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TECHNOLOGICAL AND BIOLOGICAL STUDIES ON GRAPE SEED

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ABSTRACT

This study was carried out to investigate the possibility of obtaining some phenolic compounds (as natural antioxidant) from grape seed. The physicochemical properties of the extracted oil as well as its fatty acid composition were determined. The antioxidant effect of phenolic extract was compared with the synthetic one (TBHQ) by using Rancimate method. Phenolic extract was added to sunflower oil at 0.02, 0.04 and 0.08% and TBHQ was added at 0.02%. The induction period of sunflower oil (control) was 6.07h. and it increased to 9.42, 11.5, 17.68 and 12.8 hours for the previous concentrations respectively. Sunflower oil with these different concentrations of phenolic extract and TBHQ were used for deep-frying of potato up to 18 hours to study the effect of these compounds on the stability of oil after frying. The phenolic extract constituents were identified using HPLC analysis. P. Coumaric was the major phenolic compound (64.99%) followed by O Coumaric, P.OH-benzoic acid, catechin, pyrogallic, galic acid and protocatechin. The effect of feeding on control fried sunflower oil and fried sunflower oil with the different concentrations of phenolic extract and TBHQ 0.02% on total cholesterol, LDL, HDL, triglyceride, total antioxidant, malondialdehyde. and nitric oxid in albino rats was studied, In addition the feeding on fried sunflower oil with 0.08% phenolic extract was found to enhance and improve the liver tissue followed by TBHQ when compared with fried sunflower oil and fried sunflower oil with 0.02, and 0.04% phenolic extract.

INTRODUCTION

Grape seed is considered one of the discarded residue pips from processing of grape on pilot plant scale. It contains high amounts of valuable nutrients and effective antioxidant such as polyphenols . The annual Egyptian production of grape fruits represents 1275288 tons produced from 138499 feddans of fruitful grape plants Anon (2004). Millions pounds of fruit seed are discarded yearly, resulting in disposal problems, while proper utilization of these waste products could led to an important new source of oil and meal, Kamel and Kakuda (2000). Basuny (2004) identified gallic acid, catechin, epicatechin, tannic acid and procyanidian as polyphenols in grape seed phenolic extract. The phenolics isolated from grapes and real wines had a vital role as antioxidant of human low density lipoprotein oxidation in vitro. Analogous results were obtained by Jayaprakasha *et al.*, (2001). Grapes represent a rich source of phenolic compounds, especially in the disposal seeds Bourzeix *et al.*, (1986).

Foster (1997) discussed the role of grape seeds on health promotion as antioxidants in human body by inhibiting lipid peroxidation as quencher

and scavenging free radicals and also are considered as natural antimutagenic agents . Kagan and Tyurina (1998) reported that the effect of phenolic antioxidants in protecting against oxidative stress depends on their reactivity towards reactive oxgyen species and the reactivity of the antioxidant phenoxyl radicals towards critical biomolecules. Phenolics antioxidants are a class of dietary compunds that possess anti-inflammatory properties. Robards et. al .,(1999) speculated that phenolics are considered to be most effective antioxidants since the hydroxyl phenols are good metal ion chelators , which catalyzed non-enzymatic free radicals generation that are suppressed in the presence of suitable phenolics.

Many phenols and polyphenols showed stronger antioxidant activity than vitamin antioxidants using model antioxidation of lower density lipoprotein LDL+VLDL. Phenols have also been found to enrich their lipoprotein after spiking in plasma Venson et. al., (1995).

Nutrition experiments conducted on male albino rats using the total and free phenolic fractions at concentrations of 400. 800. 1200 and 1600 ppm showed that polyphenols at 400. 800 and 1200 ppm levels had no effect on liver and kidney activities. However, the polyphenols at 1600 ppm level caused determinal effects on both origins (Basuny, 2000).

Phenolic compounds extracted from twelve different varieties of grapes showed antioxidant activity toward LDL oxidation in vitro (Mayer et al., 1997).

Phenolics in grapes and red wines have been reported to inhibit human low density lipoprotein (LDL) oxidation in vitro (Jayaprakashe et al., 2001)

The objective of the present work was to explore the role of phenolics of defatted grape seeds phenolic extract on some biological and histological properties of albino rats. Also, the physicochemical properties, fatty acid compositions, and oxidative stability of grape seed oil were determined.

MATERIALS AND METHODS

Materials:

Grape (Vitis vinifera) seeds were obtained as by-products from Ganklees factory " Wady El Natroon " -Alexandria Governorate , Egypt, season 2004. FAME standard and standard phenolic compounds were obtained from Koch-light Laboratories Ltd. Colubrook, Buckingham, Shira, England.

Artificial antioxidant Tetrabutyl hydroxyl-quinone (TBHQ) as synthetic antioxidant was purchased from Eastman Chemical Company. Sunflower seed oil was obtained from Savola Company (10th of Ramdan City, Egypt).

Fifty five kilograms of potatoes were purchased from the local market were peeled, washed and cut to chips.

Methods:

Extraction of grape seed oil:

Red grape seeds were crushed in a disintegrator. The crushed seeds were extracted with pure n-hexane at room temperature for 48 hrs then

filtered. This process was repeated for three times using fresh solvent each time to extract most of the oil from the crushed seeds. The miscella was collected, mixed and evaporated at 60°C under vacuum, then the extracted oil was dried using anhydrous sodium sulphate.

Chemical composition of grape seed:

Moisture, crude fat, crude protein, crude fibers, and ash contents were determined according to the methods of (A.O.A.C., 2000). Meanwhile, nitrogen free extract (NFE) was calculated by difference as follows: (NFE) = 100- (percent of moisture + oil + protein + fiber + ash).

Physical and chemical properties of grape seed oil:

Refractive index at 25OC, free fatty acids as oleic acid, peroxide value (m.equ/ kg. oil), iodine value and unsaponifiable matter percent were determined according to the methods described by the A.O.A.C (2000).

Preparation of the fatty acids methyl esters:

An aliquot of oil , about 100 mg , was dissolved with 2 ml heptane and then 0.2 ml of 2N KOH in an anhydrous methanol was added, and shaken, International Olive Oil Council (2001), after 30 sec. , 3 ml water was added . The organic layer, separated by centrifugation, was dried over anhydrous sodium sulfate, and then concentrated with a N2 steam to around 0.5 ml for GC analysis of fatty acids methyl esters (FAME) as described below.

Identification of the fatty acid methyl esters:

Agilent 6890 series GC apparatus provided with a DB-23 column (60m x 0.32mm x 0.25µm) was used. Fatty acids methyl esters (FAME) were directly injected into the GC. Carrier gas was N2 with flow rate of 2.2 ml/min, splitting ratio of 1:50. The injector temperature was 250°C and that of FID detector was 270°C. The temperature settings were as follows: 150° C to 225 at 5° C/min, and then held at 255° C for 20 min. Fatty acids were identified by comparing the retention time of the standard sample with that of the unknown sample.

Measurement of stability:

The oxidative stability of oils was estimated according to the method described by the Mendez et.al., (1997) using 679 rancimate (Metrohm, Herisav, Switzerland) at 100°C with air flow rate at 20L/hr.

Preparation of phenolic extract:

Phenolic extract containing phenolic compounds was prepared from 25 gm grinded grape seeds. The seed samples were grinded, and defatted by refluxing with hexane in a Soxhelt apparatus according to Kazimierz and Frank (1984), immersed in 250 ml methyl alcohol for 48 hrs. with a moderated stirring at ambient temperature . The slurry was filtered well, and the filtrate was evaporated till complete dryness of polyphenol and used to prepare fried sunflower oil to produce sample for the bioassay at the concentrations of 0.02%, 0.04% and 0.08% respectively, according to the method described by Duh and Yen (1997)

Identification of individual phenolic compounds:

Identification of individual phenolic compounds was performed on a Hewlett-Packard HPLC (Model 1100), using a hypersil C18 reserved -phase column (250 x 4.6 mm) with 5 µm particle size. Injection was by means of a

Rheodyne injection valve (Model 7125) with 50µm fixed loop. A constant flow rate of 1 ml /min was used with two mobile phase : (A) 0.5 % acetic acid in distilled water at pH 2.65 ; and solvent (B) 0.5% acetic in 99.5% acetonitrile . The elution gradient was linear starting with 100%(A) and ending with 100% (B) over 35 min, using on UV detector set at wavelength 254 nm. Phenolic compounds of each sample were identified by comparing their relative retention times with those of standards mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, and then converted to µg phenolic g1- dry weight. A11 chemicals and solvent used were HPLC spectral grade.

Frying process:

Two kilograms of sunflower seed oil mixed with various concentrations of additives were used in the frying process at 180˚C±5 for 3 hours daily for 6 consecutive days to enhance free radical generation. After frying, the oil was left to cool over night at room temperature and used for biological exterminates. Five mixtures of the heated oil were made as follows:
- sunflower seed oil (control), fried sunflower seed oil with 0.02%, 0.04% and 0.08 phenolic extract, or 0.02% TBHQ.

Designing biological experiment:

Biological experiment was designed by using thirty six male albino rats (Sprague Dowely). Rats were purchased from the National Research Center Cairo, weighting 120-123 gm and divided to 6 group each containing 6 experimental rats.

Albino rats were housed individually in stainless steal wire cages under ambient laboratory temperature, and fed on basal diet for one week as adaptation period. After that the rats were divided into 6 groups based on the various additives of the phenolic extract in rats diet. Group number one was fed on conventional basal diet, which consists of 14% casein, 10% sunflower oil, 4%salt mixture, 1% vitamin mixture, 5% cellulose and 66% starch Compbell, (1961). The rats fed on this diet formula, were assiegned as control rats. Where as the other rats that were fed on diet with 10% frying sunflower oil.

Rats groups were as follows:

- Group 1 control fed on diet containing 10% fresh sunflower seed oil.
- Group 2 fed on diet containing 10% frying sunflower seed oil.
- Group 3 fed on diet containing 10% frying sunflower seed oil with 0.02% phenolic extract.
- Group 4 fed on diet containing 10% frying sunflower seed oil with 0.04% phenolic extract.
- Group 5 fed on diet containing 10% frying sunflower seed oil with 0.08% phenolic extract.
- Group 6 fed on diet containing 10% frying sunflower seed oil with 0.02% TBHQ.
- Rats feeding were terminated after 4 weeks, then the rats were anesthetized and the blood samples were collected in solid EDTA and heparinized tubes, the plasma was separated and stored at 20°C until biological analysis.

Bioassay analytical method :

Total cholesterol and plasma triglycerides were determined according the method of Frings and Dunn (1979). High density Lipoprotein (HDL) and Low density Lipoprotein (LDL) were determined according to Roechlau et al.(1974), Assmann (1979). Total antioxidant was determined according to Koracevic et.al., (2001) Malondialdehyde (MDA) as a result of lipid peroxidation was determined according to Ohkawa et. al., (1979) .Nitric oxide (NO) was measured by the method of Montgomery and Dymock (1961).

Liver histopathology :

Rats liver was removed and kept on 10 % (V/V) neutral buffered formalin , dehydrated in ethyl alcohol and cleared in xylol .Liver was sectioned at 4-6μ thick and stained with hoematoxylin and erosin stain (H and Ex 200) according to Carlton et. al., (1967).

Statistical Analysis :

The date of bioassay were statistically analyzed using SPSS program , using one way ANOVA with Duncan test to estimate the significancy (SPSS, 1990).

RESULTS AND DISCUSSION

Chemical composition of grape seeds:

The chemical composition of grape seeds for its moisture content, crude protein, crude oil, total ash crude, fiber content and NFE are shown in Table (1). The results in Table (1) revealed that moisture content was 5.57%, lipid content was 10.77%, being in accordance with Miric et.al., (1992), the crude protein was 17.62%. The results in Table (1) also show that the crude fiber content was 50.54%, while the NFE was 12.28 in addition the ash content was that 3.22%.

Table (1): Chemical composition of grape seed on fresh weight bases.

Property	%
Moisture	5. 57
Oil content	10. 77
Fiber	50. 54
Ash	3. 22
Protein	17. 62
Nitrogen free extract*	12. 28

* By difference

Physical and chemical characteristic of the grape seed oil:

The results in Table (2) revealed that the refractive index was 1.4710 at 25°C. The results in the same Table showed low acid value being 1.1285, besides it should be stated here that, the acid value should be considered as one of the constants characterizing edible oil as it is generally affected by various factors related to both hydrolysis and oxidation of the oil. Concerning the low acid value it is quite encouraging for direct application for any purpose, without any need for neutralization. The peroxide value of the grape

seed oil was 4.06m.equ/kg.oil, indicating that no oxidatition occurred during extraction of oil. Meanwhile, iodine value was found to be 127.22. this means that it is semidrying oil, the unsaponification matter was 2.38%.

Table (2): Physical and chemical characteristics of grape seed oil.

Characteristics	Grape seed oil
Refractive index at 25° C	1.4710
Acid value (mg KOH / g oil)	1.1285
Peroxide value (m.equ/ kg. oil)	4.06
Idoine value (gm I2 absorbed by 100gm of oil)	127.22
Unsaponifiable matter%	2.38

Fatty acids composition of grape seed oil:

Grape seed oil is considered one of the untraditional oil that will be applied technologically in the present work. From the data listed in Table (3), it can be observed that linoleic acid as polyunsaturated fatty acids made up 63.22% of the total peak area through 6 fatty acids detected by GLC procedure. Oleic acid as monounsaturated fatty acid represents 22.37% of the total peak area while trace concentration of linolenic acid was detected in Table (3). Some of these results coincided with the results as is shown of Stefanie.et.al.,(2008) who found that it contains 58-78% linoleic acid, 3-15 oleic acid and minor amounts of saturated fatty acid (10%). Linoleic and linolenic acids are the only fatty acids known to be essential for complete nutrition.

Table (3): Relative percentage of fatty acids composition of grape seed oil using GLC method.

Fatty acids	Relative percentage
Palmitic acid	9.57
Stearic acid	4.11
Arachidic acid	0.19
Oleic acid	22.37
Linoleic acid	63.22
Linolenic acid	0.50

HPLC analysis of polyphenols extracted from grape seeds:

Results in Table (4) showed the chemical composition of polyphenols of grape seed determined by HPLC analysis. It could be noticed that P.coumaric was the major phenolic compounds present in grape seed, followed by O.coumaric, P.OH benzoic acid, catechin, pyrogallic, galic acid, and protocâtechin. These results are in good agreement with those reported by Bonilla.et. al.;(1999).

Table (4): Relative percentage of phenolic compounds of defatted grape seeds using HPLC procedure.

compound	Relative percentage
Pyrogallic	3.57
Gallic	1.96
Protocatechin	0.05
Catechin	5.55
P. OH benzoic	11.05
P. Coumaric	64.99
O. Coumaric	12.47

Effect of adding polyphenolic compounds from grape seed on the stability of sunflower oil:

Different concentrations of polyphenolic compounds extracted from defatted grape seeds were added to sunflower oil at concentrations of 0.02%, 0.04%, 0.08% and 0.02% TBHQ. Stability of all samples were measured by Ranicmat method at 100°C. The results were tabulated in Table (5) as induction period. The results indicated that addition of polyphenolic compounds increased stability of sunflower oil by increasing the concentration of polyphenolic compounds, as it increased from 6.07 to 9.42, 11.58 and 17.68hr. at 0.02, 0.04 and 0.08% respectively. It may be due to the effect of polyphenolic compounds extracted from the grape seed Robards et al., (1999). Also, addition of TBHQ at 0.02% increased the stability of the same oil from 6.07 to 12.8hr. It could be noticed that the addition of phenolic compound (natural materials) at 0.08% is best, the TBHQ at 0.02% (permitted concentration by food law).

Table (5): Stability and relative stability of sunflower seed oil when mixed with various concentrations of phenolic extract of defatted grape seed and TBHQ.

	Sunflower oil without antioxidant (control)	Concentration of phenolic extract %			%of TBHQ
		0.02	0.04	0.08	0.02
Stability at 100°C in hours	6.07	9.42	11.5	17.68	12.8
Relative stability*	1.00	1.55	1.89	2.91	2.1

*** Relative stability = "The stability in hours of the sunflower seed oil is 1.00"**

Effect of feeding on fried sunflower oil containing different concentrations of phenolic extract on biological parameters:

It is noticeable from results in Table (6), that rats fed on fried sunflower oil with 0.08% phenolic compounds or 0.02% TBHQ recorded the highest body weight gain and also the highest daily body weight gain since it were 172.5± 3.87 and 146.25± 22.2 for body weight gain and 6.9± 0.15 and 5.85± 0.88 for daily body weight gain respectively.

Statistical analysis showed that there were significant differences between groups regarding body weight gain and also body weight gain within groups.

Table (6): Effect of feeding on control, fried sunflower oil, and fried sunflower oil containing different concentrations of phenolic extract on body weight gain and dialy body weight gain in albino rats.

Rat group		Body weight gain (g) mean±S.D	Daily body weight gain (g) mean±S.D
1	Control	105 ± 12.24c	4.2±0.48c
2	Fried sunflower oil	113.75±7.33c	4.55±0.29b
3	Fried sunflower oil with Phenolic compounds 0.02%	90±9.48d	3.6±0.37d
4	Fried sunflower oil with Phenolic compounds 0.04%	117.5±6.70c	4.7±0.26c
5	Fried sunflower oil with Phenolic compounds 0.08%	172.5±3.87a	6.9±0.15a
6	Fried sunflower oil with TBHQ 0.02%	146.25±22.2b	5.85±0.88b

S.D. Stander Deviation
Each value within the same column followed by the same letter is not significantly different at p< 0.05.

Total cholesterol:

Results in Table (7) showed the plasma total cholesterol content of rats fed on all investigated diets. The results indicated minor increases in rats fed on either fried oil alone or those fed on frying oil with 0.02% TBHQ.

Table (7): Effect of feeding plasma total on control, fried sunflower oil and fried sunflower oil containing different concentrations of phenoilc extract on plasma total cholesterol, triglycerude, HDL and LDL.

Rats group	Plasma total cholesterol	LDL.Ch	HDL. Ch.	Triglycerides
Control	63.95 ±9.77a	17.35±8.03a	36.25±2.87a	63.20±7.43a
Fried sunflower oil	69.39±12.17a	22.10±5.42a	30.94±2.14a	81.15±14.17a
Fried sunflower oil with Phenolic compounds 0.02%	66.97±15.84a	18.71±7.29a	33.66±4.94a	73.03±18.41a
Fried sunflower oil with Phenolic compounds 0.04%	66.66±12.29a	17.68±9.87a	34.00±0.82a	68.44±10.58a
Fried sunflower oil with Phenolic compounds 0.08%	66.34±6.15a	17.45±9.08a	35.30±3.06a	67.87±6.77a
Fried sunflower oil with TBHQ 0.02%	68.58±13.25a	18.72±13.29a	31.70±4.83a	77.92±13.02a

S.D. Stander Deviation
Each value within the same column followed by the same letter is not significantly different at p< 0.05.

Low- density lipoprotein- cholesterol (LDL-C) content:

Results in Table (7) showed increase in LDL-C of rats fed on fried sunflower oil alone compared with those fed on fried oil containing 0.02% to 0.08%.

High-density lipoprotein-cholesterol (HDL-C) content:

Results in Table (7) showed a decrease in HDL-C of rats fed on either fried sunflower oil alone or with 0.02% TBHQ compared with those fed on fried oil containing 0.02% to 0.08% phenols.

Plasma Triglycerids:

Results in table (7) showed increase in triglycerids of rats fed on either fried sunflower oil alone, 0.02% TBHQ compared with those fed on fried oil containing 0.02% to 0.08% phenols.

Statistical analysis of total cholesterol, LDL-C, HDL-C and total lipids declared that there were no significant differences between all groups in these parameters.

Effect of feeding on fried sunflower oil containing various concentrations of phenolic extract on total antioxidant, malondialdehyde and nitric oxide in albino rats.

Regarding the effect of phenolic extract as antioxidant to quench the formed free radicals of fried oil, the parameters of total antioxidant, malondialdehyde and nitric oxide are listed in Table (8). The control rats group had 1.49 ± 0.189m mol/L, 1.91 ± 0.502 µmol /ml and 7.83±1.44 µmol/L of total antioxidant, malondialdehyde and nitric oxide respectively. On the other hand, rats fed on fried sunflower oil alone had 0.94 ± 0.098 µmal /L, 4.94±0.602 µmol/L and 12.72± 3.043 µmol/L of the previous parameters respectively. Rats fed on fried sunflower oil with phenolic extract at various concentrations to enhanced the antioxidant and decreases the free radical parameters.

Table (8): Effect of feeding on control, fried sunflower oil and fried sunflower oil containing different concentration of phenoilc extract on total antioxidant, malondialdehy and nitric oxide in albino rats.

Rats group	Total antioxidant m mol/L Mean ± S.D	Malondialdehyed m mol/L Mean ± S.D µmol /ml	Nitric oxide m mol/L Mean ± S.D µmol /L
Control	1.49 ±0.189a	1.91±0.502c	7.83±1.44b
Fried sunflower oil	0.94±0.098c	4.94±0.602a	12.72±3.043a
Fried sunflower oil with Phenolic compounds 0.02%	1.11±0.229bc	4.32±1.670ab	9.58±2.39ab
Fried sunflower oil with Phenolic compounds 0.04%	1.23±0.230abc	2.97±0.896bc	8.61±0.735b
Fried sunflower oil with Phenolic compounds 0.08%	1.37±0.238ab	1.99±1.024c	8.16±1.59b
Fried sunflower oil with TBHQ at 0.02	1.31±0.183ab	2.83±1.114bc	8.31±2.64b

S.D. Stander Deviation

Each value within the same column followed by the same letter is not significantly different at p< 0.05.

Statistical analysis of total antioxidant and free radical data declared that, there were a high significant differences between treated groups fed on

diets with phenolic compounds and those fed on diet with frying sunflower seed oil alone and rats fed on the diet containing the commercial antioxidant (TBHQ).

The natural antioxidant content of foods is considered a major health protecting factor by supplying the human body with exogenous antioxidant protection and reduces the content of oxidized toxic components in foods (Teissedre et. al., 1996).

Histopathological effects of defatted grape seed phenolic extract:

Our study was done on 6 groups of rats. The 1st group was fed on basal diet (Control group) this group shown no histopathological changes in hepatocytes as shown in (fig.1) (central vein and hepatocyetes arranged around it). The 2nd group included 6 rats were fed on basal plus fried sunflower oil, the histopathology of their liver showed hydropic degeneration of hepatocytes, hyperplasia of bile duct lining epithelium together with collagen fiber deposition in portal treat and portal inflammation with mononuclear inflammatory cells (fig.2a,b and,c). Meanwhile, the 3rd group which included 6 rats fed on phenolic compounds 0.02%, their liver histopathology revealed granularity of some hepatocytes and small focal area of hepatic necrosis infiltrated with mononuclear cells (fig.3a) also, some changes in that group showed heperplasia of epithelial lining bile duct, portal oedema and portal infiltration with mononuclear cells (fig. 3b). The 4th group which included 6 rats were fed on phenolic compounds with a concentrate of 0.04%, their liver showed vaculations of some hepatocytes (fig.4a) and also, portal tract infiltration with leucocytic cells (fig.4b). In relation to the 5th group which also included 6 rats fed on phenolic compounds 0.08%, their liver history was normal with no changes (fig.5). The last group (group 6) also included 6 rats fed on TBHQ 0.02%, their liver histology revealed sinusoidal leucocytosis (fig. 6).

Finally we can conclude that, the defatted grape seed is considered the main source of polyphenolics and the best concentration used was 0.08% as it showed no changes and no effect on hepatocytes and portal tracts and can be added as an antioxidant of various oil and or biologically as therapy nutrition of the human diet to protect liver from of fending agents.

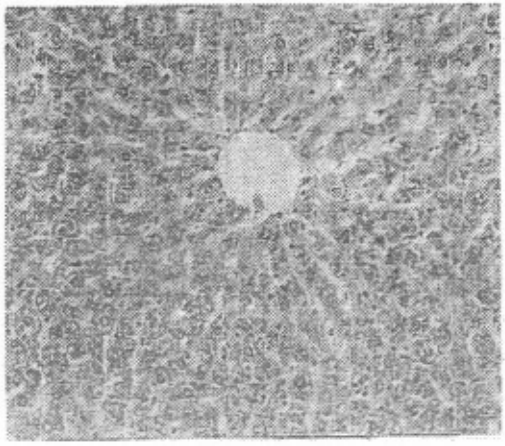


Fig. (1): (Control) liver of rat showing the normal histology of hepatic Lobule (H and E X200) (group1)

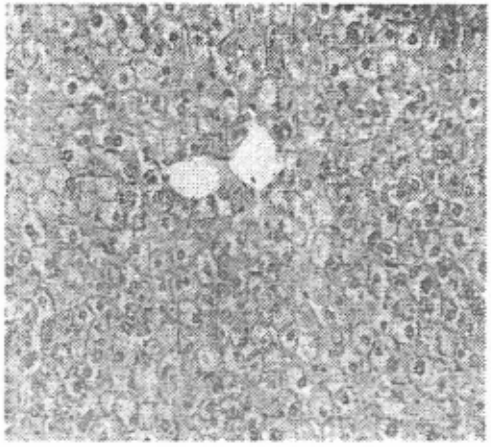


Fig (2a): (Fried sunflower oil) liver of rat showing hydropic degeneration of hepatocytes (H and E X200) (group2)

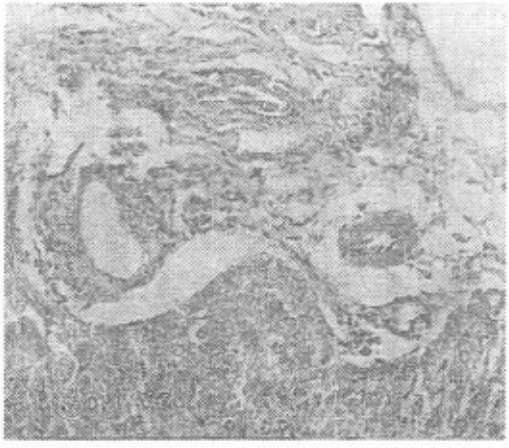


Fig.(2b) : (Fried sunflower oil) liver of rat Showing hyperplasia of epithelial lining bile duct together with collagen fiber deposition In the portal tract (H and E X200) (group2)

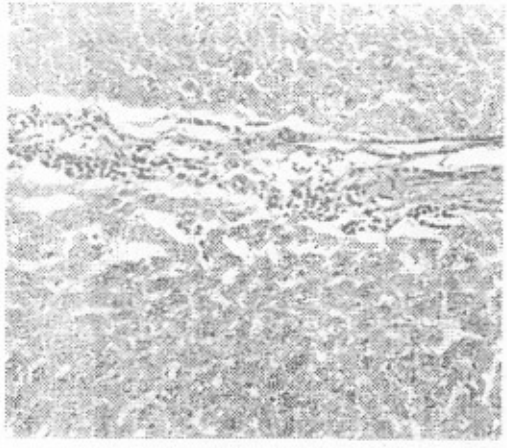


Fig.(2c): (Fried sunflower oil) Liver of rat showing portal infiltration with mononuclear inflammatory cells (H and E X200) (group2)

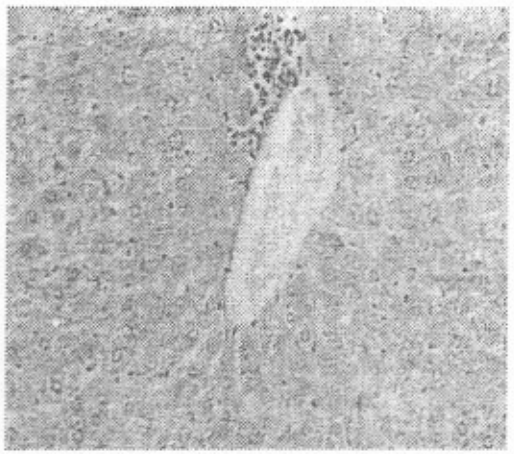


Fig.(3a): (Phenolic compounds 0.02%) Liver of rat showing granularity of some hepatocytes and small focal area of hepatic necrosis infiltrated with mononuclear cells (H and E X 200) (group 3)

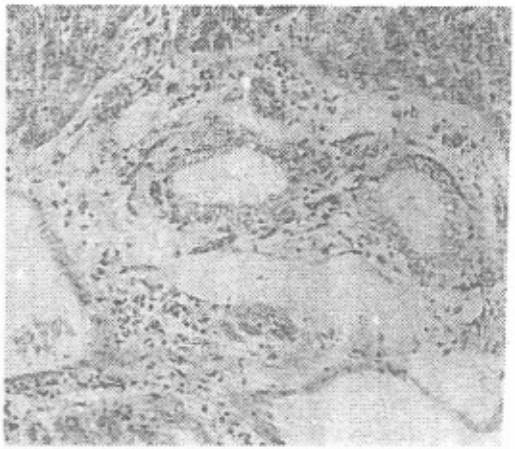
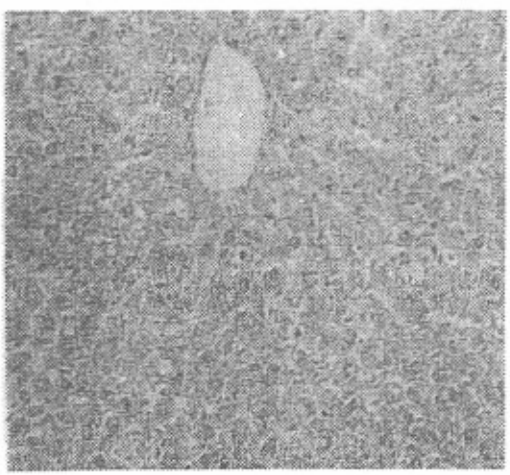
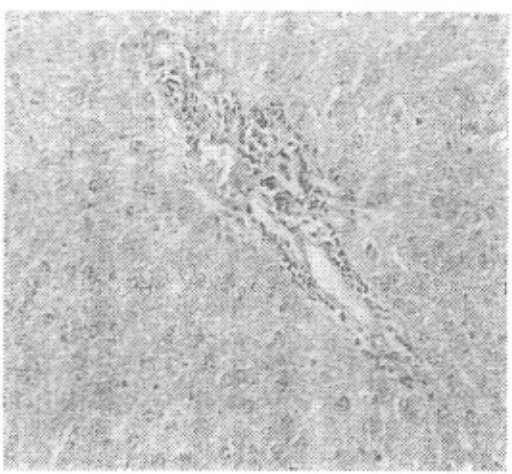


Fig 3(b): (Phenolic compounds 0.02%) liver of rat showing hyperplasia of epithelial lining bile duct, portal edema and portal infiltration with mononuclear cells (H and E X200) (group3)



Fig(4a): (Phenolic compounds 0.04%) Liver of rat showing vaculations of sporadic hepatocytes. (H and E X 200) (group 4)



Fig(4b): (Phenolic compounds 0.04%) liver of rat showing portal tract infiltration with leucocytic cells (H and E X200) (group4)

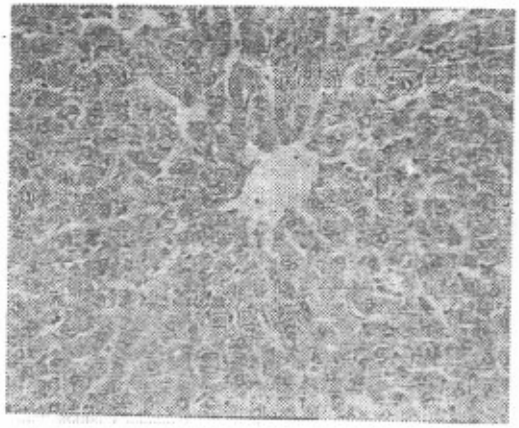


Fig (5): (Phenolic compounds 0.08%) Liver of rat showing no histopathological Changes (H and E X200) (group5)

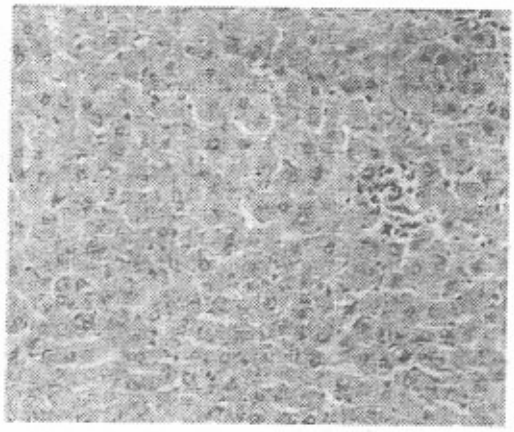


Fig (6): (TBHQ 0.02%) liver of rat showing sinusoidal leucocytosis (H and E X200) (group6)

Acknowledgment

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دراسات تكنولوجية وبيولوجية على بذور العنب

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أجريت هذه الدراسة بهدف الحصول على بعض المركبات الفينولية المضادة للأكسدة الطبيعية المستخلصة من بعض المخلفات مثل كسب بذور العنب وإمكانية إستخدامها كمضادات أكسدة طبيعية مقارنة بمضاد الأكسدة الصناعي (TBHQ) باستخدام طريقة الرانسيمات. تم إضافة المستخلص الفينولي إلى زيت دوار الشمس بتركيز ٠,٠٢، ٠,٠٤، ٠,٠٨%، TBHQ أضيف بتركيز ٠,٠٢% وكانت الفترة التحضيريه في زيت دوار الشمس (مقارنة) ٦,٠٧ ساعة وزادت إلى ٩,٤٢ , ١١,٥ , ١٧,٦٨ , ١٢,٨ ساعة لجميع التركيزات على التوالي. وتم إستخدام زيت دوار الشمس مع التركيزات المختلفة السابقة في عملية قلي البطاطس لمدة ١٨ساعة لدراسة تأثيرها على ثبات زيت دوار الشمس أثناء القلي. ووجد أن المركبات الفينولية الموجودة في كسب بذور العنب بعد تفريدها بجهاز HPLC والتي تم التعرف عليها هي باراكيوماريك، حمض أرثوكيوماريك، باراهيدروكسي بنزويك، كاتشين ، بيروجاليك، حمض جاليك وبروتوكاتشين. ثم أجريت تجربة تغذية لمعرفة مدى تأثير المركبات الفينولية المستخلصة من كسب بذور العنب على زيت دوار الشمس الذى تم إستخدامه في عملية القلي على مستويات كلا من بلازما الكوليسترول الكلي ، LDL ، HDL، والجلسيريدات الثلاثية ،مضادات الأكسدة الكلية ، المالون الدهيد، أكسيد نتريك مقارنة بالمجاميع التي تناولت زيت لم يتم إضافة مضاد أكسدة عليه وأوضحت النتائج أن تركيز ٠,٠٨% هو التركيز الأمثل للمحافظة على أنسجة الكبد يليه TBHQ.