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EFFECT OF USING LINSEED OIL AND OLIVE OIL IN RAT'S DIETS ON SERUM TOTAL CHOLESTEROL AND ITS FRACTIONS

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ABSTRACT

In a seventy three days experimental period study, diets were prepared to cause hypercholesterolemic rats. Linseed oil, a rich source of omega-3 fatty acids, and olive oil, a rich source of mono-unsaturated fatty acids were used to study their effect on serum total cholesterol and its fractions, High Density Lipo Protein (HDL), Low Density Lipo Protein (LDL) and Very Low Density Lipo Protein (VLDL).
In rats fed diets containing linseed oil, results showed decrease in the level of serum total cholesterol. Such reduction is also most important as it was accompanied with increase in HDL and decrease in LDL and VLDL.

On the other hand, rats fed diets containing olive oil showed no alteration on total cholesterol, HDL, LDL and VLDL levels.

INTRODUCTION

It has been reported (Mensink and Katan, 1989) that mono-unsaturated fatty acids (MUFAs) are negatively correlated with coronory artery disease (CAD). Grundy (1989) stated that MUFAs enriched diets reduced plasma total cholestrol concentration (T.C.) without increasing plasma triacylglycerol concentrations or reducing high density lipoprotein (HDL) which is considered as a cardioprotective fraction. This may be the reason that Kafatos and Mamalakis (1993) found the prevelance of CAD to be low in some Mediterranean regions, where diets are rich in olive oil that containing MUFAs.

Linseed oil which is rich in MUFAs, also is a good source of a poly-unsaturated fatty acids (PUFAs), namely linolenic acid which is an omega 3 fatty acid, (Simopoulos, 1991). Omega-3 PUFAs are associated with low incidence of cardiovascular disease (Wallingford and Yetley, 1991).

The object of the present study is to give an idea about the effect of substituting corn oil, or corn oil + butter by either olive oil or linseed oil on serum T.C., HDL, LDL and VLDL cholesterols.

MATERIALS AND METHODS

The experimental work of the present investigation was carried out in the Central Lab. for Food and Feed (CLFF), of the Agricultural Research Center, Egypt.

Olive oil used in the present work was purchased from the market in Cairo. It is claimed from the label to be an extra vergin oil produced by cold press technique.

Linseed oil was purchased from an old oil extraction mill called "serga", where oil is extracted using old press method.

Experimental diets:

Nine experimental diets were formulated (Table 1) according to Bobek *et al.*, (1993). Three diets were considered to be purified basal diets with some modification, where pork fat was replaced by butter or /and corn oil.

The three basal diets were formulated as follows:

Basal diet 1 (BD1): fat source is 5% butter and 5% corn oil.

Basal diet 2 (BD2): fat source is corn oil only (10%).

Basal diet 3 (BD3): same as BD1,but pure cholesterol (Winlab 99 %) was added 10g/kg on expense of cellulose content.

The six tested experimental diets were as follows :

- Corn oil was replaced in BD1 by olive oil to formulate diet BD1O and by linseed oil, to produce BD1L.
- Olive oil and linseed oil replaced corn oil in BD2 to produce diets BD2O and BD2L, respectively.
- BD3O and BD3L were prepared by substituting corn oil by olive oil and linseed oil, respectively.

Experimental design:

72 weanling albino rats were divided into 9 groups. Each group included 8 rats, 4 Males and 4 Females of approximately the same weight. During the first feeding period (1-15 day), the nine groups of rats were fed on 3 basal diets.

Groups 1, 4 and 7 were fed on BD1, groups 2, 5 and 8 were fed on BD2 and group 3, 6 and 9 were fed on BD3.

The source of fat was 5% butter and 5% corn oil in BD1, 10% corn oil in BD2, while the third basal diet BD3 contained 5% butter + 5% corn oil and 1% pure cholesterol .

The second feeding period (16-73 day), the nine groups of rats were fed as follows:

* 3 groups of rats 1, 4 and 7 continued to get basal diets BD1, BD2 and BD3, respectively as shown in (table 1).
* 3 groups of rats were fed on the 3 basal diets supplemented with linseed oil as follows:
  Group 4 (BD1L) same as BD1 but 5% linseed oil replaced corn oil.
  Group 5 (BD2L) same as BD2 but 10% linseed oil replaced corn oil.
  Group 6 (BD3L) same as BD3 but 5% linseed oil replaced corn oil.
* 3 groups of rats were fed on 3 basal diets supplemented with olive oil as follows:
  Group 7 (BD1O) got the same diet as BD1 but corn oil was replaced by olive oil.
  Group 8 (BD2O) got the same diet as BD2 but corn oil was replaced by olive oil.
  Group 9 got the same diet as BD3 but corn oil was replaced by olive oil (BD3O).

Criteria studied:

* Serum total cholesterol (T.C.) was determined using Biocon kits*.

* Serum high density lipoprotein cholesterol (HDL) was determined using Biocon kits*.
  (Biocon Diagnosemittel, GmbH Co. Produktions-KG Hecke 8, D-34516 and Vohl-Marien hagen - Germany)
* Serum low density lipoprotein cholesterol (LDL) was determined using bioMerieux sa**.
* Serum very low density lipoprotein cholesterol (VLDL) was calculated by difference.
  VLDL = TC - [HDL + LDL]
* Blood samples were drawn from individual rats on days 1, 16, 57 and 73, from Retiro orbital pluex vein. Rats were deprived from feed 12 hours before blood sampling.

Table (1): The experimental diets used throughout the experiment for different experimental groups.

Ingredients %		Basal			Basal diets supplemented with (L.)			Basal diets supplemented with (O.)		
	experimental groups	1	2	3	4	5	6	7	8	9
	experimental diets	BD1	BD2	BD3	BD1 L	BD2 L	BD3 L	BD1 O	BD2 O	BD3 O
Casein		18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Corn starch		60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00
Unsalted butter		5.00	-	5.00	5.00	-	5.00	5.00	-	5.00
Corn oil		5.00	10.00	5.00	-	-	-	-	-	-
Linseed oil (L.)		-	-	-	5.00	10.00	5.00	-	-	-
Olive oil (O.)		-	-	-	-	-	-	5.00	10.00	5.00
Cellulose		6.30	6.30	5.30	6.30	6.30	5.30	6.30	6.30	5.30
Pure cholesterol		-	-	1.00	-	-	1.00	-	-	1.00
Mineral mixture		4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
[illegible] mixture		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride		0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Bile salts		0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55

** (bioMerieux sa 69280 l'Etoile - France)

RESULTS AND DISCUSSION

Effect of linseed supplementation:

Rats fed on the three basal diets, during the first feeding period, (1-15 days), showed increase in their serum total cholesterol level on day 16. Serum total cholesterol level in rats fed BD3, the cholesterol supplemented diet, showed increase of cholesterol level on day 16 by almost 5 folds (496%) than that of the first day. When rats were fed basal diet 1 (BD1), containing butter, the increase in serum cholesterol was 33% only. Using BD2, containing corn oil only, the increase in serum cholesterol was only 18%.

The increase in cholesterol level in rats fed BD3 was accompanied by a reduction in HDL fraction of cholesterol, (reduced from 67% on first day to 9% on day 16) and increase in LDL and VLDL fractions of cholesterol from 17 to 37% and from 18 to 55%, respectively. On the other hand, the small increases in T.C. in rats fed BD1 and BD2 were accompanied by increases in

HDL and reduction in LDL and VLDL proportions of total serum cholesterol,except in LDL of BD2.

Supplementing the basal diets with linseed oil, serum total cholesterol decreased in rats fed the three diets. The greatest decrease was noticed on 57 days and 73 (22% and 21%, respectively) in rats fed the cholesterol basal supplemented diet (BD3). The decrease in total cholesterol in rats fed BD1 and BD2 ranged between 5 and 11% only.

On days 57 and 73, HDL as percent of T.C. tended to increase than percentages on day 16, due to linseed oil supplementation in all treatments. The increase in HDL percentage of total cholesterol from day 16 to day 73 was the highest with rats fed the cholesterol diet, even their HDL levels were the lowest.

LDL percent of total cholesterol tended to decrease during the days 57 and 73 compared to day 16 (start of linseed supplementation) in rats fed BD1L and BD2L. In rats fed BD3L, while total cholesterol decreased on days 57 and 73 (being 313 and 319 respectively compared to 402 on day 16). HDL % of T.C. increased on days 57 and 73 (11 and 14 %, respectively)and VLDL decreased on days 57 and 73 (being 42% and 39%, respectively). As proportion of total cholesterol the LDL percent increased on days 57 and 73 compared to day 16, (being 47% compared to 37%, respectively.

Table (2): Effect of supplementing basal diets with linseed oil on T.C., HDL, LDL and VLDL cholesterol.

	Diets[1]	Diets[2]	Basal diets		Basal diets + linseed oil (L)	
			Day 1	Day 16	Day 57	Day 73
	BD1	BD1L	74	99	88	92
Serum T.C. mg/dL	BD2	BD2L	87	97	86	92
	BD3	BD3L	82	402	313	319
	BD1	BD1L	55	64	69	65
HDL as % of T.C	BD2	BD2L	67	74	81	87
	BD3	BD3L	67	9	11	14
	BD1	BD1L	22	18	13	14
LDL as % of T.C.	BD2	BD2L	14	19	11	7
	BD3	BD3L	17	37	47	47
	BD1	BD1L	22	18	18	21
VLDL as % of T.C.	BD2	BD2L	19	7	10	6
	BD3	BD3L	18	55	42	39

[1] Diets used from day 1 to day 15 .
[2] Diets used from day 16 up to day 73 .

On linseed oil supplementation of basal diets, the concentration of VLDL was reduced slightly in rats fed BD1L and BD2L. The levels of VLDL of rats fed BD3 (cholesterol supplemented diets) on days 57 and 73 were reduced compared to day 16, the onset of linseed oil supplementation. The change on the percentage of VLDL of cholesterol in BD1 and BD2 treatment was clearly affected by the fat source of the diet. With BD3 a reduction in VLDL proportion of total cholesterol was noticed.

From the foregoing discussion, it is apparent that linseed oil affected the levels of serum total cholesterol in rats under different cholesterol

supplementation levels. Such reduction is also most important as it accompanied an increase in HDL and a decrease in LDL + VLDL. In this respect, the results obtained in the present work almost agreed with that of Ranhotra *et al.* (1993).

Effect of olive oil supplementation:

On supplementing diets with olive oil starting from day 16, no great changes on T.C. were noticed on day 73 when BD1 and BD2 were used. BD3 supplemented with cholesterol showed a great increase in T.C. concentration on 16 day. On supplementing BD3 with olive oil, no effect was noticed on day 73. High serum total cholesterol continued to exist.

High density lipoprotein cholesterol fractions (HDL) was almost the same on days 16 and 73 when diets BD1O and BD2O were used being over 60% of T.C. But when diet BD3O was used, HDL cholesterol fraction continued to be low (13 and 12% of T.C.) of days 16 and 73, respectively.

LDL and VLDL cholesterol fractions were low in case of rats fed either BD1O or BD2O (range 35-38% on da y 16 and 40-39% on day 73). When supplementing the cholesterol containing diet (BD3) with 5% olive oil, no effect was noticed in the LDL and VLDL cholesterol fractions percentage of T.C. being 87 and 88% for 16th day and 73, respectively.

From the foregoing results, no apparent effect of supplementing basal diets under study with olive oil on T.C., HDL, LDL and VLDL.

Table (3): The effect of supplementing 3 basal diets (BD1, BD2 and BD3) with olive oil.

	Diets[1]	Diets[2]	Day 1	Day 16	Day 73
	BD1	BD1O	76	98	106
TC mg/dL	BD2	BD2O	83	101	112
	BD3	BD3O	75	324	341
	BD1	BD1O	58	65	60
HDL % of T.C.	BD2	BD2O	66	62	61
	BD3	BD3O	69	13	12
	BD1	BD1O	42	35	40
LDL + VLDL % of T.C.	BD2	BD2O	34	38	39
	BD3	BD3O	31	87	88

[1] **Diets used from day 1 to day 15 .**
[2] **Diets used from day 16 up to day 73 .**

In this respect Fernandez et al. (1996) found no differences due to olive oil feeding (they used 15% olive oil in their experimental diets) on plasma T.C., HDL and LDL cholesterol in guinea-pig.

However, the benifits of using olive oil on health may be due to the presence of antioxidant phenolic compounds. The phenols in olive oil are potent inhibitors that protect lipoproteins against oxidation (Fito et al., 2000), which other wise contribute to the progression and pathogenesis of atherosclerosis.

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تأثير استخدام زيت الكتان (الحار) وزيت الزيتون فى غذاء الفئران علـــي سـيرم الكوليسترول الكلي وشقوقه

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المعمل المركزى للأغذية والأعلاف – مركز البحوث الزراعية

في دراسة لمدة ٧٣ يوما أعد فيها غذاء لزيادة الكوليسترول في سيرم دم الفئران استخدم زيت الكتان (الحار) الغني المصدر في الاحماض الدهنية او ميجا -٣ كما استخدم زيت الزيتون الغني المصدر في الاحماض الدهنية الاحادية عديمة التشبع لبيان تأثير هذان النوعان من الزيوت علي سيرم الكوليسترول الكلي وشقوقه (البروتينات الدهنية ذات الكثافة المرتفعة – البروتينات الدهنية ذات الكثافة المنخفضة – البروتينات الدهنية ذات الكثافة المنخفضة جدا) • الفئران التي تم تغذيتها علي غذاء به زيت الكتان اتضح ان له تأثير واضح علي خفض نسبة سيرم الكوليسترول الكلي وكذلك زيادة في البروتينات الدهنية ذات الكثافة المرتفعة وخفض كل من البروتينات ذات الكثافة المنخفضة والمنخفضة جدا •

علي الجانب الاخر إتضح أن غذاء الفئران الذي يحتوي علي زيت الزيتون ليس له تأثير واضح علي الكوليسترول الكلي وكذلك علي شقوقه من (البروتينات الدهنية ذات الكثافة المرتفعة او الكثافة المنخفضة والمنخفضة جدا) •