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EVALUATION OF PROMISING ANTIOBESITY ACTIVITY OF ETHANOLIC EXTRACT OF *NIGELLA SATIVA* SEED ON HIGH FAT DIET-INDUCED OBESE RATS

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ABSTRACT: *Nigella sativa (N. sativa)* (Family Ranunculaceae) is a widely used medicinal plant throughout the world. Seeds and oil have a long history of folklore usage in various systems of medicines and food. Extensive studies of literature has been revealed that *N. sativa* has to carry antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal protective, gastro-protective, antioxidant antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, anti-bacterial properties, *etc*. Due to its miraculous power of healing, *N. sativa* has got a place among the top-ranked evidence-based herbal medicines. In the present study, we are reporting the anti-obesity activity of successive solvent extract by ethanol, *Nigella sativa*. The ethanolic extract of *Nigella sativa* was orally administered in high fat, high cholesterol diet (HFHCD) obese rats for about 12-14 weeks. After four weeks of treatment, blood samples were collected for the estimation of serum lipids, glucose, Total Cholesterol, HDL, LDL, and Triglycerides. The treatment with *Nigella sativa* extracts markedly lowered total cholesterol, triglycerides in blood serum. It also showed positive effects (increase) on serum high-density lipoprotein cholesterol (HDL-c) concentrations. Presence of Thymoquinone (TQ), thymohydroquinone (THQ), dithymoquinone, thymol, carvacrol, α, and β-pinene, may be responsible for its anti-obesity activity. The results demonstrated that feeding with HFD had caused a significant increase in weight gain compared with the zero-day activity, which was significantly decreased by the co-administration of *Nigella sativa*, especially AAH, in a dose-dependent manner. Thus, the present findings emphasize that the roots of *Nigella sativa* possess potential medicinal values.

INTRODUCTION: *Nigella sativa (N. sativa)* (Family Ranunculaceae) is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha.

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Seeds and oil have a long history of folklore usage in various systems of medicines and food. The seeds of *N. sativa* have been widely used in the treatment of different diseases and ailments.

The seeds of *N. sativa* are widely used in the treatment of various diseases like bronchitis, asthma, diarrhea, rheumatism, and skin disorders. It is also used as a liver tonic, digestive, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers to fight parasitic infections and to support immune system [2-7]. Most of the therapeutic properties of this

plant are due to the presence of thymoquinone (TQ), which is a major active chemical component of the essential oil. Black seeds are also used in food like flavoring additive in the bread and pickles because it has a very low level of toxicity [8].

The most important active constituents are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpene longifolene (1%-8%) α-pinene and thymol *etc*. Seeds contain two different types of alkaloids, *i.e.,* isoquinoline alkaloids, *e.g.*, nigellicimine and nigellicimine- N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids, which include nigellidine and nigellicine. Moreover, *N. sativa* seeds also contain alpha-hederin, a water soluble pentacyclic triterpene, and saponin, a potential anticancer agent [9]. Most of the pharmacological properties of *N. sativa* are mainly due to quinine constituents, of which thymo-quinone is the most abundant. The seeds of N. sativa contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fiber (8.4%) and total ash (4.8 %), vitamins and minerals like Cu, P, Zn, and Fe, *etc*., [10] fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50-60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%); Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. α-sitosterol is a major sterol, which accounts for 44% and 54% of the total sterols in Tunisian stigmasterol (6.57-20.92% of total sterols) [11].

According to the recent WHO report, more than 1.9 billion adults, 18 years and older, were overweight in 2016; of these, over 650 million were obese [12].

Different powerful synthetic chemical drugs used against obesity are available in the pharmaceutical market; the FDA has approved five prescription medications for long-term use for the treatment of overweight and obesity, including orlistat, lorcaserin, phentermine-topiramate, naltrexone-bupropion, and liraglutide [13].

Plant-derived products (fruits and vegetables) constitute an important part of the human diet all over the world and provide nutrients that are essential for life. Plants contain non-nutrient biologically active phytochemicals, including poly-phenols and anthocyanins. *Nigella sativa* seeds have better compatibility with the human body with lesser side effects after detoxification. Looking to the importance of the plant in tradition system of medicine, it will be considered worthwhile to evaluate this plant for the importance as an anti-obesity agent. Then, the identification of the phytochemical agent responsible for the action will also be useful for the development of a new drug.

MATERIALS AND METHODS:

Selection of Plant: Antiobesity activity of *Nigella sativa* seeds has not been scientifically reported so far, and it is well known that obesity is a major lifestyle problem/ disorder nowadays. So, we selected this plant to determine and scientifically validate the effect of *Nigella sativa* seeds on obesity.

Collection, Authentication and Drying of Parts of Plant: The plant was collected from Vindhya herbals Barkheda Pathani Bhopal (M.P.) where it is widely used for commercial purposes. The plant was authenticated from the Department of Botany at Safia Science College, Bhopal (M.P.) by Dr. Zia Ul Hasan with a voucher Specimen no. 2019/Bot/ Safia/411. The seed was shade dried so as to protect its chemical constituents not to get degrade at a high temp.

Extraction: Successive Soxhlet extraction is rapid and continuous and may be employed in sparingly soluble constituents due to repeated extraction, which cannot be done by either percolation or maceration methods. Due to Soxhlet extraction's various advantages, this method was selected for the present study, using a different solvent.

Soxhlet Extraction: The moderately coarse powder of the seeds of *Nigella sativa* were subjected to Soxhlet extraction with different ethanol as solvents. The dried coarsely powdered drug was packed in Soxhlet apparatus and defatted with ethanol (40-60 °C) until complete defeating, which was insured by placing a drop by thimble on the filter paper that not exhibited any oily spot. The defatted material was removed from the Soxhlet apparatus and air-dried to remove the last traces of ethanol. The completion of the extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue

remained after evaporating the solvent. The solvent was removed by distillation, and the last traces of solvent had been removed under vacuum.

Preliminary Phytochemical Screening: *Nigella sativa* seed dried ethanolic extracts were taken for the chemical test for detection of the constituents like alkaloids, flavonoids, tannins, sterols, phenolic compounds, terpenoids, carbohydrates, *etc*. In order to detect the various constituents, present in the different extracts of *Nigella sativa*.

***In-vivo* studies:**

Animals: Healthy Wister rats of either sex (6-8 weeks) weighing 150-180 g were used for the evaluation of acute oral toxicity test and anti-obesity activity. The animals were used after an acclimatization period of 10 days to the laboratory environment. The animal was obtained from the animal house of local animal house, Bhopal. Ethical clearance for the handling of animals and procedures used in study was obtained from the institutional animal ethical committee (Approval No.-IAEC/2018/P'cology/P-03) prior to the beginning of the study. The animals were housed in a standard controlled animal care facility, in cages (six rats/cage), and maintained in a temperature-controlled room (22–25 °C, 45% humidity) on a 12:12-hour dark-light cycle. The animals were maintained under standard nutritional and environmental conditions throughout the experiment. All the experiments were carried out between 9:00 and 16:00 h, at ambient temperature. The CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) (CPCSEA no. 1413/PO/E/S/11) guidelines were strictly followed, and all the studies were approved by the Institutional Animal Ethical Committee (IAEC).

Toxicity Study: Acute toxicity studies were performed according to the Organization for Economic Cooperation and Development (OECD) 420 guidelines category IV substance (acute toxic class method). Wister rats (*n*=3) of either sex selected by the random sampling technique were employed in this study. The animals were fasted for four hours with free access to water only. The seed extracts of *Nigella sativa* were administered orally with an initial dose of 2000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in two out of three or three out of three animals, then the dose administered was considered to be a toxic dose. However, if mortality was observed in only one mouse out of the three animals, then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with a higher dose [14].

Induction of Experimental Obesity: The animals were fed with a 40% high-fat high cholesterol diet (HFHCD) for about 12-14 weeks. The standard formulation for high fat, high cholesterol diet - for 1000 gm is mentioned in the following **Table 1**.

TABLE 1: STANDARD FORMULA FOR HFHCD

S. no.	Ingredient	Quantity (in gm)	Quantity in Percentage
1	Standard powder material	180 gm	18%
2	Fructose	600 gm	60%
3	Corn oil/soya been oil	100 gm	10%
4	Coconut oil	100 gm	10%
5	Mineral mixture	25 gm	25%
6	Water	Q.S	Q.S

Test Drug Preparation: The ethanolic extract of *Nigella sativa* and standard Remonabent were taken in Sodium Carboxy Methyl Cellulose (CMC). Sodium CMC, 0.5% w/v, dissolved in distilled water was used as a media to prepare a suspension. All the drug concentrations were prepared freshly, just before administration. All the test drugs, including the standard, were given by oral canula *via* the p.o. route.

Anti-obesity Activity: Divide animals into 5 groups of 6 animals each.

Group I Normal diet and water.

Group II *Ad libitum* High Fat High Cholesterol Diet (HFHCD) & Water

Group III Receives Remonabent (10mg/kg) + HFHCD

Group IV Receive Ethanolic extract of *Nigella sativa* (150mg/kg) + HFHCD

Group V Receive Ethanolic extract of *A. heterophyllum* (300mg/kg) + HFHCD

Evaluation Parameters: The anti-obesity potential of *Nigella sativa* extracts was assessed by the following parameters - Body weight, glucose, Total Cholesterol, HDL, LDL, and Triglycerides [15].

Body Weight: The body weight of rats (g) was recorded every week for 28 days for each group, just before dosing, by using a precision balance with high sensitivity.

Biochemical Parameters:

Preparation of Serum: All mentioned parameters were tested at 0 days, 7th day, 14th day, 21st day, and 28th day Twenty-four hours after the last administration of the test drug, the animals were anesthetized under light ether anesthesia, and blood for serum preparation was collected *via* a retro-orbital puncture, using a 10µl × 20 mm (L) × 0.8 mm (2R) glass capillary, into a sterile EDTA-coated tube (3 mg/ml), for estimation. The blood was kept in wet ice for 30 min, centrifuged for five minutes at 4000 rpm, and plasma was aspirated out for the analysis of lipid profile and glucose. The serum was stored in the refrigerator for analysis of the biochemical parameters. All analyses on the serum were completed within 24 h of sample collection.

Determination of Glucose: The glucose content was determined in the samples by Accu-check digital glucometer using glucometer strips.

Evaluation of Serum Lipid Parameters:

Reagent Used: Serum lipid parameters were estimated by autoanalyzer using accurex diagnostic kit.

Serum Cholesterol: Cholesterol esters were hydrolysed by cholesterol esters hydrolase to free cholesterol and fatty acids. The free cholesterol produced and pre-existing one was oxidized by cholesterol oxidase to α 3-cholestenone and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidise, oxidizes the chromogen to a red-colored compound which is measured at 510 nm. The color of the reaction is stable for two hours if not exposed to direct light.

Microliter supernatant clear serum was taken into each test tube with the help of a micropipette, and 1ml of the working solution was added to each of the test tubes. Then these mixtures were incubated for 10 min at room temperature. On completion of the incubation period, a red color appeared from which cholesterol level was estimated by auto analyzer using parameters given above in the description.

Serum Triglycerides: Triglycerides incubated with lipoprotein lipase were hydrolyzed to free fatty acids and glycerol. Glycerol kinase catalyzes the conversion of glycerol and ATP to glycerol-3-phosphate and ADP. The glycerol-3-phosphate gets oxidized to dihydroxyacetone phosphate by glycerophosphate oxidase. The hydrogen peroxide formed in this reaction with the help of peroxides reacts with chromogens. It gives a red color complex, which is measured at 510 nm.

Ten microliter supernatant clear serum was taken into each test tube with the help of micropipette, and 1 ml of working solution was added to each of the test tubes. Then these mixtures were incubated for 10 min at room temperature. On completion of the incubation period, a red color appeared from which serum triglyceride level was estimated by auto analyzer using parameters given above in the description.

HDL-Cholesterol: After centrifugation, the cholesterol in the HDL fraction, which remained in the supernatant, was assayed by enzymatic cholesterol method using cholesterol oxidase, peroxide, and chromogen- 4aminophenozone.

Two hundred microliter of supernatant clear serum was taken into each test tube with the help of a micropipette, and 300 µl precipitating reagent was added to each test tube with a view to precipitate chylomicron, VLDL, and LDL. Each test tube was centrifuged at 3500-4000 rpm for 12-15 min to separate the clear supernatant immediately, and cholesterol content was determined using parameters mentioned above with 1ml reagent followed by 10min incubation at room temperature.

LDL-Cholesterol: Low-density lipoprotein was calculated using the following relationship:

$$LDL = TC-(HDL-C-TG/5)$$

VLDL-Cholesterol: Very low-density lipoprotein was calculated by using the following relationship:

$$VLDL = TG/5$$

RESULTS: The collected crude drug was authenticated by the botanist. The seeds were dried in the shade, and the organoleptic characteristics (*e.g.,* absence of hard and white core) [15-16]. Then the seeds drug was powdered and taken for extraction.

The percentage yield of the drug with various solvents is given in **Table 2**.

TABLE 2: PERCENTAGE YIELD OF EXTRACT WITH DIFFERENT SOLVENTS AFTER EXTRACTION

Parts	Solvent	Extract Colour	Yield (in gm)	% Yield
Seeds	Ethanol	Brown	13.9	72.1%

Preliminary Phytochemical Screening: Various preliminary Phytochemical chemical tests were performed for the qualitative determination of constituents present in the extracts of *Nigella sativa*. The tests were performed for the determination of the carbohydrates, proteins, amino acids, fats, oils, steroids, glycosides, alkaloids, phenolic compounds, tannins, and resins. The qualitative tests of the alcoholic extract of AH roots have shown the presence of protein, steroids, glycosides, alkaloids, phenolic compounds.

Animal Studies: After oral acute toxicity studies, no mortality was observed with the dose of 3000 mg/kg. Hence the two doses were selected 150 and 300 mg/kg for the study. The induction of obesity was achieved by the administration of a high fat high cholesterol diet (HFHCD) for about 12-14 weeks. The animals were divided into five groups of six animals each in a group. The Group I received the normal diet and water, Group II received HFHCD and water, Group III received standard drug Remonabent (10 mg/kg p.o) with HFHCD, Group IV received the alcoholic extract of *Nigella sativa* seeds at the dose 150 mg/kg p.o with HFHCD, Group V received the alcoholic extract of *Nigella sativa* seeds at the dose 300 mg/kg p.o with HFHCD for the evaluation of their anti-obesity potential.

Effect of *Nigella sativa* Seeds Extract on Body Weight: Present study showed the changes in body weight of all groups during the study. Feeding a high-fat, high cholesterol diet (HFHCD) 40% total fat for weeks produced significant increases (p<0.05) in body weight. Throughout the experiment, the food and water intake of each group did not differ. Rats fed on an HFHCD experienced a significant increase in body weight gain, and *Nigella sativa* seeds have anti-obesity properties on this model [13]. The alcoholic extract was more effective in higher doses. The present result clearly showed that giving *Nigella sativa* seeds is beneficial for the suppression of diet-induced obesity.

TABLE 3: EFFECT OF *NIGELLA SATIVA* SEEDS EXTRACT ON BODY WEIGHT

S. no.	Groups	Day				
		0 Day	7 Day	14 Day	21 Day	28 Day
1	Normal Control	173.19±1.02	177.21± 1.02	177.49±1.24	184.13±2.58	189.14±1.44
2	Negative Control	339.43±2.84	363.63±4.34	369±3.18	342.03±4.14	343.26±4.04
3	Standard	341.34±1.38	369.6±1.39	363.80±1.44	360.38±1.19	333.14±1.14
4	Treatment 1	356.44±1.42	363.64±1.43	363.40±1.69	360.44±1.84	334.16±1.40
5	Treatment 2	340.84±1.83	369.63±1.23	364.48±1.49	360.14±1.3	336.33±1.36

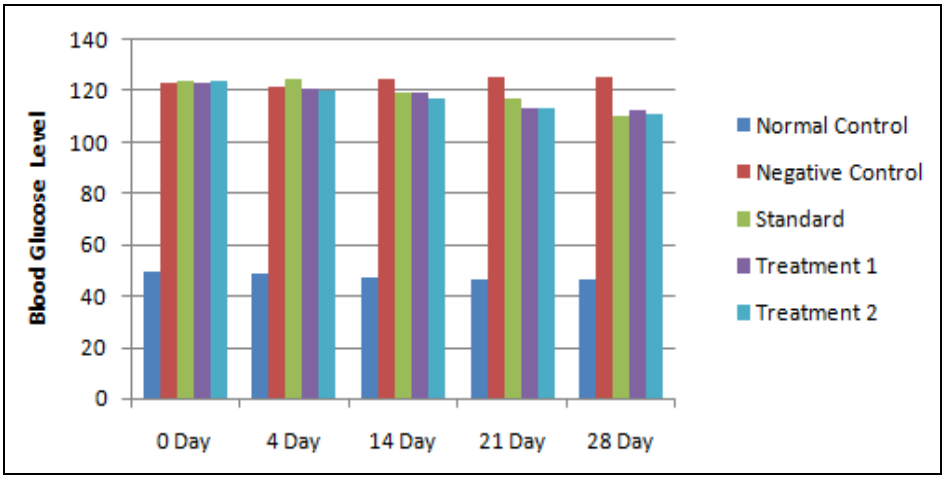
Values are Mean ± S.E.M, n = 6 animals in each group. Values in parenthesis indicate a significant increase in HDL level in treated groups Vs negative control group II. (treatment-1: 150mg/kg , treatment-2: 300mg/kg).

Effect on Blood Glucose: The effects of the *Nigella sativa seeds* on blood glucose levels were shown in **Table 4**, **Graph 1**. It showed that the *Nigella sativa seeds* produced a decrease in the blood glucose levels when compared to glucose level on zero days in animals at the dose of 300 mg/kg. It was also observed that on twenty-eighth days there is not a significant difference in the change in glucose level when compared to the negative control group.

TABLE 4: EFFECT OF ALCOHOLIC EXTRACT OF *NIGELLA SATIVA* SEEDS ON BLOOD GLUCOSE LEVEL

S. no.	Groups	Day				
		0 Day	4 Day	14 Day	21 Day	28 Day
1	Normal Control	49.23±1.20	48.57±1.03	47.14±1.37	46.33±1.24	46.49±1.27
2	Negative Control	123.11±1.32	121.97±0.23	125.03±0.13	125.72±1.23	125.23±1.15
3	Standard	124.27±1.91	124.42±2.13***	119.542±1.31***	117.07±0.21***	110.12±0.39***
4	Treatment 1	123.26±1.67	121.33±1.73	119.47±0.79**	113.12±1.09**	112.33±1.27**
5	Treatment 2	124.13±0.62	120.14±1.42**	117.37±0.75**	113.27±0.11***	111.21±1.24***

Values are Mean ± S.E.M, n = 6 animals in each group. Values in parenthesis indicate a significant decrease in blood glucose level in treated groups Vs negative control group II. Results are Mean ± S.E.M. ANOVA, Tukey's test ***p < 0.001, **p <0.01, *p<0.05.



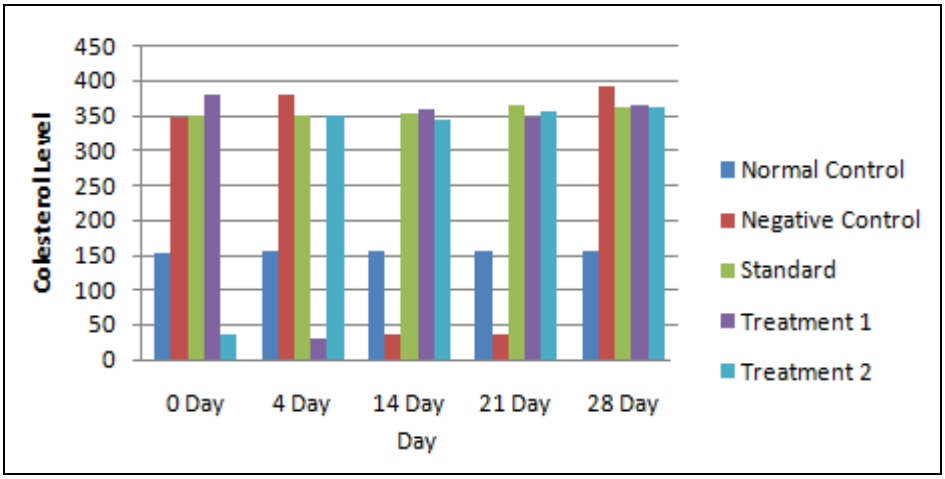
GRAPH 1: EFFECT OF ETHANOLIC EXTRACT OF *NIGELLA SATIVA* SEEDS ON BLOOD GLUCOSE LEVEL . The values are Mean ± S.D. of triplicates (P<0.001)

Effect of on Total Cholesterol: The cholesterol level was increased in the negative control significantly (345.91 ± 1.2 to 390.62 ± 1.11) and decreased in the standard group significantly (347.44 ± 0.88 to 361.51 ± 1.40) when compared to the zero-day activities. The result was shown in **Table 5**, **Graph 2**.

TABLE 5: EFFECT OF ETHANOLIC EXTRACT OF *NIGELLA SATIVA* SEEDS ON TOTAL CHOLESTEROL LEVEL

S. no.	Groups	Day				
		0 Day	4 Day	14 Day	21 Day	28 Day
1	Normal Control	153.32±1.73	155.13±1.12	154.20±0.19	154.97±0.82	154.05±0.66
2	Negative Control	345.91±1.2	379.23±1.12	385.82±0.44	387.11±0.32	390.62±1.11
3	Standard	347.44±0.88	347.42±1.23***	351.21±1.01***	363.93±1.20***	361.51±1.40***
4	Treatment 1	379.44±0.22	380.10±0.29	356.2±0.2	347.12±1.32*	363.11±1.92**
5	Treatment 2	348.53±1.05	349.71±1.3	342.42±1.54**	356.18±1.45**	361.98±1.33**

Values are Mean ± S.E.M, n = 6 animals in each group. Values in parenthesis indicate significant decrease in total cholesterol level in treated groups *vs*. negative control group II. Results are Mean ± S.E.M. ANOVA, Tukey's test ***p < 0.001, **p <0.01,*p<0.05.



GRAPH 2: EFFECT OF ETHANOLIC EXTRACT OF *NIGELLA SATIVA* SEEDS ON TOTAL CHOLESTEROL LEVEL. The values are Mean ± S.D. of triplicates (P<0.001)

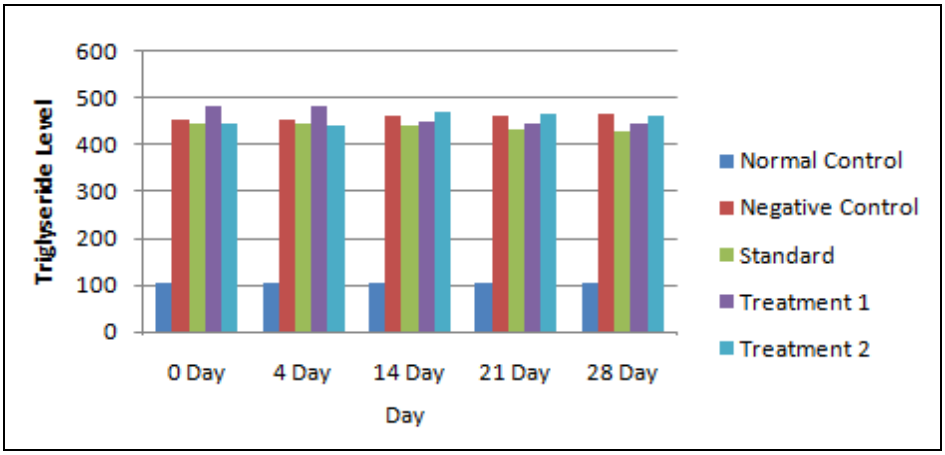
Effect of on Lipid Profile (TG, HDL, LDL):

Effect on Total Triglyceride Level: From this study, it was found that animals that received 300 mg/kg/day of dose showed a reduction in Triglyceride levels as compared to negative control or HFD group significantly (466.21 ± 0.11 to 452.16 ± 1.19). The triglyceride (TG) levels of the treated groups showed a decrease After 28 days of treatment. There is a significant decrease in triglyceride levels of the standard group at the twenty-eighth day as compared to the zero-day activity **Table 6, Graph 3**.

TABLE 6: EFFECT OF ETHANOLIC EXTRACT OF *NIGELLA SATIVA* SEEDS ON TOTAL TRIGLYCERIDE LEVEL

S. no.	Groups	Day				
		0 Day	4 Day	14 Day	21 Day	28 Day
1	Normal Control	103.23±1.24	104.22±1.96	104.42±2.33	104.33±1.42	105.19±1.04
2	Negative Control	452.10±1.09	454.85±1.44	460.93±1.14	464.11±0.47	466.21±0.11
3	Standard	444.30±1.02	444.25±0.14***	439.96±0.83***	434.27±1.94***	430.19±1.17***
4	Treatment 1	483.21±1.59	481.36±0.24	449.97±0.81*	444.29±0.67*	444.12±0.14*
5	Treatment 2	446.29±1.14	442.27±0.31	469.14±0.17*	465.34±0.144**	452.16±1.19**

Values are Mean ± S.E.M, n = 6 animals in each group. Values in parenthesis indicate significant decrease in total triglyceride level in treated groups *vs*. negative control group II. Results are Mean ± S.E.M. ANOVA, Tukey's test ***p < 0.001, **p <0.01,*p<0.05.



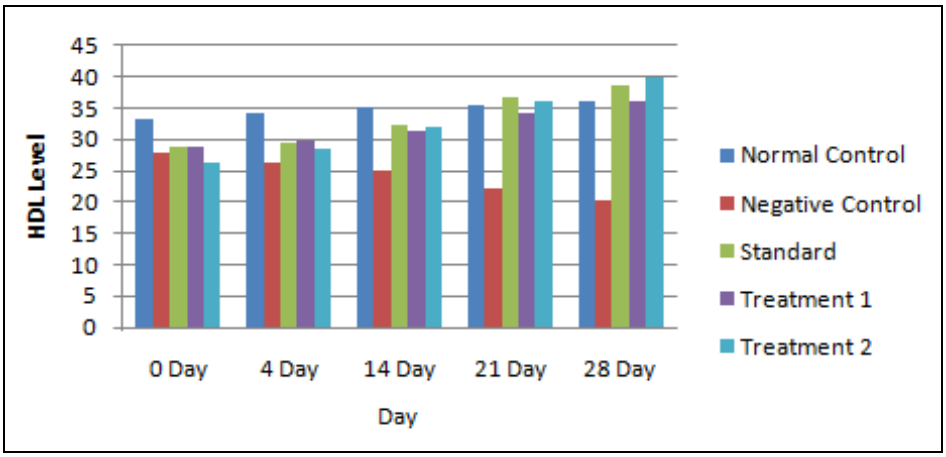
GRAPH 3: EFFECT OF ETHANOLIC EXTRACT OF *NIGELLA SATIVA* SEEDS ON TOTAL TRIGLYCERIDE LEVEL. The values are Mean ± S.D. of triplicates (P<0.001)

Effect on High-Density Lipoprotein-Cholesterol Level: High-density lipoprotein-cholesterol (HDL-C) levels showed an increase in ethanolic extract-treated animals. The extract at the dose of 300 mg/kg/day showed a significant increase in HDL (26.32 ± 0.42 to 39.84 ± 0.13) **Table 7, graph 4**.

TABLE 7: EFFECT OF ETHANOLIC EXTRACT OF *NIGELLA SATIVA* SEEDS ON HDL LEVEL

S. no.	Groups	Day				
		0 Day	4 Day	14 Day	21 Day	28 Day
1	Normal Control	33.19±1.21	34.32±1.21	35.22±1.92	35.34±1.23	36.05±1.72
2	Negative Control	27.81±0.29	26.19±1.02	24.92±1.02	22.22±1.19	20.33±1.42
3	Standard	28.83±1.09	29.46±1.91***	32.42±1.05***	36.74±1.13***	38.72±1.02***
4	Treatment 1	28.84±2.09	29.93±2.29**	31.33±1.01*	34.32±1.52**	36.22±1.02**
5	Treatment 2	26.32±0.42	28.63±1.22***	32.09±0.19***	36.11±0.34***	39.84±0.13***

Values are Mean ± S.E.M, n = 6 animals in each group. Values in parenthesis indicate significant increase in HDL level in treated groups Vs negative control group II. Results are Mean ± S.E.M. ANOVA, Tukey's test ***p < 0.001, **p <0.01,*p<0.0.



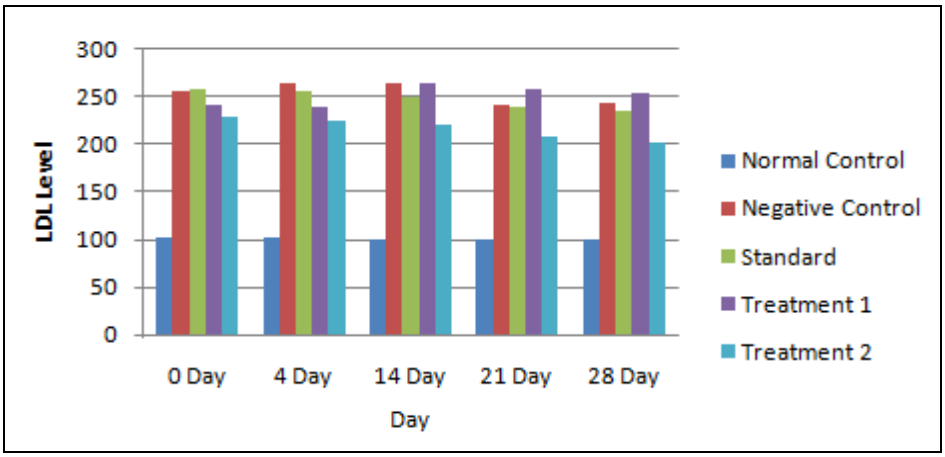
GRAPH 4: EFFECT OF ETHANOLIC EXTRACT OF *NIGELLA SATIVA* SEEDS ON HDL LEVEL. The values are Mean ± S.D. of triplicates (P<0.001)

Effect on Low-Density Lipoprotein-Cholesterol Level: The low-density lipoprotein-cholesterol (LDL-C) levels showed a reduction in value in AH treated animals as compared to HFD treated animals. HFD also induced fatty liver, with an accumulation of triacylglycerides. In the treated group, body weight, total cholesterol, and low-density lipoprotein cholesterol level were reduced significantly ($p<0.05$) as compared to the negative control group **Table 8, Graph 5**.

TABLE 8: EFFECT OF ETHANOLIC EXTRACT OF *NIGELLA SATIVA* SEEDS ON LDL LEVEL

S. no.	Groups	Day				
		0 Day	4 Day	14 Day	21 Day	28 Day
1	Normal Control	102.93±1.02	101.33±1.11	100.33±0.52	101.01±0.82	100.11±0.11
2	Negative Control	256.63±1.26	264.11±1.16	264.53±1.06	241.33±1.02	244.33±1.65
3	Standard	258.03±1.11	255.44±1.32***	249.53±1.3***	240.00±0.10***	234.94±0.12***
4	Treatment 1	241.46±1.24	240.18±1.54**	265.01±1.30**	259.00±1.36**	254.93±1.23**
5	Treatment 2	229.53±1.66	224.84±1.22**	220.84±1.32***	208.85±1.21***	201.34±1.88***

Values are Mean ± S.E.M, n = 6 animals in each group. Values in parenthesis indicate significant increase in HDL level in treated groups *vs*. negative control group II. Results are Mean ± S.E.M. ANOVA, Tukey's test ***p < 0.001, **p <0.01, *p<0.05.



GRAPH 5: EFFECT OF ETHANOLIC EXTRACT OF NIGELLA SATIVA SEEDS ON LDL LEVEL. The values are Mean ± S.D. of triplicates ($P<0.001$)

DISCUSSION: The impact of overweight and obesity from a public health perspective is enormous and continues to increase. Numerous studies have verified the association of overweight and obesity in the development of different metabolic disorders, including diabetes mellitus, atherosclerosis, cardiovascular diseases, hypertension, and cancer. It has reached epidemic proportions globally, with approximately 1.6 billion persons (aged 15 years old and above) being overweight [14]. Many attempts have been made to correct the metabolic disparity of the obesity condition, producing a number of reagents, including fibrates, Sibutramine (an anoretic or appetite suppressant), and Orlistat, but no one is free from the severe side effects. At present, because of dissatisfaction with high costs and potentially hazardous side effects, the potential of natural products for treating obesity is under exploration and this may be an excellent alternative strategy for developing an effective anti-obesity drug. A variety of natural products, including crude extracts and isolated compounds from plants, can induce bodyweight reduction and prevent diet-induced obesity [18]. Therefore, they have been widely used in treating obesity.

The present study was aimed to evaluate the effects of ethanolic extract of *Nigella sativa* seeds for 28 consecutive days, by P.O administration, on body weight, lipid profile in normal and HFD fed rats. Rats that are fed HFD (High-fat diet) are a widely used model for obesity. The so-called "High fat diet" involves feeding experimental animals a mixture of palatable commercially available supermarket foods to stimulate energy intake. The characteristic of such diets is the combination of high-fat content with high carbohydrate content. It has been suggested that rats become more obese with a high-fat diet. Ethanolic extract of *Nigella sativa* seeds is reported to have it is known as a source of thymoquinone, thymohydroquinone,

dithymoquinone, p-cymene, carvacrol, 4-terpineol, t-anethol, sesquiterpene longifolene, nigellicimine and nigellicimine- N-oxide, α-pinene and thymol etc. that may have the hypolipidemic activity. The incalculable medicinal properties and therapeutic uses of N. sativa prove its importance as a valuable medicinal plant.

Obesity is a risk factor for hypercholesterolemia and hypertriglyceridemia, which are involved in the development of cardiovascular disease [16]. Oxidative damaged LDLs are taken up by macrophages, which accumulate in the endothelial wall as lipid-laden foam cells in the initial phases of atherosclerotic fatty streak lesions. Therefore, a reduction in circulating TGs, TC and LDLs is primary in the prevention of vascular disease. In addition, the prevention of LDL oxidation by dietary antioxidants could delay the development of atherosclerosis. The ethanolic extract of *Nigella sativa seeds* has shown a significant decrease in LDL and total cholesterol level at higher doses and an increase in HDL level.

The presence of thymoquinone, α-pinene, and thymol may be responsible for its anti-obesity activity. The results demonstrated that feeding with HFD had caused a significant increase in weight gain compared with the zero-day activity, which was significantly decreased by the co-administration of The ethanolic extract of *Nigella sativa seeds* dose-dependent manner.

CONCLUSION: This study demonstrates that supplementing 300 mg/kg/day of the ethanolic extract of *Nigella sativa* seeds exhibited a decrease in body weight, total cholesterol, triglycerides, blood glucose, and increased HDL cholesterol levels. The ethanolic extract of *Nigella sativa* seeds presented thymoquinone, α-pinene and thymol which may be responsible for the anti-obesity activity. Further, the alcoholic fraction can be explored for the exact phytoconstituent responsible for the anti-obesity potential.

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AUTHORS CONTRIBUTION STATEMENT:

Payal Saiju: Carried out the experiments, handed experimental animal

Abhishek Sharma: Carried out the experiments under the guidance.

Ruchi Jain: Carried out the experiments, collection of data and to investigate [a specific aspect] and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Nilesh Jain: Contributed to the interpretation of the results and wrote the manuscript with input from all authors. Also helped supervise the project

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