J. Hum. Ecol., 23(1): 27-38 (2008)
PRINT: ISSN 0970-9274 ONLINE: 2456-6608
DOI: 10.31901/24566608.2008/23.01.05

Preparation and Effects of Cheap Salad Oil in the Management of Type 2 Rural Indian Diabetics

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KEYWORDS Type 2 diabetes. Flax oil. Chili. Fish oil

ABSTRACT Indians are prone to insulin resistance, coronary artery disease, hypertension, obesity and dyslipidaemia. Increase in omega-3 fatty acids in the diet induces beneficial changes in a diabetic reflected by changes in blood biochemistry patterns such as reduction of sugar, triglycerides and bad cholesterols in blood. Rich sources of omega-3 fatty acids are fish oils obtained particularly from sea fish and flax oil, the latter being a rich source of omega-6 fatty acids also. Both omega-3 and omega-6 fatty acids are prone to oxidation and need anti-oxidant protections whenever being consumed. Considering Indian context, particularly for rural Indians, flax oil is ideal source of omega-3 fatty acids, as it is cheap and easily available and majority of Indians are lacto-vegetarians. Chili, which is a rich source of natural anti-oxidants, is used in the study in powdered form and it provides the anti-oxidant protection of flax oil. The mixture of 15 mi of flax oil and 5g of chili powder is tasty and can be a substitute of fish oil in a diabetic.

INTRODUCTION

According to recent estimates the prevalence of diabetes mellitus is 4% worldwide and that indicate 143 million persons are affected with diabetes, which will increase to 300 million by the year 2025. The developing countries share the disease load by about 77% and most affected region is Southeast Asia (King, 1998) and where the estimated prevalence will be 79.5% by the year 2025 in comparison to 32.7% in the year 2000. Between 1995 and 2025, the number of people with diabetes in India is projected to rise from 19 to 57 million, an increase by 195%. As per a report based on National Urban Diabetic Survey (Bhowmick, 2000), published in Times of India, dated 14th February of 2005, all major cities of India recorded a high incidence of diabetes. It was found that in Hyderabad the incidence is 16.6%, Chennai 13.5%, Bangalore 12.4%, Kolkata 11.7%, Delhi 11.6% and Mumbai 9.3%. Of the diabetic populations 80% accounts for Non Insulin Dependent Diabetes Mellitus (NIDDM) or type 2 diabetes (Kahn and Weir, 1996). This is an alarmist picture and one cannot afford to ignore either the primary prevention of NIDDM or the provision of proper health services to reduce its complications.

Majority of rural Indian suffer from malnutrition and they consume more of carbohydrates, less of fats and proteins (Swaminathan, 1990). Intake of fat in India is closely related to income (Ghafoarunissa, 1996). It is observed that in both low and high income groups the omega-6 requirements are fully made but there is a distinct deficiency of omega-3 especially in high income groups as their diets are rich in omega-6 due to increased consumption of sunflower and safflower oil.

Disease and diet are inter-related. Clinical studies involving different species of animals have shown that eating less (caloric restriction) reduces the risk of cancer, diabetes, stroke and heart disease. Larsen (2004) reported that caloric restriction (CR) also benefited people on a study conducted by a group of American researchers. In their study, they compared a group of 18 volunteers who had been practicing CR for an average of six years with a group of 18 people who were eating a normal diet. The members of the CR group were found to have significantly lower levels of triglycerides, cholesterol and C-reactive protein and also significantly lower blood pressure and formation of atherosclerotic plaque in their arteries. However, eating less can result in nutrient deficiencies. Sanders et al. (1985) showed that with a low fat intake the difference in effects of omega 3 and omega-6 fatty acids are marginal. Das et al. (1984) showed that the undernourished have lower levels of plasma lipids and a favorable distribution of cholesterol among the lipid fractions from the point of view of vulnerability to development of atherosclerosis. A person on a low-fat, high-carbohydrate diet utilizes the fatty acid synthetic pathway extensively; this pathway requires citrate to leave the mitochondria to generate malonyl-CoA. The resultant high concentration of malonyl-CoA inhibits the activity of the enzyme that is the gateway to fatty acid oxidation. It is for this reason that extreme low-fat diets do not result in weight loss nearly as fast as one might expect. The burning of excess fat is actually inhibited by the high carbohydrate intake. In persons with low body fat and good lean mass, high carbohydrate intake spares the use of fatty acids and the medium chain fatty acids that are otherwise so quickly used for energy may accumulate above "normal" in membranes. New research published in the American Journal of Clinical Nutrition links diabetes with the rise in consumption of refined sugars and carbohydrates, by examining the consumption of food macronutrients (fats, proteins and carbohydrates) consumed by the population from 1909 to 1997 (http://www.newstarget.com/001054.html, 2005). Researchers from Harvard School of Public Health examined the long-term relationship between different types of dietary fat and the risk of type 2 diabetes. Polyunsaturated fatty acids (w-3 fats found in fish and flaxseed, pumpkin seed, canola, soy, and walnuts) appear to reduce the risk of diabetes while trans-fatty acids was responsible for the increase in incidence (http://www.hsph.harvard.edu/nutritionsource/fats.html, 2005).

Burr and Burr (1929) stated that essential fatty acid (EFA) include linoleic acid (18:2 w- 6) and linolenic acid (18: 3 w-3). Addition of essential fatty acids (EFA) in the diet, increase the fluidity of cell membrane and also increase the formation of desired prostaglandin from the parent EFA. It was observed that EFA's, particularly of the w-3 series are beneficial in diabetes and various diseases such as cardiovascular disease, inflammation, cancer, diabetes mellitus and renal disease (Cunnane, 1995). The diseases can be influenced through omega-6/ omega-3 ratio**.** The sharp increase in the prevalence of heart disease and diabetes mellitus in India may be due to increased use of omega-6 oils used as cooking medium and resultant lipid toxicity thereof (Raheja, 1970). Similarly in the West the increase in diabetes may be due to imbalance in omega 6 / omega-3 ratio in the western diet which may be as high as 14:1 (http://www.remedyfind.com/rem.asp?ID=6592).

Omega- 3 fatty acids produce Eicosa Pentaenoic Acid (EPA) and Docoxa Hexaenoic Acid (DHA) in the body. Epidemiological studies of Greenland Eskimos revealed that they consumed high levels of EPA and DHA, primarily in the fat of seals and coldwater fish and the component in fish that was most responsible for the beneficial effect of lowering of serum lipids was in the oils of the fish rather than in the protein or carbohydrate (Rambjor et al., 1996; Feldman et al., 1972; Bang et al., 1971 and Peifer et al., 1962**)**. Since the fish cannot make either omega-3 or omega-6 fatty acids, its tissues will have those polyunsaturated acids that it eats. Generally speaking, oils from the fish of the oceans are complex mixtures containing many fatty acids with as many as six double bonds (Lands, 1989). Harris (1989) has shown compared to other dietary oils that fish oil reduces the fasting level of plasma triglycerides while the effects on plasma cholesterol and low-density lipoprotein cholesterol have been inconclusive.

As fish oil is costly and a number of Indians are vegetarian suitable alternative of fish oil was found to be flax oil. Further, flax oil is being used widely in tribal belts of Maharastra, Chattisgarh, Jharkhand and Orissa for generations. Flax oil contains three double bonds and is highly prone to oxidation. Flax oil contains 52% of omega-3, 16% of omega-6, 20% of oleic acid, 5% of palmitic acid and 5% of stearic acid (Gill, 1987). The omega-6 and omega-3 are ultimately converted in the body to 3, 2 and 1 series of prostraglandins by the action of desaturases and elongases. Dietary 18:3 omega-6 and 20:4 omega-6 may have superior bio-potency with respect to 18:2 omega-6 (Rivers and Frankel, 1981).

The oil can be protected of lipid peroxidation by the addition of judicious quantity of anti-oxidant (Urasmus, 1986; Ornish, 1996), preferably natural due to carcinogenetic potential of synthetic antioxidants such as butylated hydroxy amisole (BHA), butylated hydroxy toluene (BHT) or tertiary butyl hydroxy quinone (TBHQ) (Ito, 1986). The oils containing omega-3 fatty acids should be used as salad oil due to its unstable structure.

Red chili (Capsicum Annum) contains large amount of natural antioxidant, mainly hot tasting capsaicin and dihydrocapsaicin, together ranging between 80-90 % and all others forming the rest 10-20 % (Govindarajan and Sathyanarayana, 1991). Nakatam et al. (1988) had isolated an antioxidant having no hot taste from a variety of chili namely Capsicum Furtescens**.** The hypo-cholesterolemic effect of capsaicin is seen at very low levels. Workers have confirmed that even levels of 70 to 80 microgram of capsaicinoids per kg body weight, when taken with a high fat diet lowers liver and serum triglycerides and serum lipoprotein triglycerides (Govindarajan and Sathyanarayana, 1991). Decrease in plasma glucose levels in response to oral administration of glucose with 14 mg % capsaicin has been also reported (Monsereenusorn et al., 1982). Nutritional evaluation of chili and its extracts at low to maximum food use levels and even five to ten times the maximum have shown to be safe. Intake at higher levels of 8 to 10 mg per kg body weight of capsaicinoids provided beneficial (dose-dependent) effects in metabolizing lipids with high fat or high carbohydrate food intake (Govindarajan and Sathyanarayana, 1991).

Flax can be easily grown in drought prone areas of India. Ermakov (1965) studied the oil content in 280 varieties of flaxseed. The oil content ranged from 34.3 to 45.4 per cent in different varieties. Sekhon et al. (1973) reported similar variation in 23 flaxseed strains from Punjab. Yellow-seeded varieties have higher oil content with more unsaturation than that of brown-seeded varieties. Photoperiod was observed to affect oil content (Sosulski and Gore, 1964). By modifying the agronomic methods, flax oil containing higher amount of omega-3 fatty acid can be obtained (Gill, 1987). Yermanos and Knowles (1962), Plessers (1966) and Yermanos (1966) determined the fatty acid composition of Linum species and found great variability among the species. Conte et al. (1979) described the fatty acid composition of flaxseed oil and reported linolenic acid content may be raised to 53.32 per cent in the oil. A positive correlation between linolenic acid and iodine value of flax oil was, perhaps, first reported by Painter (1944) and was confirmed later by McGregor and Carson (1961), Yermanos (1966) and Sekhon et al. (1973).

This paper deals with:

1. To find out a suitable neutraceutical in NIDDM affecting rural people in India where proportionate income, medical and paramedical personnel, infrastructure of health care system, educational level etc are lacking compounded by religious and ethnic belief.
2. To educate rural Indian diabetic that normal diet does not help controlling the disease.
3. To compare the efficacy of flax oil with fish oil in Indian rural context.

MATERIALS AND METHOD

Selection of Patients: 80 NIDDM patients, residing at four different villages, aged between 45 and 52 years, were divided in 4 groups containing 20 patients each. The patients were selected such that all groups from 2 homogenous sub-groups, each containing either 10 patients as volunteers to whom either flax oil with antioxidant or fish oil with antioxidant was added and 10 patients as control. All groups are kept in identical conditions to maintain homogeneity. The patients with known renal and hepatic dysfunction and lipid lowering, anti-hypertensive and anti-diabetic therapies were excluded from the study. The patients underwent clinical, anthropometrical evaluation before the study. Murray (2000) recommends 1 tablespoonful of flax oil per day is sufficient to maintain omega-3 needs of the body. To mask the insipid taste and to give anti-oxidant protection to flax oil different weights of chili powder is added to it and the mixtures are tested for palatability, taste and acceptance as salad oil and best found suitable, is tested for anti-oxidant protection. However, chili being edible and an excess of chili more than required for desired volume of oil was practically acceptable (Monsereenusorn et al., 1982). It was found that adding 5 grams of chili powder in one tablespoonful of flax oil was found to be more acceptable as a tasty material by majority of volunteers (36) for salad oil.

Extraction of Gum from Flax Seed using Water as Solvent: The undesirable cyanogenic compounds present in flax were water soluble and it was observed that long-term consumption of large quantities of flaxseed has the potential to increase thiocyanate levels in the blood and may cause enlargement of the thyroid gland, though a study with 50 g of flaxseed/day did not produce any such effect in the volunteers (Cunane, 1995). These compounds separated out along with gums when water treatments of flax seeds were performed. The solids (flax seed) and the solvent (drinking water) was put in a vessel maintaining a seed water ratio of 1:8 and continuously stirred with an agitator as degumming is time consuming and leads to stickiness. Temperature of water was noted as the extraction was dependent on temperature (Cunane, 1995). Seed sample was checked at every 30 minutes time interval to see whether the stickiness due to the gum on the surface of the flax seed exists or not. If the stickiness was not there, agitation was stopped and the time was noted. The process of filtration separate out the extract. It was then chilled in a refrigerator and the gum was separated out.

***Hydration Characteristics of Degummed Flaxseed*:** The degummed flaxseed was weighed and then heated in an oven for 2 hours at 110 °C (Das, 1992). The dried flax seed after weighing was put in a vessel maintaining a seed water ratio of 1:8 for 5 hour (Cunane, 1995). The seeds kept under water were taken out and weighed at 30 minutes interval till the seeds were properly hydrated.

Oxidation of Flax Oil: Subjecting the flax oil to high pressure either in an oxygen bomb or pumping air through the flax seed oil could carry out oxidation of flax seed oil. Passing air through it with the help of an air pump did oxidation of flaxseed oil. As the oxidation by Bomb calorimeter was a surface phenomenon, an air pump was used for passing air through the oil to carry out oxidation throughout the oil. This determines the oxidative potential of the sample used.

Extraction of Antioxidant from Chili: About 5 g of chili powder (as 5 g of chili powder forms most suitable tasty mixture with flax oil) was taken in a glass vessel and ethanol was added to it at room temperature in different volumes (w/v) such as 25 ml, 50ml, 75 ml, 100ml, 125 ml and 150 ml respectively. The mixtures were continuously stirred for 30 minutes. The solution was then filtered and the filtrate was evaporated. The residue obtained after the evaporation was the antioxidant in chili as it was soluble in ethanol. It was weighed and noted. Ethanol volume yielding maximum concentration of anti-oxidant was noted.

Solubility of Antioxidants in Flax Oil: The minimum weight of flax oil in which the weight of antioxidant, obtained after the ethanol extraction, was completely soluble was measured (Aicosoft electronic balance, Fx-300, USA). The solution obtained had an extremely dark reddish color and was unsuitable for colorimetric measurements. About 0.1 ml of the above solution was taken and mixed with 10 ml of Hexane. The resulting solution obtained was one with light yellowish color and thus suitable for colorimetric measurements. Light transmittance of this sample was measured in Monozyme India, make Innova 0.5 model of photo electric micro digital 8 filter colorimeter at filter of 490, filter number 49 and colour blue green. The above experiment was repeated a number of times by taking different weights of flax oil and a fixed weight of anti-oxidant and light transmittances of these samples were measured.

Rate of Extraction of Antioxidant from Chili Powder Using Flax Oil at Different Times of Extraction: It was necessary to keep the chili powder into the flax oil for some time so that the anti-oxidants in chili powder were extracted maximally by the oil. The different samples contained fixed weights of flax oil but different weights of chili powder (total anti-oxidant concentrations in them calculated beforehand) kept in suspension. A portion was taken from all the samples and subjected to colorimetric measurements each time at every 30 minutes interval till anti-oxidants were extracted by the oil and was completed by 8 hours. From the calibration plot the concentrations of antioxidant in the samples at definite intervals of time was determined by comparing with the standard plot as obtained in the just earlier step described above.

Influence on Peroxide Value (PV) and ThioBarbituric Acid (TBA) Values: Determination of PV and TBA values were done after 7 hours of sample collection (reflected as 0 hour in the graphs) due to transportation time delay in both the samples-fresh flax oils or when mixed with antioxidant as prepared. PV or TBA values were thereafter determined for fresh flax oil samples at 1-hour interval. To the other portion, antioxidant extracted from 5 g of chili powder (5 g of chili powder forms most suitable tasty mixture with flax oil) was added to the oil. PV and TBA values were determined of this antioxidant added sample at 5-hourly intervals unto 60 hours to get an idea of anti-oxidant protection given. To test for long standing anti-oxidant protection of the flax oil sample an experimental set up was designed to see the effects up to 6 months and was done by measuring PV and TBA values at monthly intervals.

Extraction of Oil and Adding of Anti-Oxidants of Chili to the Oil: The seed was closely inspected, cleaned from impurities and bad quality seeds were rejected. Measurement of aflatoxin in flaxseed was done by AOAC (1995) method in order to discard oil-containing aflatoxin. The oil was processed from flaxseed by village level bullock-driven *ghanis* (cold process extraction) at Balarampur Avoy Ashram, Kharagpur, as extraction process which results high temperature leads to qualitative damage of the oil To reduce the oxidative changes the oil was kept in a dark container and filled up to the brim covered by airtight leads. It was subsequently admixed with anti oxidant. It was found that adding 5 grams of chili powder in one tablespoonful of flax oil serve the purpose of antioxidant protection to all samples of flax oils used in the study (as evident from TBA values).

Administration of Neutraceuticals: 2 groups of patients were given 1 tablespoonful of flax oil (Murray, 2000) mixed with 5 g of chili powder per day as salad oil. Calculated as per recommendations of Simopoulos, (1989) daily requirement of omega-3 fatty acids, the other 2 groups were given 3 fish oil capsules /day, each capsule containing EPA180 mg and DHA120 mg, along with 5 g of chili powder.

Blood samples from the patients were taken by an indwelling catheter in the ante-cubital vein and analyzed for fasting blood glucose (FBS), triglycerides (TG), total cholesterol (TLC), high-density-lipoprotein cholesterol (HDLC), low-density-lipoprotein cholesterol (LDLC), very low-density-lipoprotein cholesterol (VLDLC). The tests were conducted as per Instruction sheets for manual assays given by Boehringer Mannheim Limited (1983) and the instrument used was photometer 4010 (Boehringer, Germany).

RESULTS AND DISCUSSION

The patients underwent clinical, anthropometrical evaluation before the study. The characteristics of the patients were:

Average age: 45.22 ± 5.30 years, (Mean ± SD)

Sex: Males: 22 Females: 18,

Average weight: 77.56 ± 5.08 kg,

Body Mass Index (BMI) = Weight (kg) / Square of height (m^2) = 21.02 ± 1.82.

Proximate analysis of the food samples and the flax seed samples were carried out using standard methods (Rangana, 1986; Stauffer, 1996; Firestone, 1990; AOAC, 1995). Commercially the oil was processed from flaxseed by village level bullock-driven *ghanis*. The cake residue was collected at the end and the oil concentration of the cake sample was measured and was found to be about 25-30%. Extraction of gum from flax seed using water as solvent showed that the extraction of gum was complete within 16 hrs 30 minutes of operation at room temperature (31 ^{O}C). Figure 1 (relationship between time of wetting degummed flax seed and water pick up) showed hydration characteristics of degummed flaxseed and about 74% of water was picked up at 210 minutes, which remained practically unaltered with increase of time. Analysis of figure 2 (relationship between volume of ethanol and weight of antioxidant extracted) showed that anti-oxidant was extracted by ethanol in the following fashion- 40 mg in 40 ml, 60 mg in 55 ml, 80 mg in 80 ml and the extraction of anti-oxidant became 105 mg and 110 mg in 90 ml and 95ml of ethanol respectively and the yield remained practically unaltered with higher volumes of ethanol.

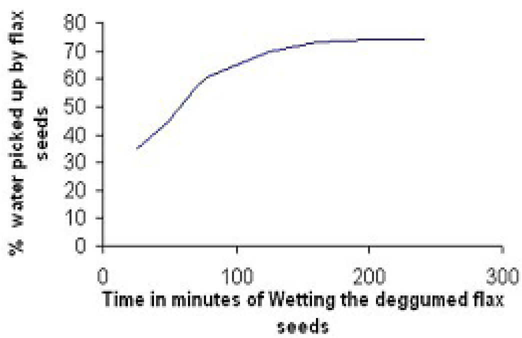

Fig. 1. Relationship between time of wetting degummed flax seed and water pick up

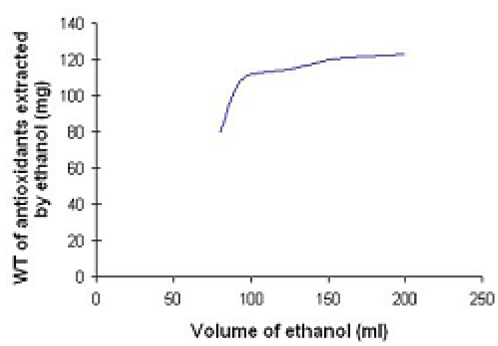

Fig. 2. Relationship between volume of ethanol and weight of antioxidant extracted

Transmittance was tested in Monozyme, India make Innova 0.5 model of photo electric microdigital 8 filter colorimeter at a filter of 490, filter number 49 and colour blue green (Figure 3 showing relationship of optical density of the sample with wave length). Figure 4 (relationship

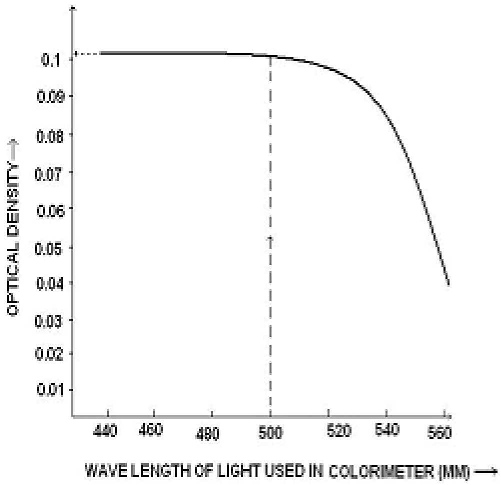

Fig. 3. Relationship of optical density of the sample with wave length

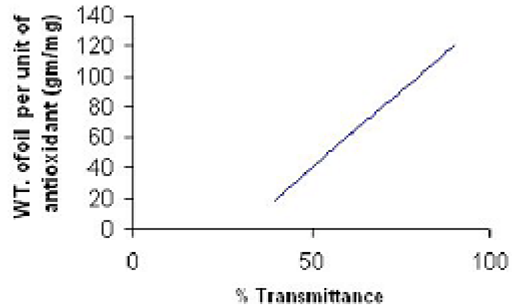

Fig. 4. Relationship between light Transmittance and weight of oil per unit weight of antioxidant

between light Transmittance and weight of oil per unit weight of antioxidant) showed with 1 mg of anti-oxidant in flax oil mixture transmittance values were, 20% with 40 g of oil, 40% with 55 g of oil, 60% with 70 g of oil, 80% with 100gm of oil of and 90% with 120gm of oil.

Antioxidant solubility in flax oil was measured by obtaining samples of different weights of flax oil mixed with a fixed weight of anti-oxidant (Figure 5 showing variation of antioxidant extracted form Chili powder by flax seed oil with time. They all had different color content. Hence they have different percentage of transmittance. The solutions thus obtained had an extremely dark reddish color, which was unsuitable for colorimetric measure-ments. So a 0.1 ml of the dark reddish colored solution was taken and mixed with 10 ml of Hexane, as oil samples were soluble in Hexane. The resulting solution obtained was one with light yellowish color and thus suitable for colorimetric measurements.

The above experiment was repeated for number of times for a different weight of flax seed oil. It was found that 1 g of flaxseed oil was soluble with 0.084 g weight of antioxidant. It was also observed that rates of extraction of antioxidant from chili powder was initially higher but gradually decreases with time and was completed by about 4 hours.

The product obtained by incorporation of Capsicum extract (anti-oxidant) in flax seed oil is found to be dull red in colour. The taste of the product is found to be hot but acceptable for use as a salad oil. The oil thus produced was tested for efficacy. TBA and PV values of flax oil mixed with the antioxidant were obtained.

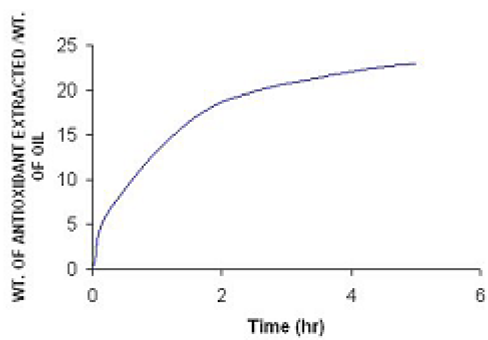

Fig. 5. Variation of antioxidant extracted form Chilli powder by flax seed oil with time by flax seed oil with time

Figure 6 (relationship between time of oxidation and TBA number of flax seed oil (mixed with antioxidant) showed the oxidative changes occurring in hours in the oil protected with antioxidant in terms of TBA values. Figure 7 (relationship between time of oxidation and peroxide value of flax seed oil) showed the peroxide value of flax oil (not protected with antioxidant) occurring in hours. Figure 8 (showing the effects of oxidation flax oil mixed with antioxidant, six months study) showed the oxidative changes of antioxidant mixed oil in terms of months. The figures 6 and 8 showed that the oil thus produced was protected from oxidation and hence can be used safely for human consumption. As both the items, flaxseed oil and chili were edible in nature

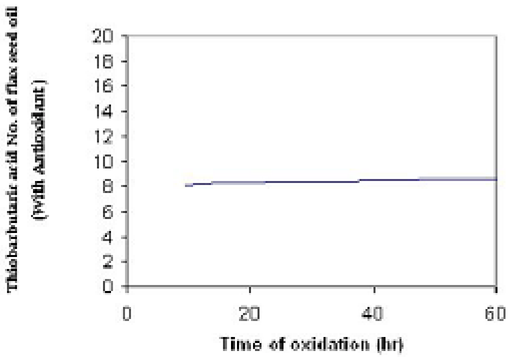

Fig. 6. Relationship between time of oxidation and TBA number of flax seed oil (mixed with antioxidant)

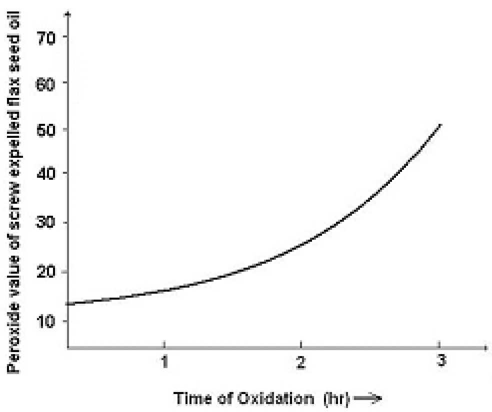

Fig. 7. Relationship between time of oxidation and peroxide value of flax seed oil

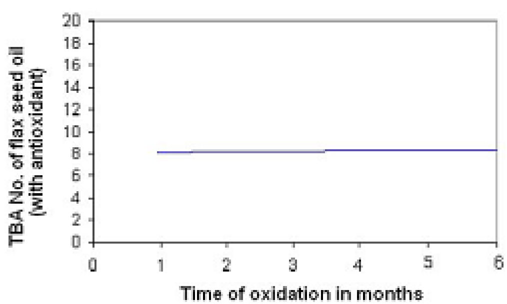

Fig. 8. The effects of oxidation flax oil mixed with antioxidant (six months study)

and was being used for human consumption since long no animal experimen-tation was required for the said purpose as per Government of India rules. The said oil was kept in a sealed container filled up to brim and it does not degrade during storage and kept for study as its efficiency to act as neutraceutical in human volunteers. Fish oil capsules were supplied by '*Merck'* and were standardized by them as per guidelines given (EPA180 mg and DHA120 mg). Written consent was taken from all the volunteers.

Flax oil Study: The above made oil (neutraceutical) was given to patients selected in 2 groups and separate studies were performed at different times in the year for 2 months (phase 1 and phase II) - in two different seasons, summer and winter respectively to nullify the effects of seasonal changes. The biochemical parameters as described earlier of the patients were observed. The results were tabulated below:

Tables 1a (Effects of the neutraceutical in NIDDM patients, phase I) and Table 2a (Effects of the neutraceutical in NIDDM patients, phase II) on being compared with Table 1b (Effects of normal diet NIDDM patients, phase I) and Table 2b (Effects of normal diet in NIDDM patients, phase II) showed that TLC, LDLC, VLDLC, TG, FBS were reduced whereas HDLC was increased for all the patients in both the phases I and phase II. The Tables also showed that neutraceutical caused remarkable reduction in TG. In phase II, the study was done in winter season, reductions in TLC and LDLC and TG were more compared with phase I (the study done in summer) while reduction in blood glucoses showed a reverse trend. Tables 1a and Table 2a being compared statistically with Table 1b and Table 2b showed that TLC ($p \leq 0.50$), LDLC ($p \leq 0.75$), VLDLC ($p \leq 0.025$), TG ($p \leq 0.05$), FBS ($p \leq 0.25$) were reduced whereas HDLC ($p \leq 0.075$) was increased for all the patients in both the phases phase and phase II. The Tables also showed that oil so developed caused remarkable reduction in TG. In phase II, the study was done in winter season, increased in TLC ($p \leq 0.075$) and LDLC ($p \leq 0.25$) and TG ($p \leq 0.025$) and blood glucose ($p \leq 0.25$) were more compared with phase I (the study done in summer). The normal diet of villagers did not produce any changes in the blood biochemistry parameters of the volunteers in both the phases during the study. Measurement of t-values and testing for null hypothesis (which was rejected) proved that the neutraceutical was effective in bringing out the desired changes in blood biochemistry parameters, that is, reduction of TLC, LDLC, VLDLC, TG and FBS as well as increase in HDLC. The reduction in FBS was more in 2nd month than in the 1st month month ($p \leq 0.025$).

Table 1a: Effects of the neutraceutical in NIDDM patients (phase I)

Time, (months)	*TLC (mg/dl)*	*HDLC (mg/dl)*	*LDLC (mg/dl)*	*VLDLC (mg/dl)*	*TG (mg/dl)*	*FBS (mg/dl)*
0	258±6	57±4	170±6	30±4	150±7	156±8
1	252±5	59±3	164±5	29±6	145±9	153±6
2	250±5	62±5	160±7	27±4	135±9	150±7

Table 1b: Effects of normal diet NIDDM patients (phase I)

Time, (months)	*TLC (mg/dl)*	*HDLC (mg/dl)*	*LDLC (mg/dl)*	*VLDLC (mg/dl)*	*TG (mg/dl)*	*FBS (mg/dl)*
0	255±7	55±5	170±5	31±6	155±5	156±8
1	255±6	55±4	170±6	31±5	153±6	156±6
2	255±7	54±4	170±6	31±4	155±6	156±7

Table 2a: Effects of the neutraceutical in NIDDM patients (phase II)

Time, (months)	*TLC (mg/dl)*	*HDLC (mg/dl)*	*LDLC (mg/dl)*	*VLDLC (mg/dl)*	*TG (mg/dl)*	*FBS (mg/dl)*
0	241±8	58±5	150±7	33±5	165±6	168±6
1	240±7	61±7	146±7	31±6	154±6	160±6
2	238±7	63±6	144±12	30±3	149±5	155±8

Table 2b: Effects of normal diet in NIDDM patients (phase II)

Time, (months)	*TLC (mg/dl)*	*HDLC (mg/dl)*	*LDLC (mg/dl)*	*VLDLC (mg/dl)*	*TG (mg/dl)*	*FBS (mg/dl)*
0	241±6	58±5	150±6	33±5	165±5	168±6
1	241±5	58±6	150±7	33±5	165±5	168±7
2	244±7	58±6	153±5	33±3	165±7	168±6

Table 3a: Effect of fish oil in NIDDM patients (phase I)

Time, (months)	*TLC (mg/dl)*	*HDLC (mg/dl)*	*LDLC (mg/dl)*	*VLDLC (mg/dl)*	*TG (mg/dl)*	*FBS (mg/dl)*
0	220±6	56±5	124±9	40±6	200±7	146±7
1	217±5	58±7	122±7	37±5	185±9	144±5
2	215±5	59±3	121±5	35±5	165±9	140±9

Table 3b: Effect of usual diet in NIDDM patients (phase I)

Time, (months)	*TLC (mg/dl)*	*HDLC (mg/dl)*	*LDLC (mg/dl)*	*VLDLC (mg/dl)*	*TG (mg/dl)*	*FBS (mg/dl)*
0	230±6	56±5	134±9	40±6	200±7	146±7
1	230±5	56±7	134±7	40±5	200±9	146±5
2	230±7	56±3	134±5	40±5	200±9	146±9

Table 4a: Effect of fish oil in NIDDM patients (phase II)

Time, (months)	*TLC (mg/dl)*	*HDLC (mg/dl)*	*LDLC (mg/dl)*	*VLDLC (mg/dl)*	*TG (mg/dl)*	*FBS (mg/dl)*
0	241±7	52±5	152±5	37±4	185±6	164±9
1	241±5	54±4	152±7	35±3	165±7	160±7
2	239±8	57±3	151±6	32±4	160±8	156±7

Table 4b: Effect of usual diet in NIDDM patients (phase II)

Time, (months)	*TLC (mg/dl)*	*HDLC (mg/dl)*	*LDLC (mg/dl)*	*VLDLC (mg/dl)*	*TG (mg/dl)*	*FBS (mg/dl)*
0	241±7	52±5	152±15	37±4	185±6	164±6
1	241±6	52±4	202±13	37±3	185±7	164±7
2	241±8	51±3	203±16	47±4	185±8	166±6

Serum insulin values was 42 ± 04µiu/ml (summer) and it was 43 ± 6 µiu/ml ml at winter and remained the same throughout the experiment.

The usual rural diet was ineffective in bringing out any changes during the period of study and may need long-term observations to obtain any conclusions.

Fish Oil Study Group 3: A study was also conducted with fish oil and was done in two groups separately at different time for 2months like earlier study (phase 1 and phase II) - in different seasons, summer and winter, to nullify the effects of climatic changes if any and also to provide identical pictures for comparing the effects of flax oil and fish oil.

Tables 3b (Effect of usual diet in NIDDM patients, phase I) and 4b (Effect of usual diet in NIDDM patients, phase II) showed the effects of normal diet. As diet being a heterogeneous item it varied, though modestly from person to person and from day to day in a single person. 3b readings were taken in summer when temperature was between 42°C-48°C and humidity 92% while 4b readings were taken in winter when temperature was between 9°C-15°C and humidity 80%. Values showed that minor variations in diet and seasonal changes did not have much effect on the disease process. Tables 3a (Effect of fish oil in NIDDM patients, phase I) and Table 4a (Effect of fish oil in NIDDM patients, phase II) on being compared with Tables 3b and Table 4b showed that TLC was marginally reduced ($p \leq 0.075$) whereas there was marked reduction in VLDLC ($p \leq 0.025$) and TG ($p \leq 0.025$). The LDLC had remained almost unaffected and the FBS was moderately reduced, whereas HDLC showed increase in concentration in blood. This confirms the findings of Rambjor et al. (1996), Feldman et al. (1972) and Haris (1989). In the group serum insulin values was 38±05µ iu/ml (summer) and it was 37± 4 µiu/ml ml at winter and remained the same throughout the experiment. Ahrens et al. (1959) demonstrated that diets that included polyunsaturated fats could lower the levels of plasma cholesterol and greatly decrease the total serum triglycerides, irrespective of the content of the omega-6 essential fatty acids in the oil. A study of 42 adults showed that those who took diet supplements with 4 grams (g) of fish oil for eight weeks lowered their levels of triacylglycerol (TAG) and consequently low incidence of heart disease. HDL values rose, ratio of LDL to HDL fell by nearly 1% among patients taking fish oil supplements. But there was no apparent effect on the ratio of total cholesterol to LDL cholesterol, a measure of heart disease risk (Adams, 2005).

Similarly, fish oil did not reduce levels of LDL in the blood, which have been closely linked to symptoms of heart disease. In conclusion, fish oil supplementation was found to partially correct the dyslipidemia (abnormal blood fats) of type 2 diabetes (Adams, 2005). In phase II it was observed that VLDLC, TG were more reduced while HDLC more increased comparing with phase I while FBS was more reduced in phase I compared to phase II. Like flax oil in fish oil also reduction in FBS was more marked in 2nd month than 1st month. On the basis of two-sample t-test, measurement of t-values and testing for null hypothesis (which was rejected) proved it is observed that fish oil cause significant reduction of TG, VLDLC and moderate increase of HDLC and also reduction TLC. Patients on rural diet showed FBS was reduced in phase I and increased in phase II, other biochemical parameters showed no changes. Analysis of variance showed that flax oil could act as a substitute of fish oil.

Siguel and Maclure (1987**),** using derivative precursor ratios, and Boustani et al (1989), using 20:3 omega-6-2H_4, demonstrated that desaturase activity operates in humans and that the desaturation/elongation enzymes have a preference for omega -3 versus omega -6 fatty acids. The "very active conversion" (Siguel and Maclure, 1987**)** of 18:3 omega-3 suggests that vegetarians can accumulate 20:5 omega -3 and 22:6 omega -3 and need not alter their diets to include fish products (which are rich in 20:5 omega -3 and 22:6 omega -3).

While science continues to confirm many health benefits of consuming omega-3 fatty acids derived from fish, recent reports suggest both fish and fish oil supplements contain high levels of contaminants that may be harmful to humans. To assure consumers that fish oil supplements are toxin free and a safe way to reduce the risk of cardiovascular disease and other health risks, Leiner Health Products commissioned the University of Southern California (USC) School of Pharmacy to test the purity of fish oil supplements, it makes for America's top retailers. The findings, documented in a study entitled "Polychlorinated biphenyls (PCBs) in dietary fish oil supplements: a comparison of EPA and ELISA methods," was presented at the Institute of Food Technologists (IFT) Annual Meeting, Las Vegas

Convention Center, July 14, 2005. According to Dr. Barry Sears, originator of the revolutionary dietary approach called '*The Zone'* and author of *The Omega RX Zone,* many of the fish find in the oceans, lakes, and streams around the world contain dangerously high levels of mercury and other toxins. The health risks of these toxins may well outweigh the potential benefits of the omega-3 obtained from fish. It is important from the standpoint to use only ultra-refined fish oil—fish oil that adheres to strict quality control standards to ensure maximum purity and the most potent and effective levels of EPA and DHA. Otherwise it may lead to increased consumption of mercury, Cadmium, dioxins, and other toxins, as well as excessive saturated fat, which can be harmful to health. In contrast, organic flaxseed oil contains twice the amount of omega-3 fatty acids (57%) without the risks associated with fish oils. Further, flax oil contain omega-6, which is entirely absent in fish oil (Lands, 1986; Chow, 1992). Gamma-linolenic acid, which is derived from omega-6, corrects most of the biological effects of zinc deficiency (Huang et al., 1981) indicating that the requirement of the Δ-5 desaturase enzyme for zinc is a first-order essential function of zinc. Dr. Sears in The Omega Rx Zone (2003) reveals a revolutionary new technological advance that helps treat chronic disease particularly diabetes by improving insulin sensitivity and improving athletic performance. Pharmaceutical-grade fish oil has the potential of a more emotionally stable, healthier person by allowing brain to work at peak efficiency and controlling the hormonal responses that ultimately govern the body and mind. Fish oil has been found to reduce levels of stress hormones, including cortisol, which increase heart rate and blood pressure. In a study conducted in France, researchers gave seven healthy men 7.2 grams a day of fish oil for three weeks. When the volunteers were subjected to mental stress, their stress hormone levels were significantly blunted. Researchers have long noted that a diet rich in omega-3 fatty acids, found in fish oil and certain other foods, is associated with reduced risks of cardio-vascular and metabolic diseases, but the mechanisms have not been completely understood (Nexus health bytes, July/August, 2004).Vegans, whose diets are totally lacking in meat, fish and dairy products, obtain omega-3 fatty acids only from plants, which are a source of ALA but not EPA and DHA. Indeed, strict vegans have low levels of omega-3 fatty acids in their red blood cells, platelets and in serum phospholipids. Moreover, they tend to have higher levels of linoleic acid, the essential omega-6 fatty acid, than omnivores. Vegetarians who add flax oil to the diet can improve the omega-3 fat content of their tissues. In vegetarian men, for example, consuming flax oil and margarine made with flax oil daily for 28 days increased the ALA, EPA and total omega-3 fatty acid content of their platelet phospholipids (Cunane, 1995). Hence the study was not conclusive that flax oil is better substitute of fish oil and needs further researches.

CONCLUSION

Flaxseed processed by cold processing, that is, by bullock driven *ghani,* mixed with natural antioxidant (chili) act as an ideal substitute of fish oil and its nutraceutical value is more when compared with fish oil as flaxseed can effectively control in all cases the dyslipidaemic pictures of diabetes. Proper health education will increase its acceptability and being widely available and very cheap and having wide medicinal value in different diseases.

ACKNOWLEDGEMENT

The author is indebted to Late Prof. S. K. Sawarkar of Department of Chemical Engineering, Indian Institute of Technology, Kharagpur, India for his valuable suggestions and encouragement throughout the work. The author deeply acknowledges the contribution of Prof. D. Bhattacharya and Prof A. K. Roy for the research project. The author is also deeply indebted to Prof. H.K. Das and Prof. S. K. Das of Agriculture and Food Engineering Department and Prof. S. S. Alam and Prof. A. K. Nanda of Mathematics Department in Indian Institute of Technology, Kharagpur, India for their kind review, comments and suggestions regarding the work. The author is also indebted to Prof. P. Chattaraj of Chemistry Department, Indian Institute of Technology, Kharagpur, India for his valuable suggestions and comments regarding the work.

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