EFFECTS OF CANOLA OIL SUPPLEMENTED WITH ATHEROGENIC ELEMENT & NIGELLA SATIVA (KALONJI) ON SERUM LIPIDS IN ALBINO RATS - AN EXPERIMENTAL STUDY

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ABSTRACT

Objective: To compare effects of canola oil supplemented with atherogenic element and Nigella sativa on serum lipids in albino rats.

Place and Duration of Study: Study was conducted at Pathology Department of Postgraduate Medical Institute, for 12 weeks.

Study Design: 'Laboratory based randomized controlled trials'.

Material and Methods: Seventy two albino rats were selected and randomly divided into six groups of twelve animals with equal number of male and female in each. Fourteen days after acclimatization to the environment and basal diet, fasting blood samples (zero week) were collected by heart puncture under ether anesthesia and experimental diets were started which were continued for 12 weeks. All parameters were measured using enzymatic colorimetric methods.

Results: Estimations of serum lipids showed increase in total cholesterol (TC) and High Density Lipoprotein Cholesterol (HDL-c) levels but fall in LDL-c concentrations in groups fed on canola oil diet. On the other hand, even atherogenic supplemented groups had decrease in cardio-protective HDL-c and raised LDL-c; although statistically non-significant. Thus canola oil diets were not hyperlipidaemic and prevented adiposity. Nigella sativa (NS) diets significantly decreased serum total cholesterol and LDL-c while HDL-c was raised but non-significantly. Thus Nigella sativa prevented deposition of lipids in tissues, thus preventing tendency to obesity and atherogenesis by decreasing LDL-c in serum.

Conclusion: Nigella sativa produces antilipidaemic and anti-obesity effects by decreasing low density lipoprotein cholesterol level which is statistically significant in two out of the three groups fed on Ns; it also increased high density cholesterol which was however non-significant in comparison with Canola oil alone.

Keywords: Atherogenicagent, Canola oil, Nigella sativa.

INTRODUCTION

Nigella sativa seed in different forms had been under use since prehistoric era for countless number of ailments by human beings[1]. Learning the saying of our Holy prophet Muhammad (PBUH) that Nigella sativa (Ns) is a remedy for every ailment except death, idea for doing experimental work against one component of the "deadly quartet", hyperlipidaemia, was instigated. There are growing evidences that patients can improve lipid levels and decrease cardio-vascular events by switching their diets from saturated and polyunsaturated fats to monounsaturated fats[2]. The change may improve endothelial dynamics, reduce oxidation of LDL and atherosclerosis and enhance thrombolytic activity[3]. Nigella sativa seeds (black cumin, kalonji) have been recommended for all ailments except death by our Holy Prophet Muhammad (PBUH) (Al-Juziah 751 Hij) The Kalonji seeds are rich in amino acids alanine, tryptophan, and leucine[4,5].

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MATERIAL AND METHODS

These Laboratory based randomized controlled trials were carried out at Pathology department of Postgraduate Medical Institute, Lahore and comprised 72 albino rats of same speciesat 8 weeks age, weighing 150 – 200 g each, placed on special diets for a period of 12 weeks duration. There were equal numbers of male and female animals. The experimental animals were collected from Pakistan Council for Scientific and Industrial Research (PCSIR) Laboratories, Lahore and kept at animal house of Postgraduate Medical Institute (PGMI), Lahore. Sick albino rats and those having a weight of more than 200 g and less than 150 g

were excluded from the study. Animals with abnormal lipid profiles were also excluded from the study. The animals were kept under recommended uniform atmospheric and hygienic conditions with food and water available all the times in the cages.The animals were randomly divided into six groups of 12 rats each, having 6 males and 6 females. The groups were given the names A, B, C, D, E and F. After over-night fasting all animals were weighed and zero week blood samples were collected[6] for fasting estimation of total cholesterol, HDL-c and LDL-c as baseline reference for comparison.

Six different types of experimental diets were prepared according to Welhe[7]. The diets contained caseine, glucose, choline / methionine, minerals mixture and vitamins mixture in quantities of 20, 10, 0.5, 3.5 and 01 grams respectively to make 100 grams of the diets as basic constituent. Group A was fed diet 1 (2.9% canola oil). Group B was fed diet 2 (2.9% canola oil and Nigella sativa (Ns) powder). Group C was fed diet 3 (20% canola oil). Group D was fed diet 4 (20% canola oil and Ns powder). Group E was fed diet 5 (20% canola oil and CCT supplement (Cholesterol, cholic acid, thiouracil supplement). Group F was fed diet 6 (20% canola oil, CCT supplement and Ns powder).

The first blood sample was collected before the start of experimental diets at zero weeks (baseline) as already mentioned and the second was collected for estimation of total Cholesterol, HDL-c and LDL-cafter 12 week of start of experimental diets. The animals were kept fasting over-night before collection of blood samples. In the morning each animal was weighed and given deep ether anaesthesia. Heart puncture was done with a 5 ml disposable syringe and 2 ml of blood was drawn very gently and slowly. The serum was preserved at -200C in freezer for lipid estimations.Statistical analysis was performed using Statistical Package for the Social Science (SPSS version 13). Data was evaluated by descriptive tests such as mean and standard deviation as shown in tables. Comparison of two means was carried out by students 't' test for parametric data while with the help of Mannwhitney-U (Wilcoxon rank-sum) test for non-parametric results (LDL-c) and baseline comparison of means for lipid profilesofall groups was carried out by analysis of variation (ANOVA) for parametric data and Kruskal

Table- 1: Comparison of TC, HDL and LDL in Groups A, B, C, D, E & F at 0 and 12 weeks.

Groups	Tests (mg/dl)	0 Week (Baseline)	12 Week	Net Mean Difference	0vs 12 weeks
A	Total Cholesterol	75.8 ± 5.6	115.0 ± 6.0	39.2	< 0.001
	HDL-c	44.4 ± 5.1	74.4 ± 6.4	30.0	< 0.001
	LDL-c	8.4 ± 3.5	15.1 ± 10.5	6.9	0.002
B	Total Cholesterol	76.9 ± 6.5	112.5 ± 6.5	35.6	< 0.001
	HDL-c	41.5 ± 4.9	77.2 ± 4.5	35.7	< 0.001
	LDL-c	11.6 ± 7.1	11.0 ± 7.0	- 0.6	0.002
C	Total Cholesterol	77.4 ± 4.9	121.6 ± 10.0	42.2	< 0.001
	HDL-c	40.9 ± 4.1	81.9 ± 4.4	41.0	< 0.001
	LDL-c	12.8 ± 5.9	10.0 ± 5.4	- 2.8	0.005
D	Total Cholesterol	76.0 ± 6.7	117.1 ± 5.2	41.1	< 0.001
	HDL-c	41.0 ± 4.3	84.7 ± 5.6	43.7	< 0.001
	LDL-c	12.1 ± 6.8	5.7 ± 2.8	- 6.4	0.002
E	Total Cholesterol	80.6 ± 5.1	120.2 ± 8.3	39.6	< 0.001
	HDL-c	40.4 ± 3.8	82.1 ± 2.7	41.7	< 0.001
	LDL-c	17.9 ± 7.2	12.8 ± 5.2	- 5.1	0.023
F	Total Cholesterol	78.4 ± 5.3	111.1 ± 4.9	32.7	< 0.001
	HDL-c	41.5 ± 4.03	88.4 ± 3.9	46.9	< 0.001
	LDL-c	13.9 ± 7.1	5.1 ± 2.4	- 8.8	0.002

Wallis test for non-parametric results. *p*-value< 0.05 was considered to be statistically significant while that of < 0.01 as highly significant.

Table-2: Comparison of change in TC, HDL & LDL between the groups.

Groups	TC	HDL	LDL
Group A	39.76 ± 7.97	30.76 ± 9.36	-5.99 ± 3.68
Group B	33.33 ± 8.83	35.95 ± 7.59	-9.88 ± 5.43
Group C	41.78 ± 6.90	42.02 ± 5.73	-5.40 ± 4.51
Group D	40.20 ± 8.73	43.60 ± 6.70	-11.42 ± 3.56
Group E	39.38 ± 6.69	34.13 ± 3.66	2.42 ± 3.57
Group F	32.65 ± 5.10	41.81 ± 6.13	-9.63 ± 3.98
p-value	0.012	< 0.001	< 0.001

Table-3: Post-hoc comparison of TC, HDL & LDL between the groups.

Group Comparisons	TC	HDL	LDL
Group A vs. Group B	0.298	0.422	0.216
Group A vs. Group C	0.986	0.002	0.999
Group A vs. Group D	1.000	< 0.001	0.026
Group A vs. Group	1.000	0.824	< 0.001
Group A vs. Group F	0.198	0.002	0.282
Group B vs. Group C	0.077	0.252	0.104
Group B vs. Group D	0.231	0.075	0.945
Group B vs. Group E	0.366	0.986	< 0.001
Group B vs. Group F	1.000	0.288	1.000
Group C vs. Group D	0.995	0.992	0.999
Group C vs. Group E	0.969	0.061	< 0.001
Group C vs. Group F	0.044	1.000	0.144
Group D vs. Group E	1.000	0.013	< 0.001
Group D vs. Group F	0.148	0.987	0.900
Group E vs. Group F	0.251	0.073	< 0.001

RESULTS

All the groups were comparable at baseline for total cholesterol (p = 0.326), HDL-c (p = 0.244) and LDL-c (p = 0.607), collectively known as 'lipid profile', as shown in tables below. Group-wise description of total cholesterol, HDL-c and LDL-c at 0 & 12 weeks after feeding the animals on special diets and net difference between the two is given in Table-1. Within-group comparison of total cholesterol, HDL-c and LDL-c is also summarized in Table-1.

Canola oil (n-3 fatty acid rich) diet in albino rats increased HDL-c and lowered LDL-c and preserved the myocardium more than the standard cholesterol-rich diets[8]. Canola oil diet containing monounsaturated fatty acids (MUFAs) decreased total cholesterol and LDL-c serum levels as compared to saturated fats[9]. (Table-2 & 3).

DISCUSSION

There was a significant rise in serum total cholesterol (TC) concentrations between week 0 and 12; after which there was a non-significant rise. The results were in conformation with Pereira et al[10] and Akman[11] but these were not in agreement with findings of Manorama and Rukmini[12]. The difference of results might be due to the age of the albino rats used. The present models also showed a rise at 12 week period. The difference of attainment of stability in TC levels might be due to feeding of different levels of Canola oil by Manorama and Rukmini[12].

Serum HDL-c concentration of various groups at week 0 and 12 showed that there was a significant rise in HDL-c levels at week 12 in all the groups. These results were in agreement with results of Pereira et al[10]. Comparison of

serum HDL-c levels of groups A, C and E (fed Canola Oil alone) revealed non-significant difference in Normal Fat Diet (NFD) and High Fat Diet (HFD)groups. These observations were in agreement with results of Cottrell[13] and Akman[11]. The observations suggested neutral effect of Canola oil on HDL-c which is consistent with reports of Marzukiet al[14] and Dahri[15]. Comparison between various groups maintained on dietary Canola oil alone and Canola oil with Ns showed that there was a rise in serum HDL-c in groups fed on Canola oil with Ns. The differences were significant in NFD group but non-significant in HFD. These results revealed that Nigella Sativa has got a weak HDL-c raising effect. The findings were in conformation with Dahri[15] and Akman[11].

Serum concentrations of LDL-c revealed variable pattern in different groups. The results were not in conformation with the study done by Pereira10. Comparison of groups maintained on dietary Canola oil without Ns showed that by increasing the levels of Canola oil in diet the serum concentrations were raised accordingly. These findings were again not in agreement with the reports of Manorama and Rukmini[12]. The reason for controversy may be difference of animal species, age of animal used, levels of fats administered and finally the method of measurement of LDL cholesterol in serum.

On comparison of groups maintained on dietary Canola oil and Canola oil with Ns showed that at different levels of oil administration, the LDL-c levels were lowered in groups maintained on Canola oil with Ns. These findings unmask LDL-c lowering effects of Ns. The results were in conformation with the works of Akman11and Dahri[15].Comparing different groups maintained on dietary Canola oil with Ns revealed that raising the concentrations of Canola oil with same level of Ns has got augmented effects on lowering of serum LDL-c levels. This finding indicates that Canola oil and Ns may act synergistically to lower the cardio-destructive LDL-c fraction. These findings were consistent with the reports of Akman[11] and Dahri[15].

CONCLUSION

Nigella sativa produces antilipidaemic and anti-obesity effects by decreasing low density lipoprotein cholesterol level which is statistically significant in two (D & F) out of three groups fed on Ns.It also increased high density lipoprotein cholesterol level which was however non-significant in comparison with Canola oil alone.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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