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A COMPARATIVE STUDY OF OMEGA-3-FATTY ACIDS OBTAINED FROM MARINE FISH AND BEZAFIBRATE ALONE AND IN COMBINATION AS HYPOLIPIDEMIC AGENTS

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ABSTRACT:

The fish oil and its constituents have been studied in detail with special reference to ailments. The discovery of ω-3 fatty acids led to a detailed investigation about its effect and role as anti-cholesterolemic agents. The effect of fish oil alone and in combination with acid derivatives was found to be a potent cholesterol and triglycerides lowering agent. Comparative studies of fish oils and bezafibrate led to the formulation of new therapeutic combination having lesser side effects and toxicity.

INTRODUCTION

Any abrupt change in various lipid parameters of blood such as increase in cholesterol, triglycerides and lipoproteins and its deposition in the arterial walls of the circulatory system may lead to atherosclerosis and arteriosclerosis. Atherosclerosis is a primary cause of cardiovascular diseases which are the major cause of death in western industrialized nations (Feinleib, 1984).

A definite correlation between atherosclerosis and hypercholesterolemia have been proposed by Gofman et al. (1956) Maclom et al. (1958) postulated that cholesterol being the major cause of atherosclerosis, triglycerides concentrations were also higher in coronary heart disease patients. Albrink and Mann, (1959) Carlson and Oro (1960) demonstrated triglycerides as an independent risk factor.

Fish oils have a great tendency to lower serum cholesterol and triacylglycerols and thus save from coronary diseases, heart attack and thrombosis (Chamberlaim and Belton 1987).

It is significant that ω-3 PUFAs of fish oil appear to be much effective in reducing hyperlipidemia. Schmidt et al. (1992) observed the effect of ω-3 PUFAs supplementation in men with moderate and severe hypertriglyceridemia and indicated that total cholesterol and triglycerides decreased significantly.

Considering the above facts and works carried out by various reserchers, (Bradford et a1., 1992 & Kim et al. 1995) the fish oil obtained from *Eusphyra blochii* (Hammer-headed Shark) and *Carcharhinus bleekeri* (Shark) were found to be rich in ω-3 fatty acids. They were tested for their antihyperlipidemic activity alone and in combination with bezafibrate which is one of the potent

drugs among fibric acid derivatives. Bezafibrate is highly effective in lowering triglycerides (Packard et al., 1986).

Comparative studies of fish oil and bezafibrate have been carried out with the aim to search out such a drug having high potency and lesser side effects. The experiments were performed on rabbits and found synergistic effects of these compounds which would be very valuable in the formulation for combination therapy that is a modem trend.

MATERIAL AND METHODS

Apparatus

(1) Electrical centrifuge 1000-4000 rpm (China)
(2) Disposable syringe 10 ml, 21 gauge B.D.
(3) Glass tubes
(4) Centrifuge tubes
(5) Pasture pipette
(6) Juster 0.1 ml to 1 ml
(7) Microlab 100 E. Merck

Reagents

All the reagents were purchased from E. Merck, Federal Republic of Germany.

Material for Feeding and Treatment Purpose

(I) Cholesterol (Cruda, USA)
(2) Butter (Lurpak, Denmark)
(3) Bezafibrate-purchased from market
(4) Fish oil isolated from two marine fish
(5) Chickpea (gram seed) local supplier
(6) Alfa Alfa (loosan or green grass) local supplier.

Animals and Feeding Schedule

The study was carried out on 25 healthy white male rabbits of 1.0 to 1.5 kg supplied by local supplier. Whole procedure from feeding schedule to the biochemical analysis was adopted as described comprehensively in the thesis (Faheem, 1995).

RESULTS AND DISCUSSION

Recently, many laboratories are attracted in fish oil research work due to its medicinal usage. It is found that serum cholesterol and triglyceride levels inhuman subjects have been decreased remarkably by ω-3 fatty acids of various chain lengths (Bronte et al., 1956, Anderson et al. 1957, Malmoos & Wigand 1957, Connor et al. 1983) and thus has a correlation with coronary heart disease. Over the past quarter of a century, many studies have shown that large amounts of PUFAs in the diet exhibited significant hypolipidemic effect, which is confirmed by the low incidence of coronary complications in Eskimo, whose major component of diet is of marine origin composed of ω-3 PUFAs (Harris and Connor, 1980). Reduction in plasma cholesterol levels can also be attributed to an increased excretion of sterols and bile acids in the feces, which

have been observed before and after consumption of fish oil (Connor & Harris, 1981). Fish oil obtained from *Eusphyra blochii* and *Carcharhinus bleekeri* were examined for their biochemical activities alone and in combination with bezafibrate. The studies were carried out on rabbits. Our observations are in support of the investigation that rabbits has propensity to develop a massive increase in plasma cholesterol levels within days when kept on a high cholesterol diet (Feet etal. 1963, Adam etal. 1964, & Petri 1987).

The work was carried out in the following manner:

1. Effect of Cholesterol and Butter rich Diet on Various Groups of Rabbits for 12 Weeks and Comparison to normal control (NC)

Normal diet supplemented with cholesterol (200 mg/day) and butter (2g/day) was given to the rabbits of all groups for 12 weeks for increasing the level of cholesterol. All the values of normal control (NC) were remained constant for the whole dietary period with slight fluctuation whereas in pathological control (PC) and treated groups, all the lipid parameters were changed significantly as compared to the normal control group.

2. Effect on Serum Total Cholesterol

In the case of fish oil, total cholesterol is decreased to 75.64% ($p<0.01$) whereas bezafibrate exhibited a decrease of 68.44% ($p<0.01$), maximum activity is achieved by the combined treatment of oil and bezafibrate, 86.57% ($p<0.01$) (Tables 1-5, Fig. 1).

3. Effect on Serum Triglycerides

Significant effect on the triglycerides level is observed in the combined treatment of fish oil and bezafibrate 83.60% ($p<0.01$) while in fish oil treatment, the triglycerides level decreased to 51.86% ($p<0.01$) in the case of bezafibrate, the level decreased upto 59.44% ($p<0.01$) among all groups, the best results were observed by the administration of fish oil - bezafibrate (Table 6-10, Fig 2).

4. Effect on Total Lipid

An overall decrease in total lipid is observed. However, maximum decrease is noted in the case of fish oil + bezafibrate 87.32% ($p<0.01$) whereas in the case of fish oil total lipid is decreased to 50.00% ($p<0.01$) and in the case of bezafibrate total lipid is decreased by 79.24% ($p<0.01$) (Tables 11-15, Fig 3).

CONCLUSION

During the course of our study we attempted to prove a better combination of fish oil + bezafibrate. The effect of bezafibrate along with ω-3 fatty acid may lead to workable formulation of a combined product reducing the risk of side effects and toxicity. It can further be proposed that ω-3 fatty acid acts in synergistic relation to that of bezafibrate.

Table – 1
Effect of Normal Diet on the Serum Total Cholesterol mg/100 ml in Group 1 Normal Control (N.C) Rabbits

Weeks	1A	1B	1C	1D	1E	MEAN	± SE	P
0	59	34	35	35	36	39.80	4.30	
4	60	44	43	40	40	45.40	3.34	
8	65	68	48	46	62	57.80	4.04	
12	77	75	52	55	68	65.40	4.57	
14	78	77	55	62	76	69.60	4.18	
16	80	80	61	65	80	73.20	3.77	
18	84	81	83	68	83	79.80	2.67	

Table – 2a
Effect of Cholesterol + Butter Rich Diet on the Serum Total Cholesterol mg/100 ml in Group 2 Pathological Control (P.C) Rabbits

Weeks	2A	2B	2C	2D	2E	MEAN	± SE	P
0	48	45	63	58	64	55.60	3.47	
4	238	198	263	245	190	226.80	12.57	
8	371	435	545	319	375	409.00	34.57	
12	529	565	714	551	495	570.80	33.72	

Table - 2b
Effect of Normal Diet*

14	490	478	689	495	395	509.40	43.32	
16	410	397	629	428	330	438.80	45.04	
18	335	310	575	340	290	370.00	46.54	

* Normal diet: Loosen (alfa alfa), Gram seeds (chick pea)

Table – 3a
Effect of Cholesterol + Butter Rich Diet on the Serum Total Cholesterol mg/100 ml in Group 3 Treated 1 (T.1) Rabbits

Weeks	3A	3B	3C	3D	3E	MEAN	± SE	P
0	65	46	85	61	45	60.40	6.55	
4	275	220	247	235	255	246.40	8.29	
8	476	334	570	361	390	426.20	38.59	
12	537	458	641	509	437	516.40	32.07	

Table - 3b
Effect of Fish Oil (71.43 mg/kg/day)

14	450	380	455	412	370	413.40	15.38	<0.05
16	300	225	205	201	290	244.20	18.96	<0.01
18	150	125	102	115	137	125.80	7.47	<0.01

Table – 4a
Effect of Cholesterol + Butter Rich Diet on the Serum Total Cholesterol mg/100 ml in Group 4 Treated 2 (T.2) Rabbits

Weeks	4A	4B	4C	4D	4E	MEAN	± SE	P
0	42	57	47	42	36	44.80	3.14	
4	110	295	363	137	163	213.60	43.87	
8	226	446	515	503	395	417.00	46.82	
12	280	594	644	713	443	534.80	69.44	

Table - 4b
Effect of Bezafibrate (8.6 mg/kg/day)

14	169	210	299	599	363	328.00	67.74	<0.05
16	137	146	211	44	281	243.89	50.40	<0.01
18	32	54	174	367	197	168.80	51.74	<0.01

Table – 5a
Effect of Cholesterol + Butter Rich Diet on the Serum Total Cholesterol mg/100 ml in Group 5 Treated 3 (T.3) Rabbits

Weeks	5A	5B	5C	5D	5E	MEAN	± SE	P
0	60	87	50	107	53	71.40	9.87	
4	253	276	602	454	193	355.60	67.44	
8	432	540	653	536	346	501.40	46.75	
12	501	648	671	922	431	634.60	75.74	

Table - 5b
Effect of Fish Oil + Bezafibrate (71.43 + 8.6 mg/kg/day)

14	300	612	360	297	275	368.80	55.83	<0.01
16	176	305	137	169	163	190.00	26.38	<0.01
18	109	174	27	40	76	85.20	23.64	<0.01

Table – 6
Effect of Normal Diet on the Serum Total Cholesterol mg/100 ml in Group 1 Normal Control (N.C) Rabbits

Weeks	1A	1B	1C	1D	1E	MEAN	± SE	P
0	47	62	64	65	56	58.80	2.99	
4	64	65	65	70	63	65.40	1.08	
8	70	77	80	76	74	75.40	1.49	
12	85	89	85	89	89	87.40	0.88	
14	87	95	91	92	91	91.20	1.15	
16	90	99	98	95	93	95.00	1.47	
18	99	108	105	102	108	104.40	1.56	

Table – 7a
Effect of Cholesterol + Butter Rich Diet on the Serum Total Cholesterol mg/100 ml in Group 2 Pathological Control (P.C) Rabbits

Weeks	2A	2B	2C	2D	2E	MEAN	± SE	P
0	22	48	59	45	58	46.40	5.98	
4	89	177	212	181	96	151.00	22.06	
8	156	231	401	245	132	233.00	42.19	
12	187	353	514	363	203	324.00	53.62	

Table – 7b
Effect of Normal Diet*

14	160	303	430	270	170	266.60	44.15	
16	145	275	350	208	130	221.60	36.76	
18	120	240	260	125	101	169.20	29.85	

* Normal diet: Loosen (alfa alfa), Gram seeds (chick pea)

Table – 8a
Effect of Cholesterol + Butter Rich Diet on the Serum Triglycerides mg/100 ml in Group 3 Treated 1 (T.1) Rabbits

Weeks	3A	3B	3C	3D	3E	MEAN	± SE	P
0	59	25	70	67	67	57.60	7.47	
4	80	58	105	78	108	85.80	8.32	
8	93	81	125	97	115	102.50	7.06	
12	102	97	145	115	131	118.00	8.01	

Table – 8b
Effect of Fish Oil (71.43 mg/kg/day)

14	85	82	110	101	105	96.60	4.97	NS
16	75	70	95	80	89	81.80	4.07	<0.05
18	61	50	75	53	45	56.80	4.69	<0.05

Table – 9a
Effect of Cholesterol + Butter Rich Diet on the Serum Triglycerides mg/100 ml in Group 4 Treated 2 (T.2) Rabbits

Weeks	4A	4B	4C	4D	4E	MEAN	± SE	P
0	52	14	37	27	17	29.40	6.21	
4	68	53	45	67	25	51.60	7.10	
8	77	65	54	85	54	67.00	5.54	
12	92	83	65	105	89	86.80	5.84	

Table - 9b
Effect of Bezafibrate (8.6 mg/kg/day)

14	83	70	51	83	76	72.60	5.30	NS
16	48	49	40	54	56	49.40	2.49	<0.01
18	47	39	20	32	38	35.20	4.01	<0.01

Table – 10a
Effect of Cholesterol + Butter Rich Diet on the Serum Triglycerides mg/100 ml in Group 5 Treated 3 (T.3) Rabbits

Weeks	5A	5B	5C	5D	5E	MEAN	± SE	P
0	45	42	10	19	25	28.20	6.00	
4	63	101	14	49	79	61.20	13.09	
8	86	125	22	73	91	79.40	14.96	
12	123	130	45	81	115	98.90	14.19	

Table – 10b
Effect of Fish Oil + Bezafibrate (71.43 + 8.6 mg/kg/day)

14	111	116	38	52	79	79.20	13.86	NS
16	28	19	28	35	58	33.60	5.91	<0.01
18	25	15	14	9	18	16.20	2.36	<0.01

Table – 11
Effect of Normal Diet on the Total Lipid mg/100 ml in Group 1 Normal Control (N.C) Rabbits

Weeks	1A	1B	1C	1D	1E	MEAN	± SE	P
0	200	195	180	200	209	196.8	4.27	
4	205	200	190	222	197	202.8	4.81	
8	230	205	200	231	225	218.2	5.85	
12	240	220	210	233	241	228.8	5.38	
14	245	223	213	241	249	234.2	6.19	
16	250	226	215	247	256	238.8	6.98	
18	269	230	217	253	259	245.6	8.59	

Table – 12a
Effect of Cholesterol + Butter Rich Diet on the Serum Total Lipid mg/100 ml in Group 2 Pathological Control (P.C) Rabbits

Weeks	2A	2B	2C	2D	2E	MEAN	± SE	P
0	200	180	180	200	210	194	5.37	
4	379	315	390	345	381	362	12.54	
8	626	543	890	569	612	646	55.09	
12	763	681	1275	691	783	838.6	99.17	

Table - 12b
Effect of Normal Diet*

14	540	585	880	560	663	645.6	55.64	
16	415	421	605	430	484	491.2	38.07	
18	303	300	360	350	401	342.8	16.91	

* Normal diet: Loosen (alfa alfa), Gram seeds (chick pea)

Table – 13a
Effect of Cholesterol + Butter Rich Diet on the Serum Total Lipid mg/100 ml in Group 3 Treated 1 (T.2) Rabbits

Weeks	3A	3B	3C	3D	3E	MEAN	± SE	P
0	170	190	185	185	180	182	3.03	
4	395	307	950	400	410	492.4	103.65	
8	615	591	1100	563	620	697.8	90.39	
12	856	687	1415	784	706	889.6	120.51	

Table – 13b
Effect of Fish Oil (71.43 mg/kg/day)

14	650	575	75	598	598	79.2	02.5	NS
16	528	490	930	425	415	557.6	85.33	NS
18	415	370	670	390	379	444.8	50.81	<0.05

Table – 14a
Effect of Cholesterol + Butter Rich Diet on the Serum Total Lipid mg/100 ml in Group 4 Treated 2 (T.2) Rabbits

Weeks	4A	4B	4C	4D	4E	MEAN	± SE	P
0	296	297	218	342	303	291.2	18.04	
4	776	1020	680	1306	1012	958.8	97.65	
8	912	1909	1650	1725	1505	1540.2	152.07	
12	1180	2628	2497	2219	2020	2108.8	228.3	

Table –14b
Effect of Bezafibrate (8.6 mg/kg/day)

14	667	995	1328	1967	1540	1299.4	199.7	<0.01
16	535	776	625	1220	720	775.2	106.05	<0.01
18	421	372	380	625	390	437.6	42.56	<0.01

Table – 15a
Effect of Cholesterol + Butter Rich Diet on the Serum Total Lipid mg/100 ml in Group 5 Treated 3 (T.3) Rabbits

Weeks	5A	5B	5C	5D	5E	MEAN	± SE	P
0	327	347	268	208	320	294	22.49	
4	915	82	2000	2223	980	1398	262.89	
8	1730	1120	2601	3415	2400	2253.2	349.29	
12	2514	1850	2730	4385	2870	2869.8	373.02	

Table - 15b
Effect of Fish Oil + Bezafibrate (71.43 + 8.6 mg/kg/day)

14	1338	1635	1550	2378	1830	1746.2	157.95	<0.05
16	892	1338	720	970	1020	988	90.53	<0.01
18	428	534	236	220	401	363.8	53.47	<0.01

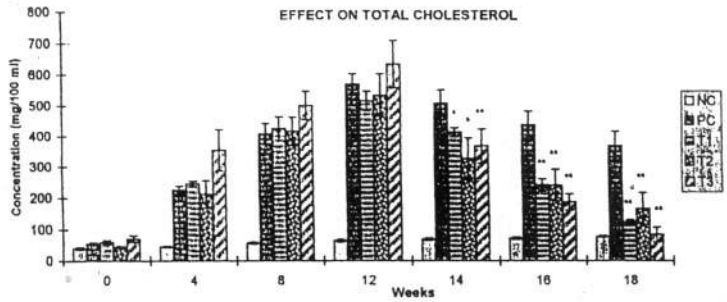

Fig. 1: Shows the effect of fish oil, bezafibrate and fish oil + bezafibrate on serum total cholesterol levels (mg/100 ml). Values are means ± S.E. (n=5). Significant differences by Newman-Keuls test. *p<0.05 and **p<0-01 as compared to 12th week treatment (onset of pathological condition) following *I-way* ANOVA.

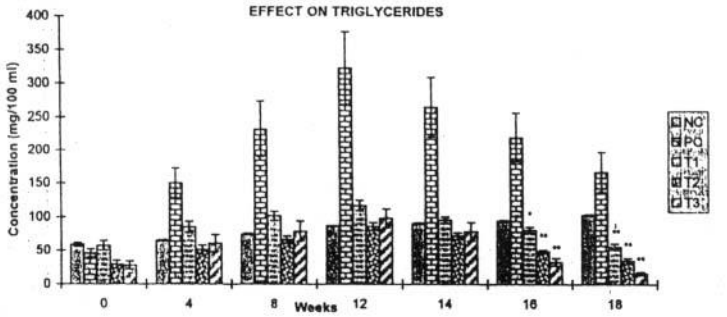

Fig. 2: Shows the effect of fish oil, bezafibrate and fish oil + bezafibmtc on scrum triglycerides levels (mg/100 ml). Values are means ± S.E. (n=5). Significant differences by Newman-Keuls test. *p<0.05 and **p<0-01 as compared to 12th week treatment (onset of pathological condition) following *I-way* ANOVA.

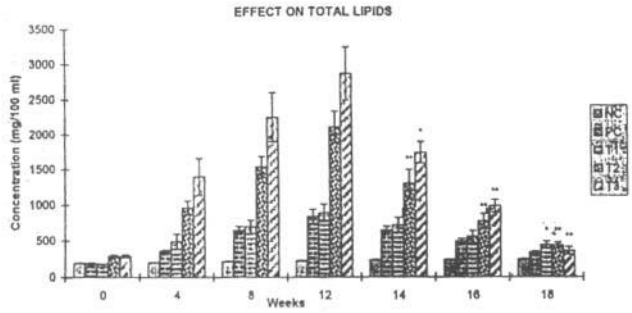

Fig. 3: Shows the effect of fish oil, bezafibrate and fish oil + bezafibrate on serum total levels (mg/100 ml). Values are means ± S.E. (n=5). Significant differences by Newman-Keuls test. *p<0.05 and **p<0.01 as compared to 12" week treatment (onset of pathological condition) following I-way ANOVA.

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