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CHEMICAL AND BIOLOGICAL STUDIES ON PRICKLY PEAR SEED OIL AND FIBER

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ABSTRACT

Prickly pear seed oil represents 10% of dry seed, and could be considered one of the important nutritional oils. The procedure of gas liquid chromatography (GLC) possessed five fatty acids based on outhentic samples. linoleic acid was the dominant monounsaturated fatty acid, it representing 68.27% of total fatty acids.

Concerning rats feeding, the analysis of blood serum declared that the addition of prickly pear seed oil and defatted seed fiber to the rich fat diet reduced cholesterol, LDL cholesterol and triglycerides. High percent of vitamin E was detected in rats serum fed on 0.5ml and 0.25ml from seed oil compared to the positive control rats fed on hyperlipidemic diet . They were 27.15 ± 2.48 mg /100ml, 24.41 ±3.54 mg/100 ml, and 15.89 ± 0.60 mg/ 100ml, respectively.

Statistical analysis revealed significant differences between groups concerning vitamin E and lipidperoxidation (MDA). Histopathological examinations of experimental rats liver were performed and observed.

The examined liver of rats fed on diet supplemented with seed oil, showed a distinect characterization like that of rats fed on basal diet (negative control rats), which showed the normal histology of hepatic lobule. From the obtained data, it can be seen that the prickly pear seed oil could be considered one of the greatest dietary seed oil which would be used to protect from several diseases caused by free radicals accumulation.

INTRODUCTION

Prickly pear (*Opuntia Spp.*) belongs to Coctacea family which is native to the arid, and semiarid regions (Benson, 1982). In Egypt 29610 tons from prickly pear fruit were produced from 2656 fruitfull feddans (Anon, 2004).

Laban (1998) analyzed prickly pear fruit, and found that the seeds represented 14.2-17.1%. The seed contains 7-15% extracted oil, and the linoleic acid as abundant unsaturated fatty acid, represented from 56.1 to 77% (Sawaya and Kahn, 1982 & Stintzing *et al.*, 2000).

The seeds contain 98.8 g/kg oil as dry weight. The high amounts of natural lipids were found to be 87% of the total lipids in prickly pear seed oil. The vitamin E level was estimated as minor concentration, whereas α-tocopherol was the predominant component in seed oil (Ramadan and Morsel, 2003). They also concluded that, the prickly pear fruit is an important, nutritional and economical fruit which could be utilized as new source of oils and functional foods.

Fat and oil play an important role in the health promoting of the human body (Chandrasekharan and Basiron, 2001). The literature of Nicolosi *et al.* (1991); Grundy (1997) and Jason *et al.* (2003) postulated that replacement of dietary saturated fatty acids by monounsaturated fatty acids reduced LDL cholesterol levels.

Linoleic and α-linolenic acids are the only fatty acids known to be essential for the complete nutrition of many species of human, and must be therefore supplied in the diet as, they are known as the nutritionally fatty acids (Fisher, 1989).

The diet rich in cereals, wheat bran, sugar beet fibers, or pea increases the fraction of unabsorbed starch, that reaches the colon (Wisker *et al.*, 1989 and Hamberg *et al.*, 1989). Soluble dietary fiber helps to improve glucose tolerance and increases sensitivity to insulin (Hamber et al., 1989).

The present study was designed to shed light on the efficiency of prickly pear seed oil phytochemicals and defatted seed fibers on some biological characteristics of experimental albino rats.

MATERIALS AND METHODS

Materials:

Prickly pear fruit (*Opuntia ficus indica*) were obtained from Giza local market, Egypt. The fruit were at the ripe stage of maturity.

Methods:

Separation of seeds:

After extraction of juice as a traditional method, the seeds were separated from the remained pulp, strained and washed well with tap water, then dried at room temperature and crushed well.

Extraction of oil:

The crushed seeds were soaked in pure n-hexane for 24 hrs., then filtered using filter paper. The solvent was evaporated using a rotary evaporator at 45°C. Anhydrous sodium sulfate was added to the collected oil, then stored at 5°C in dark brown bottles until used for analysis and rats feeding. The yield of extracted oil was 10% based on dry seeds.

Determination of fatty acids:

Gas Liquid Chromatography (Pye-Unicam PRO-GC) was used for fractionation and determination of fatty acids according to the methods reported by Zygadlo *et al.* (1994).

Biological Experiment:

Thirty six male albino rats (Sprague dowely), weighting from 130 to 140 g, were obtained from The National Research Center (Cairo). Rats were housed in wire cages under the normal conditions, and fed on basal diet for a week as adaptation period. The rats were divided into six groups (n=6). The first group was used as a negative control, fed on basal diet. The other groups were fed on hyperlipidemic diet (1% cholesterol + 0.25 g bile salt + 10% animal fat) for 40 days as follows;

Group 1: rats fed on basal diet (negative control)

Group 2: rats fed on hyperlipidemic diet (positive control)

Group 3: rats fed on hyperlipidemic diet with 0.25 ml of prickly pear seed oil (orally)
Group 4: rats fed on hyperlipidemic diet with 0.5 ml of prickly pear seed oil (orally)
Group 5: rats fed on hyperlipidemic diet with 2.5% of prickly pear seed fiber
Group 6: rats fed on hyperlipidemic diet with 5% of prickly pear seed fiber

Experimental diet:

The composition of basal diet was 14% casein, 10% corn oil, 4% salt mixture, 1% vitamin mixture, 5% cellulose and 66% starch. The compositions of salt and vitamin mixtures were applied according to (Compbell, 1961). At the end of the experiment, rats were anesthetized with diethyl ether. Blood samples were collected from Portal vein, and serum was separated.

Biochemical analysis:

Total cholesterol was determined according to Allian *et al.* (1974). Triglycerides were measured according to Jacobs and Vandenmark (1960). High density lipoprotein (HDL) was determined according to Warnick *et al.* (1983). LDL cholesterol and very low density lipoprotein (VLDL) cholesterol were participated from serum by magnesium chloride/dextran sulfate reagent. LDL was determined according to the methods of Friedewald *et al.* (1972).

Vitamin E was determined according to Baker and Frank (1968). Malondialdehyde (MDA) was measured according to the method of Ohkawa *et al.* (1979).

Histopathological examination was performanced in Pathology Department, Veterinary College, Cairo Univ. according to the method of Carlton *et al.* (1967), where (H and E X 200).

Statistical analysis: The resulted data were analyzed using ANOVA procedure (SPSS, 1990).

RESULTS AND DISCUSSION

Fatty acids composition:

To study the importance of prickly pear seed oil, Gas Liquid Chromatography (GLC) was used to know the qualitative characteristics of extracted oil. From Table (1), it could be seen that five fatty acids were detected based on the authentic samples.

Table (1): Fatty acids composition identified in prickly pear seed oil by GLC technique.

Peak No.	Ret. Time	Type of fatty acids	Conc.%
1	11.759	Palmitic acid	13.47
2	12.287	Palmitoleic acid	0.59
3	15.229	Stearic acid	3.61
4	15.777	Oleic acid	14.05
5	16.724	Linoleic acid	68.27
			Total 99.99

Palmitic acid was the abundant saturated fatty acid, representing up to 13.47% of the total fatty acids. Concerning the unsaturated fatty acids, linoleic acid was the dominant monounsaturated fatty acid, representing 68.27% of the total fatty acids. Linoleic acid and α-linolenic acids are the only fatty acids known to be essential for complete nutrition (Fisher, 1989).

The long chain ω-3 polyunsaturates are widely believed to be the active agents that can reduce the risk of coronary heart diseases and inflammatory joint diseases (Groom and Ashwell, 1994).

Feeding and weights properties:

The effect of adding prickly pear seed oil and seed fiber in the rats diet on body weight gain, daily body weight gain, daily feed intake and the ratio of food efficiency is illustrated in Table (2). From the tabulated data, it could be seen that rats fed on basal diet (negative control) had 55.0±8.94 g body weight gain, while the those fed on hyperlipidemic diet (positive control) had 70±12.65 g body weight gain. Table (2) also shows that the other groups fed on diet supplemented with oil or fiber decreased body weight gain than the rats fed on hyperlipidemic diet (positive control). This decrease may be due to from the feeding on seed oil, containing lenoleic acid as the common fatty acid as mentioned before and also to the seed fibers.

Table (2): Body weight gain, daily body weight gain, daily feed intake and food efficiency of rats fed on the diet supplemented with prickly pear seed oil and seed fiber.

Properties / Rats feed on	Body weight gain (g) Mean ± SD	Daily body weight gain (g) Mean ± SD	Daily feed intake (g) Mean±SD	Food efficiency% Mean±SD
basal diet (negative)	55.0±8.94	1.49±0.24	14.30±0.70	0.11
Hyperlipidemic (positive)	70.0±12.65	1.89±0.34	15.36±0.70	0.12
hyperlipidemic with 0.25ml oil	65.83±13.57	1.78±0.37	14.21±0.69	0.13
hyperlipidemic with 0.5ml oil	62.50±6.89	1.69±0.19	14.38±0.66	0.12
hyperlipidemic with 2.5%fiber	60.83±4.92	1.64±0.13	14.77±0.63	0.11
hyperlipidemic with 5%fiber	58.33±4.08	1.58±0.11	14.58±0.73	0.11
F value	2.00^{N}	2.01^{N}	2.28^{N}	1.23^{N}

S.D. (Standard Deviation), n=6, N (Non significant)

Girgis *et al.* (2004) reported that the rats fed on hypercholesterolemic diet contain 100% apricot kernel oil had 74.2±2.1 g body weight gain, while those fed on basal diet (negative control) had 83.3±1.95 g. The reduction in nutrient digestibility is associated with the increase of fiber (Schneem, 1987 & Sadek, 1994). There are no observed differences in food efficiency between all groups.

Relation between feeding and cholesterol, HDL, LDL, VLDL and triglycerides:

Cholesterol, HDL cholesterol, LDL cholesterol, VLDL and triglycerides are the various parameters determined in rat serum listed in

Table (3). Cholesterol plays an important role in the transportation of triglycerides and phospholipids throughout plasma (Stein, 1987). From Table (3), it can be noticed that rats fed on hyperlipidemic diet (positive control) have a high level of cholesterol which recorded 151.75±9.17 mg/dl. Meanwhile, rats fed on hyperlipidemic diet with 5% prickly pear seed fiber distinected and had the first grade as decreasing the cholesterol level, followed by rats fed on hyperlipidemic diet with 0.5 ml prickly pear seed oil. They were 68.01±5.26 mg/dl and 72.37±13.33 mg/dl, respectively. This decrease could be attributed to the role of fiber and also monounsaturated fatty acid addition to dietary saturated fatty acids found in prickly pear seed oil such linoleic, oleic and palmetic acids.

Vegetable oils rich in oleic acid lowered serum cholesterol (Kinsell *et al.*, 1953). The results of Goodnight *et al.* (1982) concluded that unsaturated fatty acids lowered total cholesterol level for about half as much as saturated fatty acids which raised it. Cassidy and Calvert (1993) reported that the increased intake of dietary fiber in human is generally associated with the increase of fecal volume and increases in the lipid content of feces. There is no difference between the addition of 0.25 ml and 0.5 ml of seed oil in the lowering of serum cholesterol.

High density lipoprotein cholesterol (HDL) was found to be 44.57±6.46 mg/dl in the case of rats fed on basal diet (negative control), while it was 31.65±8.60 mg/dl in rats serum fed on diet rich lipid (positive control) (Table 3). HDL cholesterol is negatively associated with cardiovascular disease and thus positively viewed by health educators (Geoffery, 1995). The decrease of LDL cholesterol of all supplemented diet, and the increase of HDL cholesterol is observed in Table (3) compared with the positive control rats. Mattson and Grundy (1985) suggested that the monounsaturated fatty acids have a lowering effect on the LDL fraction and are neutral in their effects on the HDL fraction. Monounsaturated fatty acids significantly decrease total cholesterol and LDL cholesterol concentrations (Shaomei *et al.*, 1995). There were significant differences between HDL and LDL cholesterol of all groups.

Very low density lipoprotein (VLDL) was 14.93±2.30 mg/dl in rats serum fed on basal diet (negative control), while it was 22.98±3.22 mg/dl in rats serum fed on rich fat diet (positive control). Observed reduction of VLDL was in rat serum fed on 0.5 ml prickly pear seed oil compared with negative control rats. But other groups fed on supplemented diet had higher percent of VLDL compared to the positive control rats fed on hyperlipidemic diet. Concerning to triglycerides, data tabulated in Table (3) show that negative control rats have 74.67±11.48 mg/dl, whereas rats fed on hyperlipidemic diet (positive control) contained 114.90±16.2 mg/dl triglycerides.

The obtained data show that rats fed on 0.5 ml seed oil have the lower percent of triglycerides, while rats fed on 0.25 ml seed oil have higher percent of triglycerides. They were 85.43±6.31 mg/dl and 115.20±7.95 mg/dl, respectively. This variation may be due to the added quantity of 0.5 ml of prickly pear seed oil, which contains high level of monounsaturated fatty acids and dietary saturated fatty acids.

Table (3) : Effect of feeding with various diets on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in the experimental rats.

Properties / Rats fed on	Cholesterol mg/dl Mean±SD	HDL-chol. mg/dl Mean±SD	LDL-chol. mg/dl Mean±SD	VLDL-chol mg/dl Mean±SD	Triglycerides mg/dl Mean±SD
basal diet (negative)	62.05±7.85^{d}	44.57±6.46^{a}	3.55±6.90^{c}	14.93±2.30^{b}	74.67±11.48^{b}
hyperlipidemic (positive)	151.75±9.17^{a}	31.65±8.60^{b}	97.12±5.85^{a}	22.98±3.22^{a}	114.90±16.12^{a}
hyperlipidemic with 0.25ml oil	76.72±7.92^{c}	42.26±7.4^{a}	11.41±4.55^{c}	23.04±1.59^{a}	115.20±7.95^{a}
hyperlipidemic with 0.5ml oil	72.37±13.33^{c}	44.79±4.56^{a}	10.49±11.59^{c}	17.09±1.26^{b}	85.43±6.31^{b}
hyperlipidemic with 2.5%fiber	88.61±6.49^{b}	36.58±4.89^{ab}	29.91±7.49^{b}	22.1±4.33^{a}	110.52±21.64^{a}
hyperlipidemic with 5%fiber	68.01±5.26^{b}	39.95±7.97^{ab}	27.79±12.85^{b}	20.61±3.67^{a}	103.07±18.37^{a}
F. value	80.51	7.89	3.25	93.92	7.89

S.D. (Standard Deviation), n=6, (P<0.05)

Data also in Table (3) reveal that five percent of added seed fibers caused reduction in triglycerides more than 2.5% seed fiber compared to negative control rat fed on basal diet. Prickly pear peel dietary fiber added to the diet rich in lipids (hyperlipidemic diet) resulted in significant reduction in the triglycerides content in the blood serum (Abd-Elwahab *et al.*, 2005). From the previous results, it could be concluded that the addition of prickly pear seed oil and defatted seed fiber to the diet reduced cholesterol, LDL cholesterol and triglycerides in rats serum.

Effect of feeding on vitamin E and lipid peroxidation:

Vitamin E is very active antioxidant, though it is very important to determine it in rats serum fed on diet supplemented with prickly pear seed oil. Vitamin E, as the major lipid-soluble chain-breaking antioxidant has been associated with a reduction in lipid (Gaziano, 1994). The role of prickly pear seed oil as a source of vitamin E is listed in Table (4). High percent of vitamin E was detected from rat serum fed on 0.5 ml and 0.25 ml seed oil. They were 27.15±2.48 mg/100 ml and 24.41±3.54 mg/100 ml, respectively, but the negative control rats fed on basal diet has 17.81±0.91 mg/100 ml. Data presented in table (4) and Fig (1) explain that the diet containing 0.5 ml seed oil surpassed on the diet containing 0.25 ml seed oil as quantity affecting. Prickly pear seed oil contains minor concentration of vitamin E, whereas α-tocopherol was the abundant component in seed oil (Ramadan and Morsel, 2003). Packer (1991) stated that vitamins such as α-tocopherol are physiological scavengers of free oxygen radicals. Vitamin E, ascorbic acid and β carotene have the capacity to react with and quench free radicals

without themselves becoming highly reactive. They thus have a free standing antioxidant effect, independent of the enzyme mechanism (COMA, 1991).

Lipid peroxidation (MDA) is one of the important procedures to detect free radicals. Malondialdehyde (MDA) is the indicator for forming lipid peroxide. Lipid peroxidation involves series of free radicals and mediated chain reaction process is associated with several types of biological damage (Esterbauer *et al.*, 1992). Table (4) and Fig (1) also indicate that lipid peroxidation (MDA) decreased in rats serum fed on basal diet (negative control rats), while the positive control rats had higher level of MDA. They were 3.91±0.77 µmol/ml in the negative and 7.11±1.22 µmol/ml in the positive, respectively. All other groups, fed on diet supplemented with oil and/or fibers having lower level ranging between 4.68±1.35 µmol/ml and 4.25±0.99 µmol/ml of malondialdehyde

Table (4): Effect of feeding with various concentrations of prickly pear seed oil and seed fiber on vitamin E and lipid peroxidation in rat serum.

Groups	Vitamin E mg/100 ml Mean±SD	Lipid peroxidation (MDA) µmol/ml Mean±SD
Rats fed on basal diet (negative control)	17.81±0.91^{b}	3.91±0.77^{b}
Rats fed on hyperlipidemic diet (positive)	15.89±0.60^{b}	7.11±1.22^{a}
Rats fed on hyperlipidemic diet with 0.25ml oil	24.41±3.54^{a}	4.68±1.35^{b}
Rats fed on hyperlipidemic diet with 0.5ml oil	27.15±2.48^{a}	4.61±0.49^{b}
Rats fed on hyperlipidemic diet with 2.5% seed fiber	17.02±1.52^{b}	4.25±0.99^{b}
Rats fed on hyperlipidemic diet with 5% seed fiber	20.04±1.38^{a}	4.58±1.15^{a}
F. value	29.88*	7.22*

S.D. (Standard Deviation), n=6, * (P<0.05)

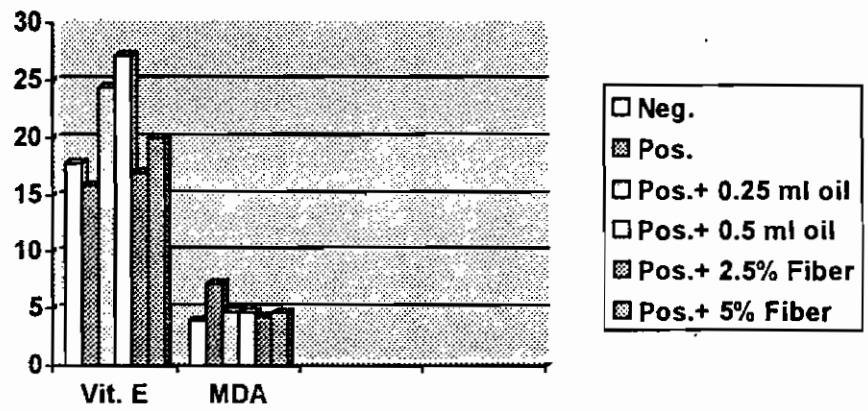


Fig. (1) : vitamin E and lipid peroxidation in rats serum fed on various concentrations of prickly pear seed oil and seed fiber

Finally the previous data ascertained the conflication between vitamin E as antioxidant and lipid peroxidation (MDA) as free radical matrix. These results are in agreement with the results of Wanger, (2001) who stated that Vitamin E has a great effect in reducing lipid peroxidation. Statistically analysis revealed significant differences between groups dealing with vitamin E and lipid peroxidation.Generally, prickly pear seed oil could be considered as one of the greatest dietary seed oil which would be used to protect from several diseases resulted from free radical accumulation.

Histopathological examination:

Histopathological examination of the experimental rat liver was performed and recorded in Figure (2).

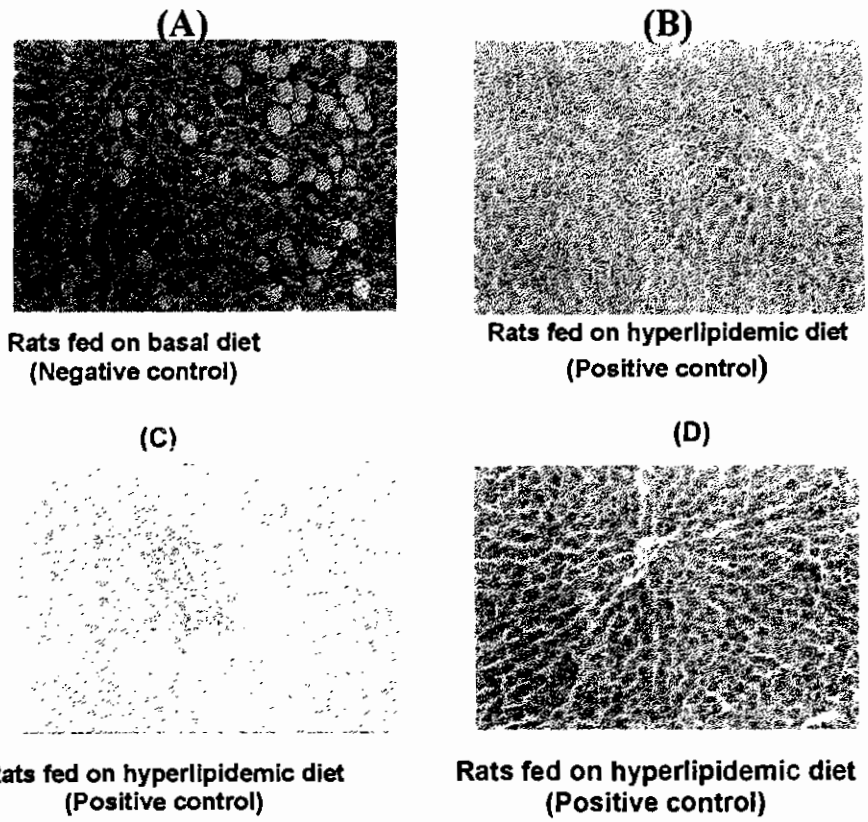


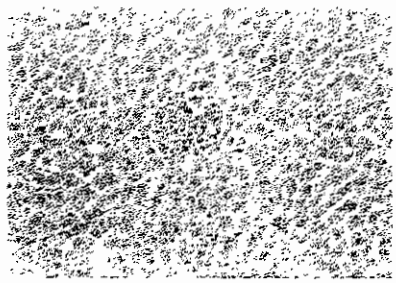
Figure (2) : Microscopical scanning of rats liver tissues.

Liver of rat fed on basal diet (negative control) showed the normal histology of hepatic lobule (figure 2A). While the examined liver of positive control which fed on hyperlipidemic diet showed the abnormalities changes in the liver such as vascular degeneration of hepatocytes (Figure 2B) and sinusolidal leucocytosis (figure 2C). Also portal infiltration with mononuclear

cells were induced (Figure 2D). These results are in accordance with Scot *et al.* (1990) who summarized that cytoplasmic vacuolation due to lipid accumulation is common following hepatic injury. Increased synthesis or decreased transport of lipid out of the cell leads to lipidosis (fatty liver). Energy and synthesis of lipoprotein are required for lipid transport, injury to these processes may lead to hepatic lipidosis.

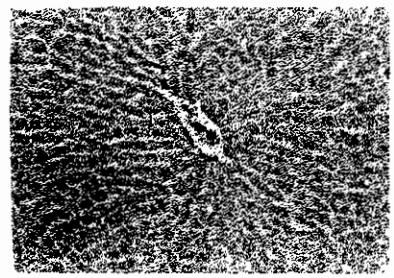
On the other hand, improvement in the histopathological picture was observed in the examined section from group (3), fed on hyperlipidemic diet supplemented with 0.25 ml of prickly pear seed oil, as the liver showed kupffer cells activation and portal infiltration with few mononuclear cells (figure 2E), and no histopathological changes were observed in liver of rats fed on the same diet with 0.5 ml of seed oil (figure 2F). Liver of rats fed on hyperlipidemic diet with 2.5% fiber (group 5) revealed local areas of hepatic necrosis (figure 2G). Meanwhile, apparent normal hepatocytes was noticed in the examined liver of group 6 fed on the same diet with 5% prickly pear seed fibers (figure 2H).

(E)



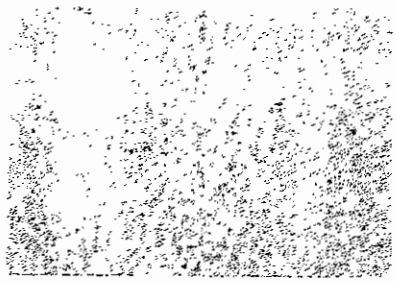
Rats fed on hyperlipidemic diet with 0.25 ml seed oil

(F)



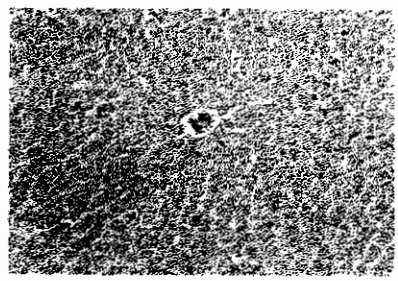
Rats fed on hyperlipidemic diet with 0. 5 ml seed oil

(G)



Rats fed on hyperlipidemic diet with 2. 5 % seed fiber

(H)



Rats fed on hyperlipidemic diet with 5 % seed fiber

Figure (2) Continue : Microscopical scanning of rats liver tissues.

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دراسات كيميائية بيولوجية على زيت وألياف بذرة التين الشوكى

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يمثل زيت بذرة التين الشوكى ١٠% من وزن البذور الجافه ، كما يعتبر من الزيوت الغذائية الهامة .
باستخدام التحليل الكروماتوجرافى (GLC) للزيت الناتج ، وجد انه يحتوى على خمس احماض دهنيه هامه ، وذلك بناء على الأحماض القياسية المحقونة فى الجهاز ، ووجد ان حمض اللينولييك هو الحامض الاحادى الغير مشبع السائد حيث يمثل ٦٨,٢٧ % من مجموع الأحماض الدهنية الموجودة بالزيت .
فيما يتعلق بتغذية الفئران، وجد ان إضافة زيت والياف البذرة الى الوجبات المرتفعة فى محتواها من الدهن ادى الى انخفاض الكوليستيرول والكوليستيرول منخفض الكثافة والجليسريدات الثلاثية فى سيرم دم الفئران .
كما وجد ارتفاع ملحوظ فى فيتامين E فى سيرم دم الفئران التى تغذت على وجبات بها ٠,٥ مل ، ٠,٢٥ مل من زيت بذرة التين الشوكى مقارنه بالفئران التى تغذت على وجبات مرتفعه فى محتواها من الدهن . حيث كانت ٢٧,١٥ ± ٢,٤٨ملجم / ١٠٠مل ، ٢٤,٤١± ٣,٥٤ ملجم/ ١٠٠مل مقارنه بالكنترول والتى كانت ١٥,٨٩± ٠,٦ ملجم/١٠٠مل على التوالى . وقد اوضح التحليل الإحصائي فروقا معنوية لكل من نتائج فيتامين E والمالون داى الدهيد وذلك بين المجوعات .
أظهرت الاختبارات التشريحية والفحص الميكروسكوبى لخلايا الكبد ان هناك تميزا فى خلايا كبد الفئران التى تغذت على الوجبات المدعمة وبخاصة على زيت البذرة وذلك عند المقارنة بالفئران التى تغذيت على وجبات مثاليه (العينة المقارنة) حيث ظهرت خلايا فصوص الكبد بصورة طبيعية . ومن خلال النتائج اتضح ان زيت بذرة التين الشوكى من أهم الزيوت الغذائية والتى يمكن عن طريقها الوقاية من أمراض عديدة والتى تنتج عن تراكم الشقوق الحرة .