was subjected to the hypercholesterolemic diet only without the administration of any extract. On the other hand, remaining groups i.e. B₁, B₂, B₃ were fed on high cholesterol diet along with various concentrations (150, 300 and 450 mg/kg B.W.) of black cumin seed extract (Khazdair, 2018).

Effect of extract on hypercholesterolemia and safety assessment

Rats were anesthetized by exposure to isoflurane/chloroform and the blood samples was collected in tubes by cardiac puncture and examined at 0 day (baseline trend), 15th day (post administration of cholesterol rich diet) and 21st day post induction of hypercholesterolemia along with administration of respective extracts to authenticate hypercholesterolemic effect of the extracts (Imran et al., 2018).

Statistical analysis

Preparation of extract

The extraction was performed in an adapted commercial kitchen microwave oven whose maximum output was 700 W with 2450 MHz of microwave irradiation frequency and a power divider of three levels (low, medium, high). In the MAE procedure, a 25g aliquot of ground black cumin seeds were placed in a 250 ml round bottom flask; 25 ml of distilled water was then added to moisturize the seeds for around 30 min. The flask was then connected to a Clevenger apparatus and heated using varied level powers of 50, 100 and 150 W for extraction time 1, 5, 10 and 15 minutes respectively. The volatile distillate was eluted out by n-hexane and dried through anhydrous sodium sulphate. The n-hexane was removed later under vacuum conditions and the extract was refrigerated prior to analysis (Liu et al., 2013).

Collected data was reported as mean value \pm standard deviation. Completely randomized design was conducted with ANOVA at a significance level of $p \leq 0.05$. The significant difference between mean values was determined by Tukey-HSD comparison test. Statistical analysis was determined by using Statistical package (Statistix 9.0) (Steel et al., 1997).

RESULTS

Total cholesterol

Statistical analysis revealed that treatments and time interval (0, 14, 28 days) have significant ($P \leq 0.05$) effect on cholesterol content of experimental rats. The mean values regarding the effect of different administrated concentrations (150, 300 & 450mg/kg B.W.) of MABCEs on the cholesterol content of experimental rats are mentioned in Figure 2. Results for total cholesterol content showed maximum (29.4%) percent reduction in group B₂ followed by B₁ (23.5%) and B₃ (22.40%). As compared to B₀ (232.65 ± 3.22 mg/dL), cholesterol content observed in groups B₁, B₂, and B₃ were as 177.79 ± 2.50 mg/dL, 164.21 ± 3.57 mg/dL, & 180.52 ± 2.80 mg/dL, respectively on 28th day of administration of MABCE. The highest reduction in the cholesterol content was observed in B₂ hypercholesterolemic group.

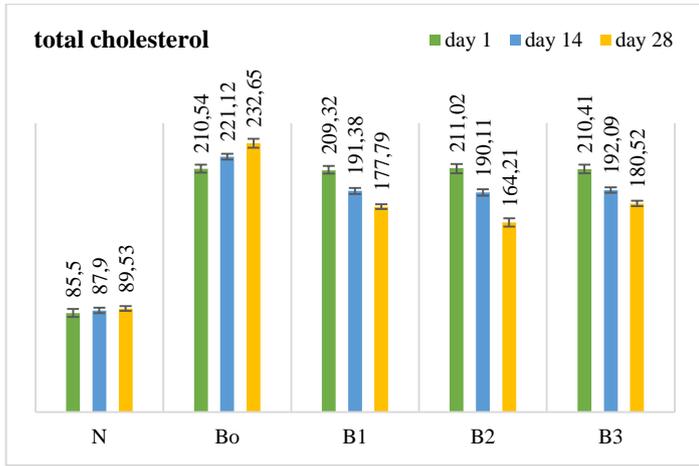


Figure 2 Effect of MAE of *Nigella sativa* (black cumin) on total cholesterol levels in hypercholesterolemic rats

N₀= Basal diet; B₀= HCD; B₁= HCD + MABCE (150mg/kg B.W.); B₂= HCD + MABCE (300mg/kg B.W.); B₃= HCD + MABCE (450mg/kg B.W.)

Total Triglycerides

Statistical analysis revealed that treatments and time interval have significant ($p < 0.05$) effect on total triglyceride concentration in experimental rats. The mean values for the concentration of triglycerides in rats are presented in Table 1. Total triglyceride content noticed in B₀ (168.67±2.80mg/dL) reduced to 151.22±2.98mg/dL, 149.57±3.55mg/dL, and 152.03±2.65mg/dL in groups B₁, B₂, B₃, respectively. On the other hand, total triglycerides content decreased from 161.10±2.49mg/dL (0 day) to 155.10±2.65mg/dL (14th day) and 149.92±3.17mg/dL (28th day), respectively. As compared to hypercholesterolemic control group (B₀), significant percent reduction of 10.34%, 11.32% and 9.87% was observed in hypercholesterolemic rats that were administrated to 150mg/kg (B₁), 300mg/kg (B₂), 450mg/kg (B₃) of microwave assisted black cumin seed extracts. The group B₂ showed the maximum percent reduction.

Table 1 Effect of MAE of *Nigella sativa* (black cumin) on triglyceride levels in hypercholesterolemic rats

Parameter	Treatments	Study intervals (days)			Means
		0	14	28	
Triglycerides – mg/dL	N ₀	76.12±1.09	77.59±2.33	80.42±2.17	78.04±1.86
	B ₀	160.36±2.75	169.23±1.77	176.41±3.89	168.67±2.80 ^a
	B ₁	161.53±3.54	150.60±2.67	141.54±2.74	151.22±2.98 ^b
	B ₂	160.50±2.69	149.45±3.69	138.76±4.28	149.57±3.55 ^b
	B ₃	162.01±2.36	151.11±2.81	142.98±2.79	152.03±2.65 ^b
Means		161.10±2.49 ^a	155.10±2.65 ^b	149.92±3.17 ^c	

N₀= Basal diet; B₀= HCD; B₁= HCD + MABCE (150mg/kg B.W.); B₂= HCD + MABCE (300mg/kg B.W.); B₃= HCD + MABCE (450mg/kg B.W.)

HCD = High Cholesterol Diet

MABCE = Microwave Assisted Black Cumin Extract

LDL

The results of the statistical analysis demonstrated significant ($p < 0.05$) effect of variables (treatments and time intervals) on the LDL content of the experimental rats. The mean values for the LDL content are presented in Table 2. The results evaluated the effect of microwave assisted black cumin extracts on hypercholesterolemic rats. The LDL concentration lowered from

133.13±2.65mg/dL at 0 day to 128.92±2.98mg/dL at 14th day and 124.99±1.94mg/dL at 28th day, respectively. However, the LDL content displayed in B₀ (138.56±2.53mg/dL) was lowered to 128.08±2.94mg/dL (B₁), 121.67±2.63mg/dL (B₂) and 127.74±2.55mg/dL (B₃), respectively. In comparison to the hypercholesterolemic control group B₀, considerable percent reduction was observed in B₁ (7.5%), B₂ (12%) and B₃ (7.8%), respectively.

Table 2 Effect of MAE of *Nigella sativa* (black cumin) on LDL levels in hypercholesterolemic rats

Parameter	Treatments	Study intervals (days)			Means
		0	14	28	
LDL cholesterol mg/dL	N ₀	49.52±1.97	50.44±2.14	47.13±1.77	49.03±1.96
	B ₀	132.42±3.21	138.92±2.34	144.34±2.05	138.56±2.53 ^a
	B ₁	134.53±1.98	127.18±3.87	122.54±2.97	128.08±2.94 ^b
	B ₂	130.21±3.45	121.47±2.76	113.32±1.68	121.67±2.63 ^c
	B ₃	135.36±2.65	128.11±3.79	119.76±1.22	127.74±2.55 ^b
Means		133.13±2.65 ^a	128.92±2.98 ^b	124.99±1.94 ^c	

N₀= Basal diet; B₀= HCD; B₁= HCD + MABCE (150mg/kg B.W.); B₂= HCD + MABCE (300mg/kg B.W.); B₃= HCD + MABCE (450mg/kg B.W.)

HCD = High Cholesterol Diet

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HDL

The statistical analysis reported significant ($p < 0.05$) effect of the treatment and time intervals (0, 14 and 28) on the HDL concentrations of rats under study. The effect of MAE of black cumin on the HDL levels in rats are mentioned in Table 3. Comparing the results of B₀ (45.627±2.12mg/dL), there was an increase noted in the values of B₁ (51.930±2.22mg/dL), B₂ (52.367±1.88mg/dL) and B₃

(52.060±2.24mg/dL), respectively. Furthermore, the HDL content was elevated from 48.790±1.89mg/dL (0 day), to 50.462±1.85mg/dL (14th day) and to 52.235±2.59mg/dL (28th day), respectively. The percentage increase noted for the treatment groups in comparison to the B₀ were 12.14, 12.88 and 12.36%; highest increase displayed by B₂ group.

Table 3 Effect of MAE of *Nigella sativa* (black cumin) on HDL levels in hypercholesterolemic rats

Parameter	Treatments	Study intervals (days)			Means
		0	14	28	
HDL cholesterol mg/dL	N ₀	50.31±2.18	49.98±2.06	46.87±1.96	49.05±2.07
	B ₀	48.21±1.98	46.83±1.67	41.84±2.71	45.627±2.12 ^b
	B ₁	49.76±1.81	51.91±2.09	54.12±2.76	51.930±2.22 ^a
	B ₂	47.65±0.82	52.23±1.84	57.22±2.98	52.367±1.88 ^a
	B ₃	49.54±2.65	50.88±1.58	55.76±2.54	52.060±2.24 ^a
Means		48.790±1.89 ^b	50.462±1.85 ^{ab}	52.235±2.59 ^a	

N₀= Basal diet; B₀= HCD; B₁= HCD + MABCE (150mg/kg B.W.); B₂= HCD + MABCE (300mg/kg B.W.); B₃= HCD + MABCE (450mg/kg B.W.)

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Catalase (CAT)

A significant ($p \leq 0.05$) effect of treatment (150, 300 & 450mg/kg B.W) and time intervals (0, 14 & 28) on the levels of catalase enzyme was reported by the statistical analysis carried out. The effect of MABCEs on catalase levels of experimental hypercholesterolemic rats are presented in figure 3. The maximum percent elevation was noted in group B₂ (25.7%), followed by B₃ (20.69%) and B₁ (19.67%). However, in comparison to the control group B₀ (11.8±0.33IU/L) the levels of catalase observed in hypercholesterolemic experimental groups were 14.69±0.83IU/L (B₁), 15.89±0.87IU/L (B₂) and 14.88±0.84IU/L (B₃), respectively on 28th day administration of MABCE. The highest elevation was observed for group B₂.

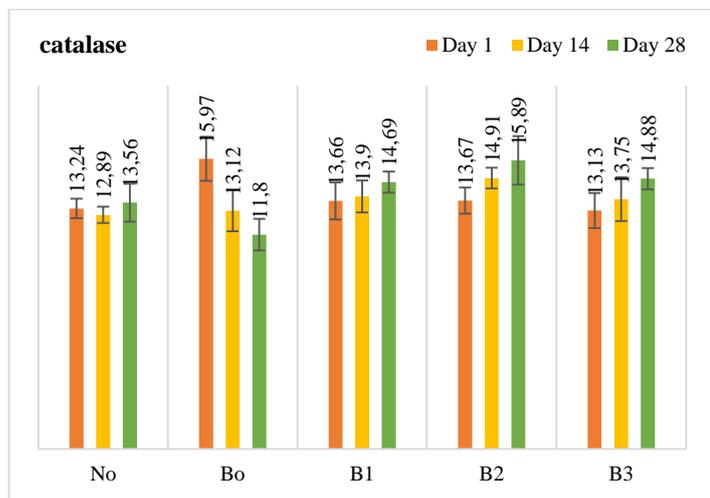


Figure 3 Effect of MAE of *Nigella sativa* (black cumin) of catalase (CAT) levels in hypercholesterolemic rats
 N₀= Basal diet; B₀= HCD; B₁= HCD + MABCE (150mg/kg B.W.); B₂= HCD + MABCE (300mg/kg B.W.); B₃= HCD + MABCE (450mg/kg B.W.)

Superoxide Dismutase (SOD)

The statistical analysis displayed a significant ($p \leq 0.05$) effect of the variables (time intervals and treatment) on the superoxide dismutase enzyme levels in the hypercholesterolemic experimental rats. The results for the levels of SOD in rats are reported in table 4. The concentration of SOD increased from 12.932±1.38IU/L on 0 day to 13.235±1.11IU/L on 14th day and 14.263±0.89IU/L on 28th day, respectively. Moreover, comparing the results of B₀ (12.293±0.74IU/L) an increase was observed in B₁ (13.493±0.98IU/L), B₂ (15.050±1.13IU/L) and B₃ (13.070±1.04IU/L), respectively. The percent elevation noted was 15.14%, 19.83% and 16.35%, showing the highest in B₂.

DISCUSSION

Cholesterol, a sterol that is synthesized in the animal tissues because of its structural importance in the plasma membrane in the tissues (Kanter et al., 2005). It being the major component in the cell system works as function for the

production of a number of hormones, bile acids and vitamin D (Kaleem et al., 2006). Moreover, in a study carried out previously determined the effect of black cumin seed extract (containing thymoquinone) at a concentration of 10 mg/kg/day for treating hyperlipidaemic experimental rats for a period of 5 days reduced the levels of cholesterol and triglycerides considerably (Bader, 2015; Aljabre et al., 2015). The results shown in the various groups of rats under study found that the hypercholesterolemic group gained the highest body weight which was due to the induction before experiment was carried out. The high cholesterol diet also increased the energy intake of the rats along with elevated fat storage in the body (Onyeike et al., 2012). The results of the current study were noted to be in accordance with the studies carried out earlier by Akbarzadeh et al., (2015) which also showed a positive association between different doses of black cumin extracts and the lowering of the body weight in the model rats. The reason behind this decrease in weight is the ability of the black seed extracts to help in decreasing the absorption of fat and other lipogenic enzymes with increased excretion of fat from body (Rains et al., 2011). A similar study reported feeding 800g/kg per day of black cumin oil orally to the rats for a period of 4 weeks displayed lowering of LDL and triglycerides with an increase in HDL (El-Dakhakhny et al., 2000). Another study administering petroleum ether extract of black cumin extract in an oral dose of 1ml/kg body weight of rats showed reduction of triglycerides (Al-Naqeep et al., 2011). The increase of triglycerides can be due to the reason of elevated expression of enzyme named acetyl CoA carboxylase along with fatty acid synthase that synthesizes triglycerides (Sukla et al., 2004). HDL is found to be involved in the transfer of cholesterol throughout the body assisting in reduction of accumulated cholesterol. It also prevented the deposition of LDL in the body (Elnaga et al., 2016). In a study earlier different formulations of black seed in form of oil, powder and methanolic extract in concentrations of 20-800mg/day, 100mg-20g/day and 3.5-20mg/day, respectively recorded lowered level of LDL cholesterol (Srinivasan, 2018).

The extract of black cumin seed displayed protective effect against the damage that may occur to kidney tissues based on total antioxidant capacity, oxidative stress and levels of superoxide dismutase and CAT (catalase) (Yildiz et al., 2010). A study showed that the concentration of 0.2ml/kg/day helps in lowering the harmful effects on liver which promoted the elevation of antioxidant enzymes such as SOD, CAT, TAC which were used to examine the antioxidant status in rats with liver injury (Demir et al., 2006; Yildiz et al., 2008). A study by Sultan et al., (2015) indicated the effect of diet on the antioxidant enzymes such as SOD and CAT. The results confirmed that the use of black cumin in diet increased the levels of antioxidant enzymes which provide the safety parameter for the liver and kidney tissues.

Hypercholesterolemia is one of the factors that contribute towards a number of complications that lead to heart diseases, diabetes, obesity etc. The unhealthy lifestyles and diet related habits presents with evidence of such disorders prevailing worldwide. The need of the hour is to cope up with the disastrous health conditions and to develop a way in order to treat these diseases through natural remedies. Foods providing with beneficial phytochemicals are very important as they combat the oxidative stress inside the body by their antioxidant properties. Nutraceutical and functional foods being an important and very recent field of study has achieved a lot of appreciation. Naturally providing with the therapeutic agents in the form of nutraceuticals is the main goal. This also makes it possible to tackle a number of diseases by these nutraceuticals available by nature. The current study addresses the issue of hypercholesterolemia which has been treated with the black cumin seed extract due to its therapeutic potentials.

Table 4 Effect of MAE of *Nigella sativa* (black cumin) on SOD levels in hypercholesterolemic rats

Parameter	Treatments	Study intervals (days)			Means
		0	14	28	
SOD (IU/L)	N ₀	12.01±0.34	12.96±1.56	13.43±0.80	12.80±0.90
	B ₀	13.72±0.89	12.04±0.78	11.12±0.56	12.293±0.74 ^b
	B ₁	12.55±1.03	13.14±0.99	14.79±0.91	13.493±0.98 ^b
	B ₂	13.54±0.98	14.72±1.09	16.89±1.32	15.050±1.13 ^a
	B ₃	11.92±1.23	13.04±1.13	14.25±0.76	13.070±1.04 ^b
Means		12.932±1.38 ^b	13.235±1.11 ^b	14.263±0.89 ^a	

N₀= Basal diet; B₀= HCD; B₁= HCD + MABCE (150mg/kg B.W.); B₂= HCD + MABCE (300mg/kg B.W.); B₃= HCD + MABCE (450mg/kg B.W.)
 HCD = High Cholesterol Diet
 MABCE = Microwave Assisted Black Cumin Extract

CONCLUSION

The black cumin seed also termed as *Nigella sativa* has a unique nutritional composition and is full of various phytochemicals thus being used as a functional food. In our current study we evaluated the microwave assisted extraction (MAE) of black cumin seed to obtain an extract that is rich in antioxidants along with other therapeutic properties. The extraction was carried out at optimum power and time to get the best yield, higher efficacy and lower use of solvent. The extract that displayed a great polyphenolic profile was used further to treat

hypercholesterolemic albino rats for a period of 28 days after induction. The administration of the extracts showed a positive outcome and was able to reduce the levels of cholesterol, triglyceride and LDL along with an increase of HDL. The study proved that the black seed can be an important source of phytochemicals which provides treatment of hypercholesterolemia in humans as well because of the close similarity between the genome of humans and rats. These seeds can easily be used in the daily diet and also provide its health benefits.

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