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Study on the Effect of Rapeseed Oils (Sarsoon and Canola) and Hydrogenated Fat on Serum Lipid Fractions of Rats

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Abstract
A feeding trial was conducted in adult albino rats to study the influence on lipid fractions of serum and various body tissues by feeding diets containing sarsoon oil, canola oil and hydrogenated fat.
The results revealed that serum cholesterol decreased in sarsoon (-14.3%) and canola oil (-9.9%) while increased in hydrogenated fat (26.1%) fed rats. Similarly serum triglycerides decreased in sarsoon (-22.6%) and canola oil (-21.3%) while increased in hydrogenated fat fed rats (24.3%). Serum HDL increased in sarsoon oil (19.30%), canola oil (35.00%) and hydrogenated fat (81.20%) fed rats while serum LDL decreased in sarsoon (-20.00%) and canola oil (-16.00%) and increased in hydrogenated fat (15.70%) fed arts. Liver cholesterol showed higher ($p<0.05$) values in sarsoon oil (426.33 + 25.03 mg/100 g) fed rats. Rapeseed oils showed lower ($p<0.01$) carcass triglycerides than hydrogenated fat fed rats. Body weight of rats decreased in sarsoon while increased in canola and hydrogenated fat fed rats.

Key words: Sarsoon oil, canola oil, hydrogenated fat, triglycerides, HDL, LDL, cholesterol, iodine value, acid value, peroxide value, rats, growth

Introduction
Vegetable oils account for 42 percent of the total fat and oil production of the world. These are rich in polyunsaturated fatty acids (PUFA) and fat soluble vitamins (Imran-ul Haq, 1990). Out of several hundred species of plants known to have oil bearing seeds, only about a dozen are significant commercially.
Rapeseed is an oilseed crop which is commonly grown in temperate areas of Pakistan. Rapeseed is a rich source of oil and protein. The seed has oil as high as 46-48 percent. The world production of rapeseed oils places its tonnage in fifth place as an edible oil after soybean, sunflower seed, groundnut and cottonseed oils (Vergroesen, 1975). Production of rapeseed in Pakistan during 1994-95 was 229.4 thousand tonnes (Anonymous, 1995). Canola is a new name for rapeseed with drastically reduced levels of erucic acid. Canola is characterized by high oil contents (approximately 41%) close to that of sunflower, and by a high level of oleic acid (58%) close to olive oil. The presence of an appreciable amount of linolenic acid gives it properties similar to soybean. Canola is considered intermediate among the vegetable oils in its level of Poly Unsaturated Fatty Acid (PUFA) (-32%), which is lower than soybean, sunflower, corn and cotton but higher than peanut and palm. It is having lower saturated fatty acids (5% palmitic acid, 2% stearic acid). The oil has a high nutritional rating which compares favorably with other major edible oils (Imran-ul-Haq, 1990),
Keeping in view, the dietary needs of fat and the problems associated with excessive intakes, it is desirable to investigate the effects of various dietary fats on lipid fractions of serum and other body tissues. The study was conducted to achieve the following objectives: 1. To estimate chemical characteristics of sarsoon oil, canola oil and hydrogenated fat. 2. To run a biological trial on adult rats by feeding the sarsoon oil (processed) canola oil and hydrogenated fat to see their effect on lipid fractions.

Materials and Methods
Chemical Characteristics: Some chemical characteristics of rapeseed oils and hydrogenated fat were determined, such as iodine value, acid value (Anonymous, 1987) peroxide value (Yu and Sinnhuber, 1957) and erucic acid contents (McGregor, 1977). Sarsoon oil was processed prior to feeding to reduce erucic acid. For this purpose Fuller's earth was selected (Mattil *et al*., 1964).

Biological Evaluation: Biological evaluation was done by feeding sarsoon oil (processed), canola oil and hydrogenated fat to adult rats for one month. Twelve adult male albino rats were divided into three groups having four rats in each and were placed separately in metallic cages. All the rats were weighed before the start of experiment and later at weekly intervals. Three diets A, B and C were prepared as shown in Table 1. Diet A contained 20 percent hydrogenated fat, while in diets B and C hydrogenated fat was replaced with sarsoon and canola oils, respectively. These diets were randomly assigned to the rats in a way that there were four rats on each diet.

Collection of Blood Samples: Of the four rats on each diet two were selected randomly and blood samples were taken under anaesthesia after weekly intervals. Serum was separated for the estimation of serum cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C). At the end of the trial all the rats were killed and their liver and carcasses were analyzed for fat contents. Serum cholesterol was determined by using acetic anhydride in the presence of Sulphuric acid by Liebermann-Burchard reaction (Stadtman, 1979). Serum triglycerides were analyzed by using kit method (cat-No.:10164) (Scheletter and Nasal, 1975). HDLcholesterol was also determined by using kit method (Cat.No:10018). LDL-cholesterol was determined by calculation method with the help of Friedewald *et al*. (1972) formula:

Conc. of LDL-cholesterol (mg/dL) = Total cholesterol-HDL-cholesterol-TG/5 (Where TG = Triglycerides)

Table 1: Composition of Experimental Diets

	Diets		
Ingredients	A (%)	B (%)	C (%)
Hydrogenated fat	20	-	-
Sarsoon Oil	-	20	-
Canola Oil	-	-	20
Skim milk	31	31	31
corn starch	19	19	19
Glucose	20	20	20
Potato starch	5	5	5
Vit. & Min. mix.	5	5	5
Total	100	100	100

At the end of the trial all the rats were killed and their liver was removed, weighed and preserved in the fornalin solution for the analysis of cholesterol and triglycerides. Rats were then placed in an oven to dry completely till constant weight was obtained. The carcasses were ground and preserved in polythene bags for triglyceride analysis.

For the estimation of liver cholesterol and triglycerides, liver samples were prepared by grinding them with 40 g of anhydrous sodium sulphate and dried in oven. One g of oven dried samples were taken and put in the flask to which 20 mL of chloroform was added. These flasks were then shaken in an electric shaker for 30 min and centrifuged. Supernatant was taken and cholesterol estimation was done using Liberman Burchard reaction (Stadtman, 1979). Liver triglycerides were determined as for serum (Scheletter and Nasal, 1975).

Carcass samples were prepared by taking 1 g of the carcass and suspending in 20 mL chloroform. This was filtered and clear solutions were taken for triglyceride estimation (Scheletter and Nasal, 1975).

The data thus obtained on various parameters was analyzed by using Minitab (10.2) computer software package (Anonymous, 1994). One way analysis of variance and general linear model (GLM) were applied for analyzing the data and means were compared by Tukey's test for confidence interval.

Results and Discussion

Chemical Characteristics of Oils: The iodine value corresponds to the average number of double bonds in the fats (Anonymous, 1987) and higher iodine value in canola than sarsoon oil and hydrogenated fat (Table 2) indicate its higher degree of unsaturation. Iodine value of 81.4 for rapeseed oil containing 43.6 percent erucic acid (Weiss, 1993) and 112 for canola oil (low erucic acid) has been reported (Ackman, 1977). The acid value of canola oil was also found higher than sarsoon oil and hydrogenated fat. However, Niewiadomski (1990) reported acid value of 1.25-2.30 of high erucic acid rapeseed oil which was lesser than 2.8 of sarsoon oil (high erucic acid), as determined in the present study. Comparatively high acid value of canola oil in the present study might be due to improper handling or storage. The peroxide value was also higher of canals oil which indicates higher proportions of double bonds (Niewiadomski, 1990). Both processed and unprocessed sarsoon oil showed high erucic acid value than canola oil. Vaisey and Eskin (1987) have also reported low erucic acid value of canola oil.

Serum Cholesterol and Triglycerides: Serum cholesterol level of the rats fed on diets containing different fat sources is sshown in Table 3. The results revealed that serum cholesterol was decreased 14.3 percent in sarsoon (processed) and 9.9 percent in canola oil, but increase by 26.1 percent from base value in hydrogenated fat (Kashmir banaspati) fed rats. The decrease observed in cholesterol in rapeseed oils (sarsoon and canola) with time was nonsignificant. However, cholesterol increased ($p<0.05$) in rats fed hydrogenated fat at 4th week (Table 3). Serum cholesterol showed non-significant difference till 2nd week in all the groups of rats. From 3rd week onward, the difference between rapeseed oils (Sarsoon and Canala) and hydrogenated fat was significant ($p<0.01$ at 3rd week $p<0.001$ at 4th week). This was due to decrease in serum cholesterol with time in rats fed on rapeseed oils while increase in rats fed on hydrogenated fat. In overall, serum cholesterol was lower ($p<0.001$) in sarsoon oil while higher in hydrogenated fat (Table 3) fed rats. Bruce and McDonald (1977) reported decrease in serum cholesterol in human adult males fed on rapeseed oil and they found increase in serum cholesterol when they were turned to mixed fat diet. Gustafsson *et al*. (1992), Nydahl *et al*. (1994) and Uusitupa *et al*. (1994) also reported decrease in serum cholesterol in individuals fed rapeseed oils. Barr *et al*. (1992) stated that saturated fatty acids causes increase in cholesterol and reduction of cholesterol without reducing saturated fatty acids in the diet is not possible.

Serum triglycerides showed a decrease of 22.6 and 21.3 percent, respectively, from the values obtained at zero day of trial when sarsoon and canola oils were fed. However, triglycerides increased by 24.3 percent when hydrogenated fat was fed. The decrease in rapeseed oils (sarsoon and canola) and increase in hydrogenated fat fed rats was nonsignificant with time. Triglycerides showed non-significant difference till 3rd week and at 4th week the levels were lower in rapeseed oils (Sarsoon, 130.47 ± 1 2.09, Canola 134.65 ± 14.09) than hydrogenated fat (206.50 ± 6.36) fed rats. This was similar in overall also (Table 3). Lasserre and Jacotot (1983) observed lowest levels of triglycerides in low erucic acid rapeseed oil than palm, corn, sunflower oil and butter fat fed individuals. Gustafsson *et al*. (1992) observed decrease in serum triglycerides in high erucic acid rapeseed oil and sunflower oil fed individuals. In present study the difference in processed high erucic acid sarsoon and low erucic acid canola oil was 1.3 percent. It probably indicates that other factors than erucic acid are involved in reducing the triglycerides level, which needs to be investigated.

Serum High Density Lipoprotein-cholesterol (HDL-C): Serum cholesterol level of the rats fed on diets containing different fat sources is shown in Table 3. Serum HDL-C during present study showed an increase of 19.3, 35.0 and 81.2 percent from the base value when sarsoon oil (processed), canola oil and hydrogenated fat were fed. The increase observed in HDL-C in rapeseed oils and hydrogenated fat with time was significant ($P<0.001$) at 2nd (hydrogenated fat), 3rd (canola oil) and 4th (sarsoon) week (Table 3). Three oils showed non-significant difference with each other except at 3rd and 4th week, where values in hydrogenated fat fed rats were higher ($P<0.05$, Table 3). The increase in HDL-C with time was much higher in hydrogenated fat than rapeseed oils fed rats. These findings were in line with those of Ahmad (1997). who also observed increase in HDL-C in rats fed on diet rich in saturated fatty acids. Srivastava (1994) reported that HDL-C is unregulated at transitional and cost-transitional levels by saturated fat.

Serum Low Density Lipoprotein-cholesterol (LDL-C): Serum LDL-C during present study showed a decrease of 20and 16

Table 2: Chemical characteristics of rapeseed oils and hydrogenated fat

Characteristics	Samples			
	Sarsoon oil Processed	Sarsoon oil unprocessed	Canola oil	Hydrogenated fat
Acid Value	2.80	-	3.90	1.70
Iodine value	104.00	-	109.00	74.00
Peroxide value	3.60	-	5.10	3.00
Erucic acid (%)	39.00	43.00	7.00	-

Table 3: Serum lipids (mg/100 mL) in rats fed on diets containing different oils at weekly intervals

	Diets		
(Weeks)	Sarsoon oil	Canola oil	Hydrogenated fat
Cholestrol			
0	223.0±30.45a	243.15±13.63a	234.45± 16.04Aa
1	207.85 ± 11.10a	238.50 ±20.51ab	252.00i- 32.53ABb
2	204.00 ± 8.49a	227.50 ±34.65ab	260.00 ± 7.07ABb
3	191.00 ±14.14a	223.00 ±2.83a	276.00 ±9.90ABb
4	191.67±8.08a	219.00 ±17.94a	295.00 ±8.44Bb
Means	204.14 ± 19.74a	230.47 ±18.77b	258.65 ± 26.51c
Triglycerides			
0	168 ± 20.52	171.75 ± 24.80	166.50 ± 22.72
1	165.00 ± 18.38	170.50 ± 13.44	187.50 ±17.68
2	152.50 ± 12.02	160.50 ± 38.89	181.50±28.99
3	147.00± 19.80	156.50 ± 17.68	193.0 ±14.14
4	130.47 ±12.09a	134.65 ± 14.09a	206.50 ±6.36b
Means	152.03 ± 20.35a	157.19 ± 24.34a	183.58 ± 22.11b
HDL-C			
0	34.650±0.786Aa	33.03 ±2.7Aa	32.15 ±4.199Aa
1	37.17 ±0.382Aa	34.46 ±3.45Aba	34.93 ± 1.259ABa
2	38.88 + 1.018Aba	39.954 3.323ABCa	47.30 ± 4.384BCa
3	40.41 ±0.891Aba	43.465 ±2.42Bca	55.59 ± 5.64Cb
4	41.76±1.47Ba	44.59±1.92Ca	58.23 +5.72Cb
Means	38.57 ± 2.94a	39.30 ± 5.43a	43.39 ± 11.89a
LDL-C			
0	154.79 ± 33.72	175.15 ± 2.6 6	169.00 ± 19.01
1	137.68 ± 15.16	167.50 ± 14.85	179.57 ± 34.80
2	134.40 ± 5.37	155.45 ± 30.19	176,40 ± 5.66
3	121.19 ± 18.99a	148.24 ± 3.94ab	181.90 ± 12.87b
4	123.75 ± 11.58a	147.60 ± 15.82a	195.45 ± 12.99b
Means	135.18 ± 21.56a	159.53 ± 18.27b	178.55 ± 18.17c

Values of cholesterol in a column with different capital letters are significant at $p<0.05$, while in a row with small letters at 3rd week are significant at $p<0.01$ and at 4th week and overall at $p<0.001$.

Values of triglycerides in a row with small letters are significant at $p<0.01$, while in a column with different capital letters at $p<0.001$

Values of HDL-C at 3rd week and 4th week with different small letters are significant at $p<0.05$ and $p<0.01$, respectively Values of LDL-C in a row with different small letters at 3rd week are significant at $p<0.05$ and at 4th week at $p<0.01$ and overall means with different small letters at $p<0.001$

Table 4: Total liver cholesterol, triglycerides and carcass triglycerides in rats fed on diets containing different oils

	Liver Cholesterol (mg/100 g)	Liver Triglyceride (mg/100 g)	Carcass Triglyceride (mg/100 g)
Sarsoon	426.33 ± 25.03a	339.35 ±46.67	55.063 ± 5.820a
Canola	413.25 ± 33.64a	412.38 ± 42.63	49.330 ± 9.487a
hydrogenated fat	332.00 ± 35.30b	354.64 ± 49.85	84.045 ± 3.557b

Values in a column with different small letters are significant at $p<0.05$

Table 5: Effect of diets containing oils on weights of rats

Diets	Initial weight (g)	Final weight (g)	Weight gain (g)
Sarsoon	321.16	301.10	-20.06
Canola	292.00	318.00	26.00
Hydrogenated fat	288.00	305.00	17.00

percent, respectively, from the base value when sarsoon and canola oils were fed. However, LDL-C showed increase by 15.7 percent when hydrogenated tat was fed. The decrease observed in LDL-C in rapeseed oils (each in sarsoon and canola) with time was non-significant. Similarly, the increase in LDL-C with time in hydrogenated fat was also non-significant with time (Table 3). Overall, LDL-C was lower ($p < 0.001$) in sarsoon (135.18 ± 21.56) than canola (159.53 ± 18.27) and hydrogenated fat (178.55 ± 18.17 mg/ 1OO mL) fed rats. The lower value of LDL-C in sarsoon oil fed rats was probably due to the lower value of LDL-C at zero day of the trial (Table 3). Nydahl *et al*. (1994) reported decrease in LDL-C in patients with hyperlipidaemia when given diets enriched with monounsaturated fatty acids and in diets enriched with PUFA. Similarly, Mattson and Grundy (1985) reported decrease in LDL-C when sarsoon and canola oil diets were used. Gustafsson *et al*. (1992) also reported 16 percent decrease in LDL-C in 95 subjects with hyperlipoproteinemia when given diets containing rapeseed oil.

Liver Cholesterol and Triglycerides: Significant ($p < 0.05$ difference with higher liver cholesterol was observed in rapeseed oils than hydrogenated fat fed rats (Table 4). This agreed with the findings of Zhang *et al*. (1990). They also reported higher liver cholesterol in rats given rapeseed oils than other fats.

Carcass Triglycerides: Rapeseed oils exhibited lower ($p < 0.01$) carcass triglycerides than hydrogenated fat (Table 4). However, non-significant differences were observed in rats fed sarsoon (55.06 ± 5.82) and canola (49.33 ± 9.49) oils. Appelqvist and Ohlson (1972) made similar observations and reported that rapeseed oils reduces the fat in the carcass compared to other diets.

Effect Of Different Oils on Growth: It is obvious from the Table 5 that sarsoon oil had a growth depressing effect. This may be associated with high erucic acid content of sarsoon oil. It has been reported that the oil containing low erucic acid is well digested by rats and the addition of saturated fatty acids in the diet improves the food intake and weight gain (Niewiadomski, 1990).

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