

***Chemical ,Biological and histopathological effects
of fortified bread with flax and grape seeds on
acute liver diseases in rats***

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Abstract

This study was performed to investigate the effects of diet containing fortified bread with 10% flax seeds (FS), grape seeds (GS) and their combination on rats suffering from acute liver disease. Different levels of diet containing fortified bread with (5 , 10, and 20%) flax seeds, grape seeds and their combination were used in preparing of tested breads which were subjected to sensory evaluation. The best level of fortified bread after sensory evaluation was 10%. Chemical analysis of this level (10%) was determined and used in the biological experiment . Twenty-five male albino rats were randomly divided into two main groups, the first main group (n=5 rats) fed on basal diet containing un-fortified bread and kept as a control negative group. The second main group (n=20 rats) were injected with single dose of carbon tetrachloride (CCL₄) in paraffin oil 50% V/V (4ml/kg b.wt) subcutaneous injection to induce acute damage of the liver, after that the injected group was divided into 4 subgroups as the following , subgroup 1 fed on basal diet containing

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un-fortified bread as positive control group, subgroups 2 ,3 and 4 fed on diet containing fortified bread with(10% flax seeds),(10% grape seeds) and (10% mix of them), respectively.The obtained results revealed that the injected groups with 10% FS or GS or mixture of them (FS& GS) had significant decrease in serum levels of total cholesterol , triglycerides , LDL-c , VLDL-c , blood urea nitrogen , uric acid , creatinine , aspartate aminotransferase (AST) , alanine aminotransferase (ALT) , Glucose , alkaline phosphatase (ALP) enzymes and organs weight/ body weight% but body weight gain% and serum levels of HDL-cwere increased significantly,when compared with those of positive control group, butnon significant in feed intake which nearly returned toward the positive control group.Partially improvements in liver structures and kidney compared to those of positive control group.The best improvements of all the biochemical parameters and histological structures of liver and kidney which were tended toward normal results were observed in group was treated with mixture of 10%(FS&GS).In conclusion,the present findings suggested that regular intake from mixture of 10%(FS&GS) may be useful in improving liver and kidney functions and may protect against(CCL₄) which induce acute damage of the liver in rats.

Introduction

Hepatitis is most caused by viral and toxic agents. Deemed chronic when persisting for longer than 6 months, hepatitis triggers an ongoing inflammation that often leads to fibrosis and eventually cirrhosis, with a concomitant increased risk of hepatocellular carcinoma(**Centers for Disease Control and Prevention ,1998**).

Flaxseed or linseed (*Linum usitatissimum L.*) has been used in food and medicines in many countries. Flaxseed comes from the flax plant. It has been used in various forms such as flour, oil and seed.

Flaxseed and flaxseed oil is considered as healthy due to presence of various bioactive compounds in it (**Pourafsharet al.,2010**). The composition of flaxseed are 41% fat, 20% protein, 28% fiber, 7.7% moisture and 3.4% ash (**Charuet al.,2012**).

Flaxseed is rich in plant-derived omega-3 (n-3) polyunsaturated fatty acids (PUFAs), mainly α -linolenic acid (ALA, 18:3 n-3). Alpha-linolenic acid (ALA) is a fatty acid antioxidant. A key metabolite in mitochondrial energy production and a potent free radical scavenger, ALA is used medicinally in many European countries, primarily to treat liver disorders and neuropathy. ALA also helps recycle and regenerate other antioxidants, including vitamins E and C (**Hagen et al., 1999**).

Hepatobeneficial effect of increased levels of γ -glutamyltranspeptidase (γ GT) in the livers of both male and female rats with using a flax seed supplemented diet (**Hemmings and Barker, 2004**).

Lignans represented by the principal lignan from flax seed, secoisolariciresinoldiglycoside and the so-called mammalian lignans, enterolactone and enterodiol (**Westcott and Muir,2003**).

Low intake of polyunsaturated fatty acid (PUFA) is associated with increased prevalence of non-alcoholic fatty liver disease NAFLD (**Mussoet al.,2003**). Type of dietary PUFA strongly influences liver outcomes. A diet rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) provided the greatest protection against steatosis and hepatic inflammation. Animals provided with high dietary ALA had lower liver lipid masses and were slower to develop hepatic inflammation (**Monteiroet al.,2013**).

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Flaxseed is one of the richest plant lignans. pretreatment with flaxseed extract reduced the extent of CCL4-induced liver necrosis. Lignans included in flaxseed appear to modulate oxidative damage through antioxidative effects in the liver (**Daijiet al.,2002**).

Grape (*Vitisvinifera*) member of family vitaceae, is one of the widely cultivated and most important fruit crops in the world (**Satishaet al.,2008**). Chemical composition of seeds of red grape based on dry weight components % are moisture content (6.93), total solids (93.07) , protein (18.89) , fat (7.39) , fiber (48.90) Ash (0.33), nitrogen free extract (17.56). Some minerals and vitamins contents of seeds (mg/100 gm) are calcium(75.16),magnesium (25.10),potassium (8.30),phosphor (6.50),iron(12.50), vitamins (A) (8.10 mg/100 gm), vitamins (C) (23.40 mg/100 gm) ,Total phenols (73.59 mg/100 gm) (**Salem and Saltana ,2015**).

The beneficial health effects promoted by consumption of grape products are attributed to polyphenolic compounds and flavonoids are considered have biological properties, including antioxidant, anti-inflammatory and hepatoprotective activities (**Vasilet al.,2014**).Grape seed extract (GSE) has a preventive role in production of extra-cellular matrix elements which cause hepatic fibrosis **AyhanAtasever and DuyguYaman,(2014)**.

Therefore,the present work was conducted to study the effect of fortified bread with flax seeds,grape seeds and their combination on rats suffering from acute liver diseases.

Materials and Methods

Materials:

- Casein, vitamins, minerals, cellulose, choline chloride and carbon tetrachloride(CCL₄) were purchased from El-Gomhoreya Company, Cairo, Egypt.
- Oil and starch were purchased from local market, Cairo, Egypt.
- Twenty-five male albino rats (Sprague Dawley Strain) were obtained from Helwan farm.
- Flax seeds and grape seeds were purchased from local market, Cairo, Egypt.

Methods:

- The seeds were milled to coarse powder and used for fortified the bread .
- **Sensory evaluation of pan bread fortified with different levels of flax and grape seeds.**

Sensory evaluation was performed by invited ten panelists of staff members from Faculty of Home Economics, Helwan University. Each panelist was asked to evaluate unfortified and fortified bread samples with 5, 10 and 20% (flax seeds, grape seeds and their combination), according to color, odor, taste, volume, texture tenderness and general acceptability (***Abd El-Latif 1990***).According general acceptability the best level of fortified bread was used in biological experiment

- **Chemical analysis of un-fortified and fortified bread with 10% flaxseeds, grape seeds and their combination.**

Chemical analysis of un-fortified and fortified bread with 10% flaxseeds, grape seeds and their combination including protein,

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carbohydrate, oils, moisture and ash were determined in Food Technology Res. Institute according to the method described by the (A.O.A.C., 2003).

The biological assay:

Male albino rats SpargueDawley Strain (25 rats) weighing (200 ±10 g) were kept in individual stainless steel cages under hygienic conditions and fed one week on basal diet according to **Reeves et al ., (1993)** for adaptation at ad libitum in the animal house of Faculty of Home Economics , Helwan University. After this period, the rats divided into two main groups, the first main group (n=5 rats) fed on basal diet containing (250g/kg diet) un-fortified bread and kept as a control negative group. The second main group (20 rats) treated with single dose of CCl₄, in paraffin oil (50 % v/v 4 ml/kg) subcutaneous injection to induce acute damage in the liver **Jayasekhar et al., (1997)**. after that, the injected group was divided into 4 subgroups as the following , subgroup 1 fed on basal diet containing (250g/kg diet) un-fortified bread as positive control group, subgroups 2 ,3 and 4 fed on diet containing (250g/kg diet) fortified bread with (10% flax seeds), (10% grape seeds) and (10% mix of them), respectively.

During the experimental period (28 days), the diets consumed and body weights were recorded twice weekly to determine the feed intake and the body weight gain %

At the end of the experiment, the animals were fasted overnight, then the rats were weighed, anaesthetized and sacrificed, then blood samples were collected from the aorta. The blood samples were centrifuged and serum was separated to estimate some biochemical parameters, i.e. serum cholesterol (**Allainet al.,**

1974), triglycerides (*Foster and Dumns., 1973*), HDL-c (*Lopes – Virellaet al., 1977*), LDL-c and VLDL-c (*Fried walDET al., 1972*), glucose (*Trinder., 1969*), aspartate amino transferase (AST) and alanine amino transferase (ALT) (*Reitman and Frankel., 1957*), serum alkaline phosphates (ALP) (*Belfield and Goldberg., 1971*), uric acid (*Fossatiet al., 1980*), urea nitrogen (*Patton and Crouch.,1977*), Creatinine(*Bartels and Bohmer.,1971*),

Liver and kidneys were separated from each rat and weighted to calculate organs to body weight % and examined histopathologically.

The data was presented as means \pm SD statistically analyzed using one way ANOVA test, $p < 0.05$ was used to indicate significance (*Steel and Torri., 1980*).

Results and Discussion

Sensory evaluation of pan bread fortified with different levels of flax and grape seeds.

The results in **Table(1)** indicated that bread fortified with 5% flax seed , grape seed and mix of(flax seed& grape seed) had a non-significant in(external color , internal color , taste , odor , volume , texture , tenderness and general acceptability) in comparing of control un-fortified bread and showed bread fortified with 10% FS , GS and mix of(FS&GS) had a non- significant in external color , internal color , taste , odor , volume , texture and tenderness in comparing of control un-fortified bread except general acceptability in all types of fortified bread, in comparing of control un-fortified bread. Bread fortified 20% FS and GS had non significant in external color except in internal color , taste , odor , volume , texture ,

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tenderness and general acceptability. On the other hand the results illustrated that bread fortified 20% mix of (FS&GS) recorded lowest significantly in external color , taste , odor , volume , texture , tenderness and general acceptability in comparing of control un-fortified bread.

Therefore fortified bread with 5% and 10% FS,GS and their combination recorded the best results in sensory evaluation, and will be used the three types of fortified bread with 10% in the biological study.

Chemical analysis of un-fortified and fortified bread with 10% flax seeds, grape seeds and their combination.

Chemical composition of un-fortified bread fortified bread with 10% (flax seeds, grape seeds , flax and grape seeds) were determined and illustrated in **Table(2)**. The tabulated results it could be noticed that, carbohydrate and protein contents recorded the highest amounts on nutrients in un-fortified and fortified bread with 10% (flax seeds, grape seeds and the combination of them). The three types of fortified bread showed increase in moisture than that of the un-fortified bread. The values of moisture , oils , ash and fiber contents in fortified bread with 10% (flax seeds, grape seeds , flax and grape seeds) were (9.7 , 7.7 and 9.7%); (1.67 , 0.47 and 0.90 %) ; (0.6 , 0.79 and 0.72 %) and (0.34 , 0.62 and 0.42%) respectively. while , in un-fortified bread the amounts of these contents were (6.4 , 0.19 , 0.53 and 0.20%).

Effect of diet containing fortified bread with flax seeds, grape seeds and their combination on serum glucose and liver enzymes of rats suffering from acute liver diseases.

The obtained results in **Table(3)** illustrated that control positive group recorded highest significantly in glucose , AST , ALT and ALP comparing with negative control group. Animals treated by diet fortified bread with flax seeds , grape seeds and their combination recorded significant decrease in serum glucose , AST , ALT and ALP as compared to the positive control group. The results are in agreement with **Kanbur et al., (2009)** who reported that, grape seeds (GS) application reduced alkaline phosphatase (ALP) activities closer to control group level ($p < 0.01$). These result showed that GS can be effective on Carbon tetrachloride (CCl_4) induced hepatic damage. Additionally, **(Al-Bishri ,2013)** revealed that, essential fatty acids in flax seeds, could improve the liver and kidney dysfunctions in the hypertensive condition. **Tsuda, (2012)** who found that polyphenols in grape seeds may be reduce type 2 diabetes, by acting as antioxidant and anti-inflammatory agents. **Pourafshar, et al.(2010)** reported that flours extracted from some other edible grains like flaxseed can provide an ample quantity of good quality protein and dietary fiber and contribute effectively in the reduction of diabetes. Another study by **Sunita Mishra and Pooja Verma,(2013)** revealed that the components in the present in flaxseeds, α -linolenic acid (ALA), lignans, and fiber helps in the prevention of diabetes and constipation. The phenolic compounds of flaxseed help in reduction of the fasting plasma glucose levels.

Effect of diet containing fortified bread with flax seeds, grape seeds and their combination on lipid profile of rats suffering from acute liver diseases.

The obtained results in **Table(4)** showed a marked significant increase in serum Cholesterol (CH), Triglycerides (TG) ,Low density lipoprotein-Cholesterol (LDL-c) and Very low density lipoprotein-Cholesterol (VLDL-c) levels and significant decrease in serum High

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density lipoprotein-Cholesterol (HDL-c) levels in positive control group compared with those of the normal rats (negative control group). Comparing treated rats with diet containing fortified bread with grape seeds 10% and their combination 10% revealed a marked significant decrease in serum levels of CH , TG , LDL-c and VLDL-c and significant increase in serum HDL-c , bread fortified 10% (flax and grape) seeds which nearly returned toward the normal levels in serum TG , HDL-c and VLDL-c. Animals treated with bread fortified with 10% flax seeds recorded highest significantly in serum HDL-c and showed lowest significantly in serum LDL-c except serum CH , TG and VLDL-c had a non-significant by comparing of positive control group.

Our results agree with *Liu et al. (2001)* who cleared that the high content in unsaturated fatty acids (around 85–90%) are considered to be responsible for health promoting effects like reduction of cholesterol in serum. Also *Krithika et al., (2015)* said that phenolic compounds in the grape seeds extract and its antioxidant properties could be reduce the concentration of LDL-c in plasma. *Lucas et al., (2004)* reported that flax seed decrease plasma cholesterol, it appears that secoisolariciresinoldiglucoside (SDG) and other lignans may be effective in lowering risk factors for coronary artery disease including hypercholesterolemic atherosclerosis. Another study by *Lemay et al. (2002)* revealed that flax seeds treatment reduced LDL-c, glucose and insulin levels but increased HDL-c and apolipoprotein levels also attenuated menopausal symptoms.

Effect of diet containing fortified bread with flax seeds, grape seeds and their combination on kidney functions of rats suffering from acute liver diseases.

Results of uric acid , urea nitrogen and creatinine of experimental rats are presented in **Table(5)**.The mean values of serum uric acid , urea nitrogen and creatinine of positive control group were increased significantly, compared with those of the normal rats on the other hand , treated rats which feeding on diet containing fortified bread with flax seeds , grape seeds and their combination had significantly decreased in uric acid , urea nitrogen and creatinine compared with those of positive rats. The best result recorded for bread fortified with 10% (flax and grape) seeds in serum creatinine which nearly returned toward the normal levels,in this respect,**Nagib,(2014)** found that, The rats treated with grape seeds powder ,extract and oil cleared significant improvement of the levels of serum creatinine, urea, uric acid, albumin/globulin ratio ,kidney tissue and malondialdehyde (MDA).

Table(6): Effect of diet containing fortified bread with flax seeds, grape seeds and their combination on feed intake, body weight gain% and organs weight/body weight% of rats suffering from acute liver diseases.

The obtained result in **Table(6)** showed a marked significant decrease in feed intake (FI) and body weight gain (BWG) and significant increase in organs weight/ body weight% in positive control group compared with those of the normal rats (negative control group).In contrast, rats feeding on supplemented diet with bread fortified 10% flax seeds, grape seeds and the mix from (flax &grape)seeds had significantly decreased in organs weight/ body weight% and increased of body weight gain% when compared them with those of positive control group. But non significant in feed intake which nearly returned toward the positive control group.**Bloedon and Szapary,(2004)** confirmed that, diets high in flax seeds have been recommended for weight loss, because of its

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components besides fiber have an effect on body mass index (BMI). **Nagib, (2014)** who reported that, The rats treated with grape seeds powder showed a non significant difference in weight gain ,food intake compared with healthy group but cleared a significant increase in body weight gain compared with control(+ve) group.

Histopathological Examinations

Histopathological examination of Liver:

Microscopically, liver of rats from control negative group revealed the normal histological structure of hepatic parenchyma **(photo. 1)**. On the other hand, liver of rats from control positive group revealed steatosis of hepatocytes **(photo. 2)**, focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration and fibroplasia in the portal triad **(photo. 2)**. Meanwhile, liver of rats from group fed on fortified bread with 10% flax seeds showed marked improvement, as examined sections revealed slight activation of Kupffer cells and steatosis of sporadic hepatocytes **(photo. 3)**.

Moreover, liver of rats from group fed on fortified bread with 10% grape seeds revealed no histopathological changes except slight activation of Kupffer cells **(photo. 4)**, binucleation of hepatocytes and hydropic degeneration of focal hepatocytes.

However, liver of rats from fed on fortified bread with 10% mix of flax seed and grape seeds revealed small vacuoles in the cytoplasm of sporadic hepatocytes **(photo. 5)**, slight activation of Kupffer cells **(photo. 5)** and slight dilatation of hepatic sinusoids. Our results agreed with **Rodriguez-Leyvaand et al.(2010)** stated that improved liver architecture and pathological changes were observed in flaxseed treated diabetic rats and protective effects of these treatments against the hepatic changes. Additionally, **Lazzeet**

al. (2003) reported that grape seeds are rich in phenolic compounds such as anthocyanins that is protect against DNA damage in rats hepatoma cellsdemonstrating potential anticarcinogenic properties.Alpha-linolenic acid (ALA) which rich in flax seeds oils preventing hepatic steatosis and inflammation. ALA can act on risk factors associated with the development of fatty liver disease (*Monteiroet al.,2013*).

Histopathological examination of kidneys:

Microscopical examination of kidneys of rats from control negative group revealed the normal histological structure of renal tissue (**photo. 1**). On the contrary, kidneys of rats from control positive group revealed vacuolar degeneration of epithelial lining renal tubules (**photo.2**), perivascular inflammatory cells infiltration (**photo.2**), thickening of the parietal layer of Bowman's capsule and distension of Bowman's space with filtrate. Meanwhile, some examined sections from group fed on fortified bread with 10% flax seeds showed vacuolar degeneration of epithelial lining some renal tubules and focal mononuclear cells infiltration, whereas, other sections from group revealed no histopathological alterations (**photo. 3**). Moreover, some sections from groups fed on fortified bread with 10% grape seeds & fed on fortified bread with 10% mix of flax seed and grape seeds showed vacuolar degeneration of epithelial lining some renal tubules, whereas, other sections from these groups revealed no histopathological alterations (**photo. 4 & 5**).The present result was in accordance with *Velasquez et al.(2003)* who showed that flaxseed meal was more effective in reducing proteinuria and kidney histologicabnormalities. In another study, confirmed that The rats treated with grape seeds powder demonstrated significant improvement of kidney tissue and significant decrease in kidney functions (*Nagib, 2014*).

Conclusion

In conclusion the effect of diet containing fortified bread with 10%(flax seeds , grape seeds and their combination had effect and exhibited improvement in liver and kidney functions against injury induced by carbon tetrachloride (CCL₄).The mixture consisting of 10% (flax seeds and grape seeds) provide the best improvement in all biochemical measurements and histological structure. Hence, regular intake of them or using it for enriching food product as functional foods may help to improve health status.

Table (1): Sensory evaluation of pan bread fortified with different levels of flax and grape seeds.

Characters Samples	External Color 5	Internal Color 5	Taste 10	Odor 5	Volume 5	Texture 5	Tenderness 5	General Acceptability 10
Control (un-fortified bread)	4.00 ^a ± 0.707	4.400 ^a ± 0.894	9.00 ^a ± 1.00	4.200 ^a ± 0.836	4.800 ^a ± 0.447	4.600 ^a ± 0.547	4.200 ^a ± 0.836	9.600 ^a ± 0.547
Bread fortified with 5% FS	3.800 ^{ab} ± 0.447	4.00 ^a ± 0.707	8.600 ^a ± 0.547	4.00 ^a ± 0.707	4.400 ^a ± 0.547	4.200 ^{ab} ± 0.447	4.00 ^{ab} ± 0.707	9.00 ^{ab} ± 0.707
Bread fortified with 10% FS	3.800 ^{ab} ± 1.095	3.600 ^a ± 0.894	8.400 ^a ± 0.547	3.600 ^a ± 0.894	3.800 ^{ab} ± 0.836	4.200 ^{ab} ± 1.095	3.600 ^{abc} ± 0.547	8.400 ^b ± 0.547
Bread fortified with 20% FS	3.400 ^{ab} ± 0.547	2.400 ^b ± 0.547	6.600 ^b ± 0.547	2.600 ^b ± 0.547	3.200 ^{bc} ± 0.440	3.200 ^{bc} ± 1.100	3.00 ^{bcd} ± 1.00	6.400 ^c ± 0.547
Bread fortified 5% GS	3.800 ^{ab} ± 0.836	3.800 ^a ± 0.836	8.600 ^a ± 1.341	4.200 ^a ± 0.836	4.400 ^a ± 0.894	4.200 ^{ab} ± 1.090	4.00 ^{ab} ± 1.00	8.800 ^{ab} ± 1.090
Bread fortified 10% GS	3.400 ^{ab} ± 0.547	3.600 ^a ± 1.140	8.400 ^a ± 1.340	3.800 ^a ± 0.836	3.800 ^{ab} ± 0.836	4.200 ^{ab} ± 1.303	3.600 ^{abc} ± 0.894	8.400 ^b ± 0.547
Bread fortified 20% GS	3.00 ^{ab} ± 0.707	2.200 ^b ± 0.447	6.00 ^b ± 1.00	2.400 ^b ± 0.547	2.800 ^{bc} ± 0.836	2.800 ^c ± 0.836	2.600 ^{cd} ± 0.547	6.00 ^c ± 0.707
Bread fortified 5% mix. of FS&GS	4.00 ^a ± 1.00	3.800 ^a ± 1.095	8.600 ^a ± 1.140	4.00 ^a ± 0.700	4.400 ^a ± 0.547	4.200 ^{ab} ± 0.830	4.00 ^{ab} ± 0.707	8.800 ^{ab} ± 1.303
Bread fortified 10% mix. of FS&GS	3.600 ^{ab} ± 0.547	3.800 ^a ± 1.00	8.400 ^a ± 1.673	3.800 ^a ± 0.447	3.800 ^{ab} ± 1.095	4.200 ^{ab} ± 0.800	3.400 ^{abcd} ± 0.894	8.400 ^b ± 1.140
Bread fortified 20% mix. of FS&GS	2.800 ^b ± 0.836	2.200 ^b ± 0.447	5.600 ^b ± 0.547	2.200 ^b ± 0.440	2.400 ^c ± 0.547	2.400 ^c ± 0.547	2.400 ^d ± 0.547	5.600 ^c ± 0.547

Values are expressed as means ± SD.

Values at the same column with different letters are significant at P<0.05.

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Table (2): Chemical analysis of un-fortified and fortified bread with 10% flaxseeds, grape seeds and their combination(g/100g).

Samples Nutrients	Un-fortified Bread	Fortified bread with 10% flax seeds	Fortified bread with 10% grape seeds	Fortified bread with 10% flax and grape seeds
Moisture	6.4	9.7	7.7	9.7
Carbohydrate	82.08	77.29	79.82	77.66
Protein	10.6	10.4	10.6	10.6
Oils	0.19	1.67	0.47	0.90
Ash	0.53	0.6	0.79	0.72
Fiber	0.20	0.34	0.62	0.42

Table (3): Effect of diet containing fortified bread with flax seeds, grape seeds and their combination on serum glucose and liver enzymes of rats suffering from acute liver diseases.

Groups	Parameters Glucose mg/dl	AST	ALT	ALP
		U/l		
Control (-ve)	139.875 ^e ± 2.626	95.187 ^e ± 5.302	47.275 ^e ± 3.754	139.345 ^e ± 2.174
Control (+)	264.010 ^a ± 7.055	245.682 ^a ± 11.891	194.832 ^a ± 5.328	356.550 ^a ± 9.179
Bread fortified with 10% flax seeds	205.062 ^b ± 12.054	166.100 ^b ± 8.454	144.232 ^b ± 8.268	316.800 ^b ± 8.054
Bread fortified 10% grape seeds	186.925 ^c ± 11.054	149.300 ^c ± 2.771	104.650 ^c ± 8.978	252.650 ^c ± 6.639
Bread fortified 10% (flax and grape) seeds	157.555 ^d ± 5.404	125.232 ^d ± 4.878	70.527 ^d ± 6.257	155.550 ^d ± 8.961

Values are expressed as means ± SD.

Values at the same column with different letters are significant at P<0.05.

AST: Aspartate Amine Transaminase

ALT: Alanine Amine Transaminase

ALP: Alkaline Phosphatase

Table (4): Effect of diet containing fortified bread with flax seeds, grape seeds and their combination on lipid profile of rats suffering from acute liver diseases.

Parameters Groups	Ch	Tg	HDL-c	LDL-c	VLDL-
	mg/dl				
Control (-ve)	76.292 ^d ± 5.179	93.410 ^c ± 7.369	42.870 ^a ± 2.664	14.740 ^e ± 2.323	18.682 ^c ± 1.473
Control (+)	206.815 ^a ± 7.342	184.850 ^a ± 7.496	19.752 ^c ± 3.621	150.092 ^a ± 10.282	36.970 ^a ± 1.499
Bread fortified with 10% flax seeds	200.347 ^a ± 4.350	180.505 ^a ± 9.254	33.750 ^b ± 3.280	130.496 ^b ± 7.540	36.101 ^a ± 1.850
Bread fortified 10% grape seeds	175.280 ^b ± 9.785	153.166 ^b ± 8.269	36.122 ^b ± 0.978	108.524 ^c ± 10.951	30.633 ^b ± 1.653
Bread fortified 10% (flax and grape) seeds	105.850 ^c ± 9.525	98.137 ^c ± 6.726	43.860 ^a ± 2.280	42.362 ^d ± 6.934	19.627 ^c ± 1.345

Values are expressed as means ± SD.

Values at the same column with different letters are significant at P<0.05.

Ch: Cholesterol Tg: Triglycerides HDL-c: High density lipoprotein-Cholesterol
 LDL-c: Low density lipoprotein-Cholesterol VLDL-c: Very lowdensity lipoprotein-Cholesterol

Table (5): Effect of diet containing fortified bread with flax seeds, grape seeds and their combination on kidney functions of rats suffering from acute liver diseases.

Parameters Groups	Uric acid	Urea nitrogen	Creatinine
	mg/dl		
Control (-ve)	1.305 ^c ± 0.141	45.975 ^d ± 6.704	0.560 ^c ± 0.039
Control (+)	2.247 ^a ± 0.212	118.125 ^a ± 12.277	1.157 ^a ± 0.188
Bread fortified with 10% flax seeds	1.512 ^b ± 0.097	75.002 ^b ± 9.746	0.762 ^b ± 0.084
Bread fortified 10% grape seeds	1.557 ^b ± 0.082	65.580 ^{b c} ± 5.445	0.752 ^b ± 0.053
Bread fortified 10% (flax and grape) seeds	1.525 ^b ± 0.095	58.662 ^c ± 3.707	0.592 ^c ± 0.033

Values are expressed as means ± SD.

Values at the same column with different letters are significant at P<0.05

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Table(6): Effect of diet containing fortified bread with flax seeds, grape seeds and their combination on feed intake, body weight gain% and organs weight/body weight% of rats suffering from acute liver diseases.

Parameters Groups	Feed intake (g/day/each rat)	Body weight gain %	organs weight / body weight%	
			Liver	Kidney
Control (-ve)	17.327	36.241 ^a ± 5.615	2.890 ^c ± 0.323	0.591 ^c ± 0.066
Control (+)	16.00	19.417 ^c ± 2.565	3.906 ^a ± 0.105	0.970 ^a ± 0.104
Bread fortified with 10% flax seeds	16.432	24.381 ^b ± 1.969	3.310 ^b ± 0.047	0.708 ^b ± 0.097
Bread fortified 10% grape seeds	16.545	23.850 ^b ± 1.815	3.249 ^b ± 0.061	0.705 ^b ± 0.073
Bread fortified 10% (flax and grape) seeds	16.00	27.642 ^{bc} ± 2.142	3.136 ^b ± 0.030	0.665 ^{b c} ± 0.049

Values are expressed as means ± SD.

Values at the same column with different letters are significant at P<0.05.

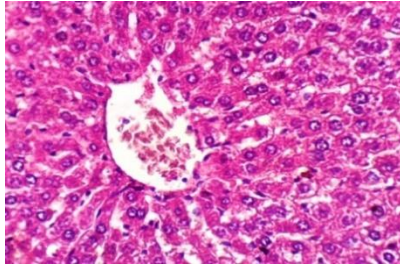


photo. (1):

Liver of rat from control negative group showing the normal histological structure of hepatic parenchyma (H & E X 400).

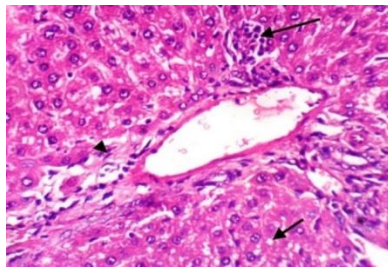


photo. (2):

Liver of rat from control positive group showing steatosis of hepatocytes (short arrow), focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration (long arrow) and fibroplasia in the portal triad (arrow head) (H & E X 400)

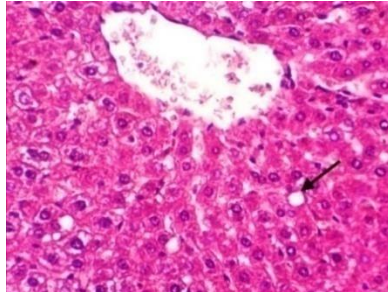


photo. (3):

Liver of rat from group fed on fortified bread with 10% flax seeds showing steatosis of sporadic hepatocytes (H & E X 400).

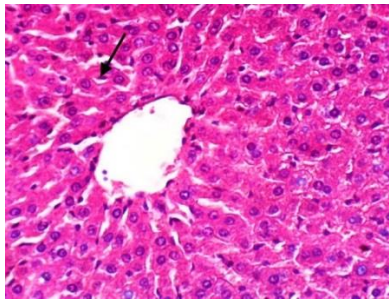


photo. (4):

of rat from group fed on fortified bread with 10% grape seeds showing slight activation of Kupffer cells (H & E X 400).

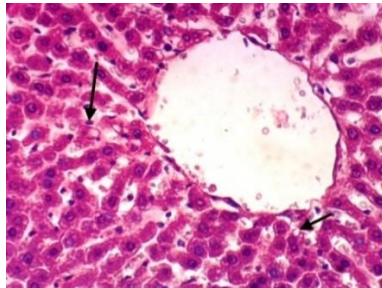


photo. (5):

Liver of rat from group fed on fortified bread with 10% mix of flax seed and grape seeds showing small vacuoles in the cytoplasm of sporadic hepatocytes (short arrow) and slight activation of Kupffer cells (long arrow) (H & E X 400).

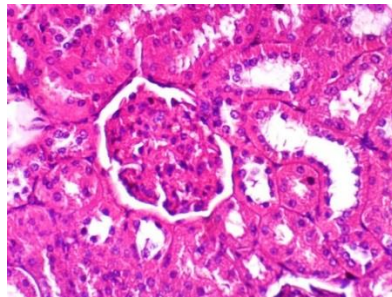


photo. (1):

Kidney of rat from control negative group showing the normal histological structure of renal tissue (H & E X 400).

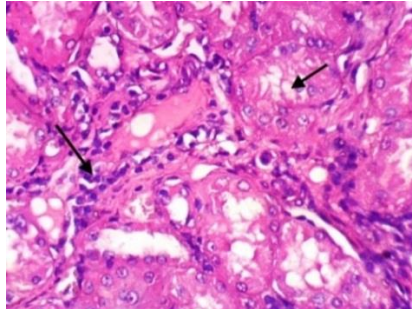


photo. (2):

Kidney of rat from control positive group showing vacuolar degeneration of epithelial lining renal tubules (short arrow) and perivascular inflammatory cells infiltration (long arrow) (H & E X 400).

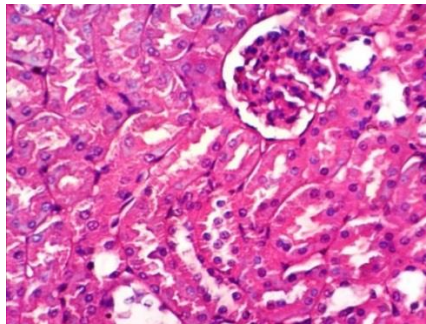


photo. (3):

Kidney of rat from group fed on fortified bread with 10% flax seeds showing no histopathological alterations(H & E X 400).

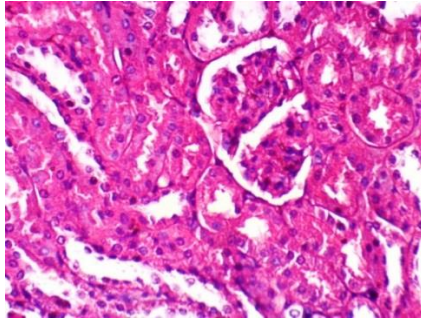


photo. (4):

Kidney of rat from group fed on fortified bread with 10% grape seeds showing no histopathological alterations(H & E X 400).

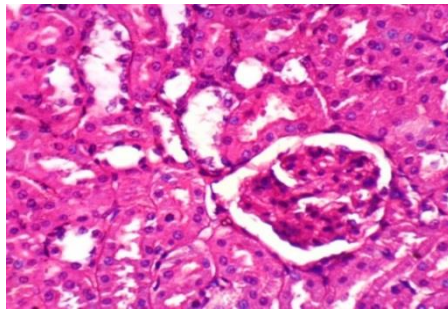


photo. (5):

Kidney of rat from group fed on fortified bread with 10% mix of flax seed and grape seeds showing no histopathological alterations(H & E X 400).

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التأثيرات الكيميائية والبيولوجية والهستوباثولوجية للخبز المدعم ببذور الكتان و العنب على أمراض الالتهاب الكبد الحاده فى الفئران

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الملخص العربى

أجريت هذه الدراسة لدراسة تأثير النظام الغذائي الذي يحتوي على الخبز المدعم بنسبة ١٠٪ من بذور الكتان وبذور العنب وخليطهم على الفئران التي تعاني من أمراض الكبد الحادة. تم استخدام مستويات مختلفة من بذور الكتان والعنب وخليطهم بنسب (٥ ، ٢٠ ، ١٠٪) في أعداد الخبز المختبر الذي خضع للتقييم الحسي. استخدمت أفضل عينة ١٠٪ من حيث التقييم الحسي وتم أيضا إجراء تحليل كميائي للخبز المدعم بهذه النسبة (١٠٪). استخدمت هذه الدراسة خمسة وعشرون جرذاً من ذكور الألبينو تم تقسيمهم بشكل عشوائي إلى خمس مجموعات تحتوى كل منها على خمس فئران. المجموعة الاولى تم تغذيتها على النظام الغذائي الاساسي كمجموعة ضابطة سالبة (الفئران الطبيعية) و تم حقن باقي الفئران الأخرى (٢٠ فأر) بواسطة رابع كلوريد الكربون (CCl₄) المعلقه بزيت البارافين بنسبة ٥٠ ٪ (حجم/حجم) وتم الحقن مرة واحدة بجرعه ٤ مللى /كجم من وزن الجسم لاحداث الاصابة بالتهاب الكبدى الحاد. تم تقسيم الفئران المحقونه الى اربع مجموعات فرعية كالتالى المجموعة الفرعية (١) تغذت على الغذاء الاساسي المحتوى على خبز غير مدعم كمجموعة ضابطة موجبة(المجموعة المصابة). وتمت تغذية المجموعات الفرعية ٣ و ٤ و ٥ بالغذاء الذي يحتوى على الخبز المدعم بنسبة ١٠٪ من بذور الكتان وبذور العنب وخليط منها ، على التوالي. وقد أظهرت النتائج أن المجموعات التي تغذت على خبز مدعم ب ١٠ ٪ بذور الكتان أو بذور العنب أو خليط منهما قد احدثت انخفاض بشكل ملحوظ في مستويات الكوليسترول الكلي ، الدهون الثلاثية ، الليبوبروتينات منخفضة الكثافة LDL-C ، نيتروجين اليوريا في السيرم ، حمض اليوريك ، الكرياتينين ، ووظائف الكبد (AST ، ALT ، ALP) ، الجلوكوز فى سيرم الدم هذا بالاضافه الى وزن الأعضاء / وزن الجسم. كما أظهرت النتائج حدوث ارتفاع فى النسبة المؤثرة للزيادة فى وزن الجسم و الليبوبروتينات مرتفعة الكثافة HDL-C عند مقارنتها بالمجموعة الضابطة الموجبة. وعدم وجود فروق معنوية تذكر بالنسبة للطعام المأخوذ بالنسبة

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للمجموعات المعالجة بالمقارنة بالمجموعة المصابة. وقد لوحظ تحسن جزئي في انسجة الكبد والكلى مقارنة بتلك المجموعة الضابطة الموجبة. أفضل النتائج البيوكيميائية والهستوباثولوجي للكبد والكلى التي كانت قريبه من النتائج الطبيعية كانت للمجموعة التي تغذت على الخبز المدعم ب ١٠% من خليط بذور الكتان و بذور العنب معا. في النهاية ، تشير النتائج الحالية إلى أن الاستخدام المنتظم من هذا المخلوط ١٠% (بذور الكتان و بذور العنب) له تأثير مفيد في تحسين وظائف الكبد والكلى وبعدها عمل وقائي ضد الاصابة بالتهاب الكبدى الحاد المحدث بأستخدام رابع كلوريد الكربون في الفئران.