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EFFECT OF DIETARY RAPESEED OIL ON LIVER, KIDNEY FUNCTION AND FERTILITY IN THE RATS

BY

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ABSTRACT

Feeding diet with 20% unheated or heated rapeseed oil to rats resulted in a significant reduction of growth weight, body gain and relative growth rate compared with rats received basal diet plus 20% corn oil.

The same rapeseed diet caused a significant increase of serum trans aminases [Serum glutamic pyrovic transaminase (SGPT) and serum glutamic oxalacetic transaminase (SGOT)], alkaline phosphatase, urease, total proteins, albumine, glubuline, creatinine and cholesterol of rats. While sperm motility and sperm abnormality (primary, secondary and total) significantly decreased when compared with those feed diet with 20% corn oil. Fatty acid composition of the livers lipids changed to reflect the fatty acid composition of the diet.

INTRODUCTION

In Egypt, there is a great shortage in edible oils and hence large amounts are annually imported to cover the shortage in the local consumption market. During the past few years, rapeseed has been introduced to Egypt as a cheap source for edible oil. High and low erucic acid varieties were cultivated and experimented under Egyptian environmental conditions. However, up till now, the use of rapeseed oil for human consumption has not been yet confirmed and still its use is a big question for scientist to answer.

There are general agreement that diets containing rapeseed oil high in erucic acid content cause lipidosis and necrotic lesions of the myocardium in several laboratory animal species (Aaesjorgensen, 1972 and Rocquelin *et al* 1973). Engfeldt (1975) reported that rats fed on diet containing high levels of rapeseed oil high in erucic acid were affected by decreased digestibility depressed growth rate, intracellular lipid accumulation in the heart muscle followed by necrosis and fibrosis and depresseon of fertility of male rats due to spermatogenesis.

Vles *et al.* (1978) stated that rats fed low erucic acid rapeseed oil have higher incidence and severity of myocardial lesions than those fed other vegetable oils. They explained such phenomenon on the basis of the ratio of saturated to unsaturated fatty acids in rapeseed oils. Farnworth *et al.* (1982) observed growth depression in rats fed high erucic acid rapeseed oil (HEAR) lower digestibility, prolonged absorption time and inefficient energy utilization that led to relatively depression of organs weight (liver. kidneys. spleen. adrenals and testes). On the other hand,

Kramer *et al.* (1983) mentioned that erucic acid is poorly metabolized. and consequently their triglycerides could be accumulated in heart muscle. adrinal gland and overian tissues.

Farage *et al.* (1986) stated that there is a considerable risk in using rapeseed oil for human consumption owing to its unbalanced fatty acids content. Kramer and Sauar (1987) mentioned that the toxicity of rapeseed oil might be also attributed to the hard imbalance of the nutritional fatty acid in this oil.

Therefore, the following study was designed to evaluate the effects of dietary rapeseed oil on liver and kidney function and on fertility of male rats.

MATERIALS AND METHODS

Fourty male albino rats (at average weight 70-95 g) were divided randomly into four groups each included 10 rats. The first group given diet without oil (control), the second group received diet containing 20% w/w of unheated rapeseed oil. the third group received diet containing 20% of heated rapeseed oil. The fourth group received diet containing 20% corn oil. to evaluate the effect of feeding rats on rapeseed and corn oils and the experiment was finished at 8 weeks (Table 1).

- Rapeseed oil (French variety. Lasira 145) was obtained from Central Agricultural Research. Giza, Egypt.
- Corn oil was obtained from Misr oils and soap company. Zagazig Factory. Egypt.
- Heated rapeseed oil: about 1500 g of rapeseed oil were placed in 2000 ml beaker and heated at 180±10°C for 8 hrs.

The clinical manifestation and the effect of oils on growth were recorded. At the end of the experiment. rats were slaughtered and serum was isolated for the biochemical analysis. Semen was collected for semenl evaluation. Different organs were weighed. Specimens of liver were kept in a deep-freezer at -20°C until chromatographic analysis of their fatty acids.

The activity of serum glutamic pyrovic transaminase (SGPT) and serum glutamic oxalacetic transaminase (SGOT) were determined colorimetrically according to the method described by White (1970); serum alkaline phosphatase (SAPA) (Roy, 1970); total proteins (Wooton, 1964); serum albumin (Bartholonew and Delancy, 1966); serum globulin (Colos, 1974); cholesterol (Freidwald, 1972); total bilirubin (Jandrassik and Grof, 1938); Creatinine (Schirmeister *et al.*, 1964) and urea (Chaney and Marbach 1962).

Total lipids of rats liver were determined according to A.O.A.C. (1975). The fatty acids were isolated and treated with diazomethane to prepare their methyl esters as reported by Vogel (1975). The fatty acid methyl esters were analyzed using a Pye Unicum Series 304 GC. with flame ionization detector under the following conditions: column, PEGA 10%, nitrogen 30 ml/min.; chart speed, 1 cm/2 min.; injector temperature, 250°C, detector temperature, 300°C; 140°C for 4 min.; rate 10°C/min.; final temperature, 180°C for 22 min.

Identification of fatty acids was performed by comparing the relative retention time of each peak with the authentic fatty acids. Statistical analysis was performed using the methods of Snedecor and Cochran (1967).

Table (1): Composition of experimental diets.

Ingredient (%)	Group I	Group II	Group III	Group IV
Casein	20	20	20	20
Sucrose	30	20	20	20
Corn starch	40	30	30	30
Vitamin mixture*	1	1	1	1
Salt mixture	4	4	4	4
Alpha floc	5	5	5	5
Unheated rapeseed oil	-	20	-	-
Heated rapeseed oil	-	-	20	-
Corn oil	-	-	-	20

* Ingredients (mg/kg diet): Thiamin HCl 10, riboflavin 10, pyridoxine HCl 10, pantothenate 30, inositol 500, niacin 50, aminobenzoic acid 100, biotin 0.2, vitamin B_{12} 20, folic acid 2, vitamin A palmitate (500,000 IU/g), ergocalciferol (500,000 IU/g) and vitamin E acetate (500 IU/g)

RESULTS AND DISCUSSION

Fatty acids composition of unheated rapeseed, heated rapeseed and corn oils are presented in Table (2). The obtained data revealed that the ratios between total unsaturated to total saturated acids (TU/TS) for unheated

rapeseed and heated ones amounted (13.9:1) and (9.5:1), respectively. These values simply mean that the unsaturated fatty acids of unheated rapeseed oil are about 14 folds that of the saturated ones. Such values are higher than that values recommended for corn oil which showed in general low ratios of (TU/TS) i.e. (1.8:1). Oleic acid with one double bond resembles the highest amount of unsaturated fatty acids which is not an essential fatty acid and amounted to 53.4% in rapeseed oil, while belnoleic amounted only to 15.1%. On the other hand, Linoleic acid ($C_{18:2}$) which is the major essential fatty acid of corn oil amounted to (46.6%). The obtained results are in good agreement with those reported by Kramer *et al.* (1983). and concluded that rapeseed oil is considered as imbalanced edible oil.

Data in Table (3) showed a significant reduction in different parameters in first group which might be due to the unbalanced ration deficient in energy source. On the other hand body gain and daily body gain were slightly affected in 2nd and 3rd group. Also, relative growth rate in the 2nd experiment was highly affected and its value 30.27 was less than other values. Therefore different values of 2nd and 3rd experiment were obtained which might be attributed to the presence of erucic acid which amounted to 7.9% in the fatty acid composition of unheated and 6.2% in heated rapeseed oil. This acid effect on the nutritional quality of dietary oil (low digestibility. prolonged absorption time. inefficient energy utilization). Farnworth *et al.* (1982).

Also. Table (3) showed insignificant variance between different organs (testes, epididimes. seminal vesical, kidneys. liver. heart and spleen) in 2nd and 3rd group when compared with 4th group. These results agreed with those reported by Engfeldt (1975).

The data presented in Table (4) showed a significant increase in trans aminases (SGOT and SGPT). alkaline phosphatase. urease. total proteins. albumine, globuline. creatinine in serum of rats fed ration without oil (1st group) or rats fed basal diet plus 20% unheated or heated rapeseed oil (2nd or 3rd group) in comparison with rats fed basal diet plus 20% corn oil (4th group). The unbalanced ration of diet is responsible for significant increase of these parameters in 1st group. While in 2nd and 3rd group of rats. The increment in liver and kidney enzymes may be attributed to the presence of high amount of long chain monoenoic fatty acid (oleic and erucic acids) and to the deficiency of essential fatty acids in rapeseed oil. Kramer *et al.* (1983) reported that most enzymes of B-oxidation e.g. GOT were less active in the presence of very long chain monoenoic fatty acids (such as erucic acid) Consequently. these intracellular enzymes are liberated in blood stream.

Table (2): Fatty acid composition of dietary oils.

Dietary oils	Fatty acid percentage							TU/TS
	Palmetic $C_{16:0}$	Stearic $C_{18:0}$	Oleic $C_{18:1}$	Linoleic $C_{18:2}$	Linolenic $C_{18:3}$	Arachidic $C_{20:0}$	Cadoleic $C_{20:1}$	Erucic $C_{22:1}$	
Unheated rapeseed oil	4.4	1.7	53.4	15.1	8.9	0.6	8.0	7.9	13.9:1
heated rapeseed oil	6.9	1.8	53.8	17.3	6.7	0.8	6.5	6.2	9.5:1
Corn oil	17.8	2.4	31.2	46.6	1.4	0.6	-	-	1.8:1

Table (3): Effect of dietary rapeseed and corn oils on rate of growth and weight of different organs of rat.

Parameters		Group I	Group II	Group III	Group IV
Initial weight (g)		71.70±14.94	96.14±14.69	88.57±16.25	74.36±15.52
Final weight (g)		99.20±10.57	125.34±14.66	117.86±13.85	106.01±11.79
Body gain		27.50	29.20	29.24	31.65
Daily body gain		0.49	0.52	0.52	0.57
Relative growth rate (%)		38.35	30.27	33.01	42.56
Liver weight (g)		4.64±0.17*	6.17±0.37	6.14±0.34	6.47±0.42
Heart weight (g)		0.50±0.07*	0.59±0.04	0.60±0.05	0.64±0.04
Spleen weight (g)		0.38±0.02*	0.64±0.03	0.50±0.05	0.61±0.04
Kidney weight (g)	R.	0.56±0.05*	0.63±0.04	0.67±0.03	0.80±0.05
	L.	0.50±0.03*	0.57±0.03	0.67±0.04	0.79±0.04
Test weight (g)	R.	0.96±0.13	1.13±0.12	1.29±0.07	1.30±0.14
	L.	0.98±0.13	1.16±0.14	1.31±0.08	1.30±0.14
Epidedmes weight (g)	R.	0.54±0.15	0.50±0.05	0.59±0.07	0.59±0.06
	L.	0.46±0.12	0.43±0.05	0.56±0.05	0.57±0.07
Semenal vesical		0.26±0.06	0.19±0.05	0.11±0.01	0.26±0.05
		0.18±0.03	0.19±0.03	0.10±0.01	0.30±0.05

Group I received basel diet (no added oil).
Group II: received 20% unheated rapeseed oil in diet.
Group III: received 20% heated rapeseed oil in diet.
Group IV: received 20% corn oil in diet.

Table (4): Effect of dietary rapeseed and corn oils on liver and kidney function in rats.

Parameters	Group I	Group II	Group III	Group IV
Serum GOT µ mol/ml	30.60±0.46**	34.20±1.04**	35.20±0.87**	26.20±0.52
Serum GPT µ mol/L	55.60±0.67**	56.60±1.76**	63.20±2.52***	50.40±0.92
Serum APA µ mol/L	192.78±2.25*	227.15±1.59**	229.25±1.56*	180.71±3.02
Serum urea mg/L	21.44±0.36*	21.35±0.49*	20.90±0.36	19.41±0.59
Total bilirobin mg/100 ml	1.35±0.09**	0.82±0.05	1.13±0.02	0.59±0.02
Cholesterol mg/100 ml	80.93±4.99	184.44±6.05**	196.69±21.72***	80.53±5.21
Total protein g/100 ml	27.00±1.10	35.6±0.96	37.00±1.67	22.2±1.91
Albumine g/100 ml	22.31±1.04	30.99±0.95	32.04±1.73	16.98±1.92
Globuline g/100 ml	4.69±0.09	4.61±0.05	4.96±0.07	5.22±0.18
Creatinine mg/100 ml	1.45±0.03**	1.15±0.02**	0.99±0.32*	0.56±0.32

Group I: received basel diet (no added oil).
Group II: received 20% unheated rapeseed oil in diet.
Group III: received 20% heated rapeseed oil in diet.
Group IV: received 20% corn oil in diet.

Also. Table (4) showed a significant increase of cholesterol in 2nd and 3rd group. There are two main reasons for the accumulation of cholesterol in rats fed rapeseed oil in the diet. First the activity of cholesterol ester hydrolase enzyme was inhibited owing to the presence of long chain fatty acid. Secondary. cholesterol erucate ester. which accumulates in the organs of rats fed rapeseed oil was slowly hydrolyzed by the enzyme. Beckett and Boyd (1975).

Table (5) showed significant decrease of sperm motility in 1st. 2nd and 3rd group compared with rats received diet with 20% corn oil (4th group). The decrease in sperm motility in rats fed basal diet without oil may be due to unbalanced ration. On the other hand, erucic acid in the diet of rats showed an impair of spermatogenesis which is due to degeneration of tubules of testes. Also. erucic acid may act directly through an antagonism or competition with fatty acids essential for normal testicular function particularly since the testes is rich in highly unsaturated fatty acid, Carroll and Noble (1957). Also the deficiency in lenolic acid in this type of this oil i.e. rapeseed affects hardly on the synthesis of arachidonic acid which is a vital precurser for the formation of prostoglandin. The latter are ubiquitous cellular hormones with many local functions. Guyton (1981).

Therefore, rats feed on diet containing 20% unheated or heated rapeseed oil showed a significant decrease in their sperm motility. According to the unbalanced diet (1st group) or the presence of erucic acid in the diet (2nd and 3rd groups) results in Table (5) showed a significant increase in sperm abnormality compared with 4th group.

The lipid content and fatty acid compositions of the livers of rats received different diets are shown in Table (6). Higher levels of lipids were demonstrated in the livers of rats fed 20% heated or non-heated rapeseed oils compared to those fed control diet or diet containing 20% corn oil. These increment of lipids in livers may be attributed to the decrease of most enzymes of β-oxidation which act on very long chain monoenoic fatty acids. Such criteria was occurred because of the accumulation of erucic acid as long chain fatty acids in ones in liver. Kirschner and Harris (1961).

From the data presented in Table (6). it appears that the liver fats of all treatments contain many fatty acids but four usually comprise more than 93% of the fatty acid composition. The acids are palmitic and stearic of the saturated acid series and oleic and linoleic of the unsaturated series. In this study. lipids of rats liver fed 20% unheated or heated rapeseed oil contained higher levels of palmitic and oleic acids and lower level of linoleic acid and distinguished by the presence of erucic acid comparing with liver lipid of rats fed 20% corn oil.

Table (5): Effect of dietary rapeseed and corn oils on physical properties of semen of rats.

Parameters	Group I	Group II	Group III	Group IV
Sperm motility (%)	17.00±4.60	18.57±4.41	18.33±0.41	48.0±2.68
Sperm abnormality:				
Primary abnormality (%)	6.80±0.72	5.00±0.49	3.20±0.44	2.20±2.68
Secondry abnormality (%)	3.60±0.96	4.00±0.69	5.40±1.04	3.80±0.87
Total abnormality (%)	10.40±1.56	9.00±0.57	8.60±1.43	6.20±0.66

Table (6): Fatty acid composition of rats liver lipids.

	Lipids %	Fatty acid %						
		$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{22:1}$
Group I	4.06±0.37	0.63±0.20	33.05±1.79	1.41±0.10	23.60±0.96	37.46±3.97	5.77±1.98	-
Group II	6.60±0.94	0.78±0.38	38.96±2.40	3.58±0.31	11.98±1.30	35.99±2.10	6.66±1.61	2.10±0.32
Group III	5.01±0.14	0.85±0.05	32.64±1.68	2.50±0.24	17.98±2.42	39.93±1.04	7.48±1.81	1.34±0.18
Group IV	4.55±0.47	0.82±0.10	28.77±1.26	1.30±0.20	15.54±3.78	26.79±5.00	26.17±5.68	-

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تأثير التغذية بزيت بذر اللفت على وظائف الكبد والكلى والخصوبة فى الفئران

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تم دراسة إضافة زيت الشلجم (المسخن وغير المسخن) مقارنة بزيت الذرة الي عليقة الفئران وتأثير ذلك على الخصوبة ونشاط انزيمات الكبد والكلية.

وجد ان اضافة زيت الشلجم المسخن أو الغير مسخن بنسبة ٢٠٪ الى عليقة الفئران أدى الى انخفاض معنوى فى وزن الفئران وكذا فى معدل الزيادة اليومية كما أدى الى زيادة معنوية فى درجة نشاط أنزيمات الكبد (SGPT، SGOT) وكذا زيادة معنوية فى نشاط الكلى (Alkaline phosphatase, urease) وزيادة معنوية فى الكرياتينين وكذا زيادة كبيرة فى كوليستيرول الدم. كما لوحظ انخفاض معنوى فى الـ Sperm motility .

وأظهرت النتائج أن إضافة ٢٠٪ زيت ذرة إلى العليقة أدى إلى زيادة معنوية فى وزن الفئران وكذا فى معدل الزيادة اليومى وإنخفاض معنوى فى نشاط إنزيمات الكبد والكلى وأيضا إلى انخفاض معنوى فى كوليستيرول الدم وزيادة معنوية فى Sperm motility.

أظهرت النتائج أن إضافة ٢٠٪ زيت شلجم (مسخن أو غير مسخن) إلى عليقة الفئران أدت إلى زيادة كبيرة فى محتوى الكبد من الدهون وكذا زيادة فى محتوى حمضى البالمتيك والأوليك مقارنة بالتغذية بزيت الذرة الذى أدى إلى إنخفاض ملحوظ فى محتوى كبد الفئران من الدهون وكذا إلى زيادة فى محتوى لحمض الليبوليك وإنخفاض معنوى فى نسبة حمض البالمتيك.