ORIGINAL Professional Med J Dec 2008; 15(4): 500-507. / **PROF-1258**

SERUM LIPID PROFILE;
CORRELATION OF NIGELLA SATIVA AND SUNFLOWER OIL DIET INTAKE IN ALBINO RATS.

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Article received on: 12/06/2007

Accepted for publication: 08/11/2008

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ABSTRACT... Objective: To determine the effects of Nigella sativa and sunflower oil diet intake on serum lipid profile in albino rats. **Material & Methods:** Eighty four albino rats with equal number of males and females were selected for the study, they were divided into six different groups, Control groups1,111,V,were given low fat diet(3%),high fat diet(20%), high fat diet supplemented with bile salt (1% colic acid) and antithyroid drug (0.5% propylthiouracil). The Experimental groups were given the above diets with supplemented Nigella sativa. Low fat diet increased all the lipid fractions significantly when given at12 and 24 weeks duration as compared to 0 week. **Results:** The high fat diet when given at different intervals decreased all lipid fractions significantly as compared to baseline level. The high fat diet with propylthiouracil and bile salt also increased all the lipid fractions and the increase was more as compared to previous groups. The supplements of Nigella sativa in the groups decreased all the lipid fractions significantly as compared to the control groups except HDL-c, which was significantly increased in all the experimental groups as compared to control groups. **Conclusion:** On the basis of these findings conclusions are made, that Nigella sativa has got TG,TC, and LDL-c lowering and HDL-c raising effects.3% sunflower oil low fat diet has got TG,TC,HDL-c, and LDL-c raising effects.20% sunflower oil high fat diet has got TG,TC,HDL-c and LDL-c lowering effects. Both Nigella sativa and sunflower oil have got low atherogenic index (TC/HDL) and may be recommended in hyperlipidaemic patients or normal individuals.

INTRODUCTION

Coronary heart disease (CHD) is a major global health problem. High intake of fat is a risk factor for the development of CHD[1]. Excess eating of sweets and fatty foods, lack of exercise and excess sleep leads to obesity. Honey, herbs, guggul, silageet and certain fruits, in association with exercise may be protective[2].

High plasma cholesterol was positively related to the risk of CHD[3,4]. Saturated fats and cholesterol in the diet play a major role in the aetiology of hypercholesterolemia and act as a risk factor for CHD[5] (National institute of health consensus 1985[6]). Grundy[7] reported the elevation of plasma cholesterol is usually due to an increase in the level of low-density lipoprotein cholesterol (LDL-c).

Polyunsaturated fat diet decreases plasma cholesterol level and beta lipoprotein when substituted for saturated

fats[8,9,10].

Saturated fats and cholesterol in the diet cause rise in serum cholesterol while diets low in saturated fat and cholesterol decrease cholesterol level in human beings[11], while low fat, low cholesterol or polyunsaturated fat diet decrease plasma cholesterol level[12]. Polyunsaturated fatty acids (PUFA) lower triglyceride (TG), very low density lipoprotein cholesterol (VLDL-c), low density lipoprotein cholesterol (LDL-c) and also high density lipoprotein cholesterol (HDL-c)[13].

Tayyab et al[14] observed that saturated fats raised the serum total cholesterol (TC), mono-unsaturated fatty acids (MUFA) increased HDL-c; PUFA decreased both TC and HDL-c.

Elevated LDL-c and decreased HDL-c in plasma have been independently attributed to be associated with increased risk for CHD in man.

Obese people tend to have relatively high triglyceride (TG) and low HDL-c. Obesity raises LDL-c levels High level of serum TG is also considered a major risk factor in the pathogenesis of CHD[15]. The Nigella sativa has innumerable effects, cholerectic activity[17].

It has been reported our Holy Prophet Hazrat Muhammad Salallah-u-alai-hi wasillam Said, that the black seeds (Nigella sativa) is a remedy for every disease except death[16]. Hence a study was planned to assess the effect on lipids in albino rats fed on high sunflower oil diet and Nigella sativa. If significant reduction of lipids is observed, this will be of great help to the patients as well as physicians.

MATERIAL AND METHODS

PLAN OF STUDY

The albino rats were obtained from the Pakistan conical of scientific and industrial Research (PCSIR) laboratories complex, Lahore. Eighty-four albino rats, with equal number of males and females were selected for the study. The weights of each rat ranged from 150-200 grams and their ages at the start of the study were 8 weeks. They were divided into six groups, each group consisting of equal number of male and female rats. Both sexes were kept in separate cages in the animal house of Postgraduate Medical Institute, Lahore. Each albino rat was weighed at the zero, 12th and 24th weeks of the study. Each group of animals was given separate diet starting at zero week and continued for a period of 24th weeks, hygienic conditions and optimum temperature (242 $_{o}$C) was maintained for all albino rats. These animals were provided prepared diets and fresh drinking water daily. Blood samples were collected under deep either anesthesia by heart puncture with sterile disposable syringes at zero, 12th and 24th weeks.

EXPERIMENTAL DIETS

Six different diets were prepared. These included low fat diets (A and B) and four high fat diets (C to F). The control diet A contained 3% sunflower oil and experimental diet B contained 3% sunflower with Nigella sativa. The diet C contained high 20% sunflower oil, diet D included 20% sunflower with powdered Nigella sativa. The diets E contained 20% sunflower oil, 1% cholic acid and 0.5% propythiouracil in diet, and diet F contained 20% sunflower oil, 1% cholic acid, 0.5% propythiouracil with Nigella sativa. Minerals and vitamins mixtures were prepared and mixed with diet according to the recommendation. The prepared diets were stored in refrigerator at -4° C in clean closed glass containers. Weighed quantity of diet was placed in each cage container daily in the morning and evening throughout the study period. The tap water in the bottles was also changed daily. Nigella sativa in a dose of 30% mg/kg body weight of albino rat.

Weighed respective diets, weights at 0,12th and 24th

weeks of study were recorded.
Six different diets were kept in twice daily at 9 AM and 9 PM. Changes daily in clean bottles.

BLOOD COLLECTION

Blood samples 2ml were taken by heart puncture with a sterile disposable syringe after 12 hours overnight fasting, in the morning after giving ether anesthesia to each albino rat First sample was collected at the start of experimental diet, 2nd after 12^{th} weeks and the last after 24^{th} weeks.

INVESTIGATIONS

Following estimations pertaining to lipid profiles were done:

1. Serum triglyceride (TG)
2. Serum total cholesterol (TC)
3. Serum high density lipoprotein (HDL-c)
4. Serum low density lipoprotein (LDL-c)

The results of lipid profile of albino rats are given as follows:-

SERUM LIPID PROFILE

The serum lipid levels in different groups were expressed as mean ±SD mg/ dl at 0,12 and 24 weeks.

Triglycerides (TG)

This study revealed that the albino rats (group I) fed on low fat diet (3% sunflower oil only) showed significant increase in TG level as compared to the baseline level (0 week). However, the albino rats in-group II fed on low fat diet supplemented with Nigella sativa showed significant reduction in TGs level.

Albino rats in group III fed on high sunflower oil fat diet (20%) showed significantly reduction in serum TG level, contrary to group I in which TG level was raised.

Interestingly when nigella sativa was given with high fat diet in group IV there was significant reduction in TG level. This finding indicates that high fat diet (20% sunflower oil) in combination with Nigella sativa has significant TG lowering effects as compared to combination of Nigella sativa and low fat diet (3% sunflower oil)It was observed that when albino rats were given 20% sunflower oil (high fat diet) alongwith 1% cholic acid and 0.5% propylthiouracil (atherogenic element) in group V, there was increase in serum TG level. However, when nigella sativa was given alongwith above diet in-group VI, there was slight reduction in TG effect of cholic acid and propylthiouracil which were used as atherogenic element. Similar findings 24 weeks experiment and Nigella sativa had similar hypotriglyceridaemia effect. These findings in the present study are consistent with those reported by Shabir. This hypotriglyceridaemic effect of Nigella sativa is possibly due to the cholerectic activity of Nigella sativa as reported by El-Dakhakhany and Brunton. The results are in agreement with the results obtained by Shephered et al and Hostmark, who observed that polynusaturated fat have hypotriglycridaemic effect. Our study indicates that low fat sunflower oil diet increases and high fat polyunsaturated oil decreases the plasma TG level probably due to antagonism. The results are in confirmation with Hostmark .

Total Cholesterol (TC)

The albino rats in group II who were fed on low fat diet supplemented with Nigella sativa showed a significant reduction in total cholesterol level as compared to group I. This observation clearly showed that Nigella sativa has hypocholesterolaemic effect. Also showed that albino rats in group III who were fed on 20% sunflower oil (high fat diet) showed significant reduction in serum cholesterol level. Interestingly, when Nigella sativa was added to high fat diet in group IV 20% sunflower oil (high fat diet) there was significant reduction in serum cholesterol level. High fat diet i.e. 20% sunflower oil in

combination with Nigella sativa has significant hypocholesterolaemic effect.

When albino rats in group V were given 20% sunflower oil (high fat diet) along with 1% cholic acid 0.5% propylthiouracil, serum cholesterol level. When Nigella sativa was given along with the above diet in-group VI, there was significant decrease in serum cholesterol level. This decrease was non-significant possibly due to cholic acid and propylthiouracil. Exactly similar findings were recorded at 24 weeks of experiment and Nigella sativa had similar hypocholesterolaemic effect. This hypocholesterolaemic effect of Nigella sativa is possibly due to cholerectic activity of Nigella sativa as reported by El-Dakhakhany. The cholerectic function of Nigella sativa is either by reducing the synthesis of cholesterol by hepatocytes or decreasing its fractional reabsorption from small intestine and thus following the serum cholesterol level.

High Density Lipoprotein Cholesterol (HDL-c)

The albino rats in group II who were fed on low fat diet with Nigella sativa showed very significant raised level of HDL-c, as compared to group I.

Albino rats in group III, who were fed on 20% sunflower oil (high fat diet) only showed significant decrease of HDL-c level Grundy , when linoleic acid (polyunsaturated fatty acid) was given in large amount, then HDL-c was lowered. Nigella sativa was mixed with high fat diet in group IV, there was significant increase in HDL-c level.

These findings clearly show that Nigella Sativa Causes Significant increase in HDL level which is protective. These results are in conformity with Bonanoma.

It was observed that when albino rats group V were given 20% sunflower oil (high fat diet) along with 1% cholic acid and 0.5% propylthioracil, there was elevation in HDL-c which was statistically significant. It may be due to deposition of cholesterol in the presence of cholic acid and propylthiouracil. Added to the above diet in group VI, HDL-c level. These results regarding the effects the effects of sunflower oil are in agreement with low fat diet increase HDL-c Grundy (1987) while high fat diet decrease HDL-c (Bananoma) and Rader .

Low Density Lipoprotein Cholesterol (LDL-c)

The albino rats in group II who were fed on low fat diet with Nigella sativa there was non-significant reduction of LDL-c. It may be due to low fat diet with combination of Nigella sativa. This observation shows that Niella sativa has LDL-c lowering effect.

Group III fed on high fat diet (20% sunflower oil) showed highly significant reduction of LDL-c Nigella sativa was mixed with high fat diet group IV there was highly significant decreases and this decrease in LDL-c was more Nigella sativa has got a better LDL-c lowering effect when diet (20% sunflower oil) in combination with Nigella sativa has highly significant lowering effects on LDL-c. High fat diet along with colic acid and propylthiouracil, there was increased level of LDL-c in both but it is somewhat lesser in experimental group VI which was given Nigella sativa.

The decrease in serum LDL-c in experimental group II, which were given Nigella sativa showed significant decreased in LDL-c level. Similar effect of Nigella sativa on LDL-c level was previously reported by the other workers, (Shaikh).

When Nigella sativa is given in combination with higher concentration of polyunsaturated fat like sunflower oil (20%), it significantly lowers serum cholesterol LDL-c, and serum TG level, while it significantly increases HDL-c level. All these effects are beneficial for patients with CHD and those individuals who are predisposed to this problem or have familial tendency.

Table-I. Lipid profile comparison between group-I (3% sunflower oil diet with nigella sativa) at 0,12 and 24 weeks

Estimation	Group-I			Group-II		
	0 week	12 weeks	24 weeks	0 week	12 weeks	24 weeks
TG	82.35 ± 4.92	86.9 ± 5.0	92.31 ± 5.10	81.68 ± 4.76^{+}	85.5 ± 4.66^{+}	92.40 ± 6.81^{+}
TC	73.37 ± 3.63	78.13 ± 3.88	85.1 ± 3.36	71.18 ± 5.26^{+}	74.2 ± 5.17^{+}	85.42 ± 5.62^{+}
HDL-c	21.6 ± 3.28	39.6 ± 3.58	24.93 ± 2.29	21.51 ± 2.12^{+}	23.8 ± 2.13^{+}	27.36 ± 1.37^{+}
LDL-c	35.29 ± 2.34	39.6 ± 3.58	41.65 ± 2.52	33.25 ± 2.34^{+}	33.4 ± 9.93***	39.13 ± 4.27^{+}

$^{+}$P>0.10 Not Significant **P<0.05 Significant* ****P<0.001 Very Highly Significant*

Key: TG: Triglycerides HDL-c: High-density lipoprotein cholesterol

TC: Total cholesterol LDL-c: Low density lipoprotein cholesterol

Table-II. Lipid profile comparison between group-III (20% sunflower oil diet) an experimental group IV (20% sunflower oil diet with nigella sativa) at 0,12 and 24 weeks (Values are expressed in mg/dl (mean ± SD)

Estimation	Group-III			Group-IV		
	0 week	12 weeks	24 weeks	0 week	12 weeks	24 weeks
TG	83.24 ± 4.74	79.8 ± 4.14	74.19 ± 4.56	79.16 ± 3.67^{+}	73.4 ± 3.01**	71.2 ± 3.88*
TC	74.56 ± 4.17	67.5 ± 3.73	60.95 ± 4.13	73.7 ± 4.57^{+}	66.6 ± 4.23^{+}	60.36 ± 3.10^{+}
HDL-c	22.70 ± 2.41	20.6 ± 2.23	18.38 ± 2.83	22.15 ± 1.85^{+}	24.3 ± 2.58^{+}	21.64 ± 2.48*
LDL-c	35.16 ± 2.59	30.9 ± 2.24	27.67 ± 2.39	35.73 ± 3.56^{+}	27.6 ± 4.95*	24.35 ± 2.40*

$^{+}$P>0.10 Not Significant **P<0.05 Significant* ***P<0.01 Very Highly Significant*

Key: TG: Triglycerides HDL-c: High-density lipoprotein cholesterol

TC: Total cholesterol LDL-c: Low density lipoprotein cholesterol

Table-III. Lipid profile comparison between control group-V (20% sunflower oil, 1% cholic acid, 0.5% propylthiouracil) and experimental group VI (20% sunflower oil, 1% cholic acid and 0.5% propylthiouracil with nigella sativa) at 0,12 and 24 weeks (Values are expressed in mg/dl (mean ± SD)

Estimation	Group-V			Group-VI		
	0 week	12 weeks	24 weeks	0 week	12 weeks	24 weeks
TG	81.76 ± 7.9	104.60 ± 7.86	128.9 ± 9.05	79.5 ± 1.29^{+}	103.92 ± 9.1^{+}	127.01 ± 8.81^{+}
TC	71.69 ± 4.97	129.34 ± 12.69	186.81 ± 12.3	72.62 ± 3.46^{+}	126.31 ± 3.80*	183.43 ± 17.09*
HDL-c	22.33 ± 2.06	33.83 ± 4.59	49.29 ± 0.35	22.53 ± 3.12^{+}	35.45 ± 4.2*	49.62 ± 4.72^{+}
LDL-c	37.41 ± 12.05	74.59 ± 6.5	111.26 ± 10.22	36.3 ± 7.36^{+}	70.08 ± 5.36*	108.5 ± 12.99*

$^{+}$P>0.10 Not Significant **P<0.05 Significant*

Key: TG: Triglycerides HDL-c: High-density lipoprotein cholesterol

TC: Total cholesterol LDL-c: Low density lipoprotein cholesterol

DISCUSSION

Enriched fatty diets usually cause elevation of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c) development of atherosclerosis and leads to coronary heart disease (CHD) (Apgar et al, Grundy Havel and Rapport (1995). Cholesterol (TC) has received too much importance because of its strong and consistent association with CHD.

The significant elevation of LDL-c is positively associated while elevation of high density lipoprotein cholesterol (HDL-c) is negatively associated with the development of CHD (Segal 1993). High total cholesterol and LDL-c levels, increase the risk of cardiovascular disease (CVD) (McNamara and Howel 1992, Klag et al 1993). Low HDL-c are necessarily at risk of premature CHD (Rader et al 1993) Ascherio et al (1995). Fish intake, Long-chain fatty acids, abundant in fish, reduce plasma VLDL-C concentrations, increase vesodilation, platelet aggregation.

Six experimental models of albino rats were made (groups I-VI). All the albino rats various groups were weighed at 0, 12th and 24th weeks of experiment. In the present work estimations included TG, TC, HDL-c and LDL-c in all albino rats.

Out of these the albino rats in control group I was given 3% sunflower oil (low fat diet), group III was given 20% sunflower oil (high fat diet) and group V was given 20% sunflower oil with 1% cholic acid and 0.5% propylthiouracil (high fat diet with atherogenic element). Other three experimental groups (group II, group IV and group VI) were given the above diets supplemented with Nigella sativa seed in powdered form.

CONCLUSIONS

Coronary heart disease (CHD) which most commonly follows atherosclerosis is a worldwide life threating problem. Atherosclerosis is, in turn, is a disease entity based upon hyperlipidaemias, hypercholesterolaemia. These metabolic states are associated with multiple factors including dietary habits. Various methods such as intake of cholesterol sequentrants medicines which increase the excretion of cholesterol or decrease the synthesis of cholesterol have emerged for lowering

cholesterol levels in body. Also have various side effects Ingredients are under trial pertinent to cholestrol lowering effects Nigella sativa is curative remedy for all diseases, except death is the saying of our Holy Prophet Hazarat Muhammad Salalah-o-allaihe- w-salam (peace be upon him).Having studied the uncountable effect of Nigella sativa e.g. its use as a medicine for treatment starting from simple cold to jaundice, from expulsion of kidney stones to abortion was tried to study its hypolipidaemic and hypocholesterolaemic effects.

To investigate the effect of Nigella sativa and various concentration of sunflower oil on serum lipids fatty diets were given to 84 albino rats. The rats were divided into six groups, three control as well as three experimental groups. The control groups were given, low fat diet (3%), high fat diet (20%) and high fat diet supplemented with 1% colic acid and 0.5% antithyroid drug (propylthiouracil). The experimental groups were given the above diets with supplemented Nigella sativa. The low fat diet increased all the lipid fractions significantly when given at 12 and 24 weeks duration as compared to 0 week.

The high fat diet when given at different intervals decreased all lipid fractions significantly as compared to baseline levels. The high fat diet with propylthiouracil and bile salt also increased all the lipid fractions and the increase was more as compared to previous groups. The supplements of Nigella sativa in the groups decreased all the lipid fractions significantly as compared to the control groups except HDL-c, which was significantly increased in all the experimental groups as compared to control groups. These observation confirm that Nigella sativa decreased total cholesterol, LDL-c and triglycerides levels, while it increases the HDL-c level. The above effects will be beneficial for patients with CHD. On the basis of these findings following conclusions are made:

Nigella sativa has got TG, TC and LDL-c lowering and HDL-c raising effect.

3% sunflower oil (low fat diet) has got TG, TC, HDL-c and LDL-c raising effects.

20% sunflower oil (high fat diet) has got TG, TC, HDL-c and LDL-c lowering effects.

Both Nigella sativa and sunflower oil have got low atherogenic index (TC/HDL) and may be recommended in hyperlipidaemic patients or normal individuals.

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