



EFFECT OF RUSK CONTAINING SOME PLANT OILS ON HYPERCHOLESTEROLEMIC RATS

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ABSTRACT: Plant oils as sunflower, sesame and fenugreek oils has been successfully used in food technology. They have multi purpose for human health as safe and natural additives. Thus, this research was carried out to study the fatty acids composition of sunflower, sesame and fenugreek oils. Chemical composition and sensory evaluation of rusk containing these plant oils were studied. Also, Thirty-six male rats were divided into 6 groups to study the effect of feeding hypercholesterolemic rats, on rusk containing these plant oils, for six weeks on lipid profile, kidney functions, liver functions and histopathological examination of liver. The results showed that fenugreek oil had the highest total unsaturated fatty acids 86.19% followed by sesame and sunflower oils revealing 85.05% and 81.86%, respectively. Rusk product manufactured from studied oils at ratio 1:1:1 W:W:W (T3) had the highest content of moisture, crude protein, fat and ash. While, it had the lowest content of carbohydrate. Moreover, as a result of feeding hypercholesterolemic rats on rusk products fortified with plant oils caused a significant decrease in Triglyceride (TG), Total cholesterol (TC), Low density lipoprotein (LDL), Very low density lipoprotein (VLDL), LDL/HDL ratio and a significant increase in High density lipoprotein (HDL) after 6 weeks of feeding compared to the positive control group. The results indicated that liver enzymes and kidney functions of hypercholesterolemic rats were improved significantly as a result of feeding on rusk products. So, the group feeding on rusk product fortified with mixture of sunflower, sesame and fenugreek oils at ratio 1:1:1 W:W:W (T3) had the lowest levels of all these parameters compared to the positive control group. It could be recommended that adding plant oils as sunflower, sesame, fenugreek oils at ratio 1:1:1 W:W:W during the manufacture of Egyptian bakery (rusk) product can improve the health and nutritional benefits of this product and also, have positive effects on hypercholesterolemia.

Key words: Rusk, fatty acids, hypercholesterolemic, liver and kidney functions, lipid profile and histopathological examination.

INTRODUCTION

Plant oils have a great importance in food technology which affect the quality of products from a nutritional and functional perspective (Hedjazi *et al.*, 2011). Cardiovascular diseases cause the highest percentage of death in human beings and there is a relation between development of cardiovascular diseases and lipid metabolism which are affected by diet, especially the quantity and types of fatty acids. α -Linolenic acid and linoleic acid in oils may prevent

coronary heart diseases, hypertension and reduce cholesterol levels (Simopoulos, 2011).

Sunflower seeds oil (*Helianthus annuus*) is a source of unsaturated fatty acids and fat soluble vitamins. Sunflower oil is rich in linoleic acid, which is an essential n-6 polyunsaturated fatty acid. The biological effects of the n-6 fatty acids are mediated by their conversion to n-6 eicosanoids, n-6 prostaglandins and leukotrienes, which are hormones that act at different levels in human metabolism, especially on the inflammatory response (Simopoulos, 2002). So, the intake of

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polyunsaturated fatty acids has a great effect on levels of lipoprotein cholesterol in the body, which play an important effect on the incidence of cardiovascular disease (**Erkkilä *et al.*, 2008**). Sesame seed oil (*Sesamum indicum* L.) is extracted by pressing roasted seeds without further refining. Chinese, Korean and Japanese are using it in cooking because of its pleasant nutty flavour and a long shelf life and can be blended with others oils to improve their stability and shelf life. Moreover, it is highly resistant to oxidation and plays several medicinal effects (**Kochhar, 2000**). Fenugreek (*Trigonella foenum graecum*) is belong to the leguminous family and considered as medicinal herbs which used for more than 2500 years due to its food and medicinal properties. It is reported to be native to West Africa but now cultivated in Asia and Latin America (**Jiang *et al.*, 2007**; **Shi *et al.*, 2017**). The seeds and leaves have a healthy beneficial purposes as anti-diabetic (**Bahmani *et al.*, 2016**), anti-microbial (**Subhapiya and Gomathipriya, 2018**), anti-inflammation (**Tavakoly *et al.*, 2018**), anti-cancer (**El Bairi *et al.*, 2017**). Fenugreek seed oil is rich in omega-6 fatty acids (linoleic acid), which plays an important role in prevention of coronary heart diseases, inflammation and cancer (**Akbari *et al.*, 2018**). Fenugreek essential oil from the seeds play an important role in the control of cholesterol metabolism (**Strömgaard and Nakanishi, 2004**; **Tigrine-Kordjani *et al.*, 2011**).

Hypercholesterolemia is a risk factor for the development and progression of atherosclerosis and cardiovascular diseases. A high cholesterol diet is a major environmental contributor to unbalanced lipid metabolism and associated with an increase in prevalence of coronary heart disease (**Libby, 2008**). **WHO (2008)** reported that, cardiovascular disease (CVD) causes a major risk to worldwide deaths, from 17.1 million in 2004 to 23.4 million in 2030. CVD develops slowly due to long-term exposure to change in life style such as cigarette smoking, lack of exercise, constant stress, overweight and consuming diet with high saturated fat contents (**Yokoyama *et al.*, 2008**; **Neylon *et al.*, 2013**). So, **Gustafsson *et al.* (1994)** demonstrated that dietary oils has good effect on lipid peroxidation, which lead to favourable changes in the lipid status for hyperlipidemic patients.

Functional foods, the most natural and safest source of health ingredients which providing health benefits beyond basic nutrition and becoming popular in use for reducing the risk of CVDs (**Han *et al.*, 2018**). Recently, unsaturated fatty acids are considered as functional ingredients and nutraceuticals which it has antiatherogenic, antithrombotic, anti-inflammatory, antiarrhythmic, and hypolipidemic effect resulting in cardioprotective. Also, reducing the risk of serious diseases like cancer, osteoporosis, diabetes and other health promotion activities following from their complex influence on concentrations of lipoproteins, fluidity of biological membranes (**Mišurcová *et al.*, 2011**; **Mobraten *et al.*, 2013**). Rusk is a type of bakery product attributed with prolonged shelf-life beneficial in certain diets. Rusk is traditional hard in South Africans they love to dunk it in tea or coffee in the morning. (**Fliiopovic *et al.*, 1991**). So, the aim of this work was to study the fatty acids composition of sunflower, sesame and fenugreek oils, chemical composition and sensory evaluation of Egyptian bakery product (rusk) fortified with tested plant oils. The effect of feeding hypercholesterolemic rats on rusk products fortified with tested plants oils on lipid profile, liver and kidney functions were studied.

MATERIALS AND METHODS

Materials

Sesame oil (*Sesamum indicum* L.) and fenugreek oil (*Trigonella foenum-graecum*) were purchased from El-Captain Company (CAP PHARM) Al-Obour city, Kalubia Governorate, Egypt. While, sunflower oil (*Helianthus annuus*), wheat flour (72% extraction), sugar powder, baker's yeast, salt, corn oil, and starch were purchased from the local market at Zagazig city, Sharkia Governorate, Egypt.

Adult thirty six male albino rats, weighing 110-120 g, were purchased from breeding unit of Helwan Experimental Station.

Cholesterol powder and kits (total cholesterol, triglyceride and total high -density lipoprotein cholesterol (HDL-C), total protein, ALT, AST,

creatinine and urea) were purchased from Sigma-Aldrich (MO, IL USA). Reagents and chemicals used were of the highest purity.

Methods

Chemical Analyses

Fatty acids composition of plant oils

Saponification and esterification of different oils were performed according to **Hartman and Lago (1973)** and then analyzed by gas chromatography according to **AOAC (2005)**. The instrument used was HP6890 with flame ionization detector. Column was DB-23, 30m, 0.32 mm ID and 0.25 µm film thickness. Identification of the peaks was carried out by their retention time.

Preparation of rusk

Local Egyptian bakery product (rusk) were prepared by mixing all dry ingredients, 100 g of white flour with 0.5% baker's yeast, 1% salt, 30% sunflower oil and water as needed dough in a mixer (Ka 5ss, Kitchen Aid, st. Joseph, mt) at speed 2 at room temperature for 3 min. The dough was mixed well for about 2min at speed 2 and another 1min at speed 4, then the dough was covered by plastic sheet and allowed to rest for 5 min at room temperature. After that, the dough was cut manually to small round pieces. The dough cut in pieces 9 cm thickness. Control sample (C) rusk containing 30% sunflower oil, (T1) rusk containing 30% sesame oil, (T2) rusk containing 30% fenugreek oil, (T3) rusk containing sunflower, sesame and fenugreek oils at ratio 1:1:1 W:W:W. The dough was mixed well for about 5 min in mixer and was baked in the oven at 200°C, approximately for 10 min, until light brown colour according to standard **AACC (2002)** baking procedure. After cooling at ambient temperature, it was packed in low-density polyethylene bags until it was used for the analysis according to **Filipovic et al. (2012)**.

Chemical composition of rusk

Moisture, crude protein, fat, and ash contents of rusk fortified plant oils were determined by the standard procedures described in the **AOAC (2005)**. Total carbohydrates were calculated by difference according to the following equation:

$$\text{Total carbohydrates} = 100 - [\text{moisture (\%)} + \text{crude protein (\%)} + \text{crude fat (\%)} + \text{ash (\%)}].$$

Sensory evaluation for rusk

The sensory evaluation of rusk fortified with plant oils was done to determine the acceptability of the product. Rusk was evaluated for (appearance, colour, flavour, taste, texture and over all acceptability) by 15 panelists from the staff members of Food Science Department, Faculty of Agriculture, Zagazig University according to **Olaoye and Onilude (2008)**. The hedonic scale has 9 levels: the first four levels (1-4) show the positive sensations and the last four (6-9) show the negative sensations. The results are statistically analysed using multiple correspondence analyses.

Biological Experiment

Experimental animals

Thirty six healthy adult male albino rats weighting between 110-120 g, were housed in institutional experimental animal laboratory in Biochemistry Department, Faculty of Agriculture, Zagazig University, Egypt. The rats were kept in cages at room temperature. The rats were housed in stainless steel cages with screen bottom in a controlled environment with 12 hr., light and 12 hr., dark cycles. Water was available over period.

Experimental design

The rats were randomly classified into two main groups as follows: the 1st group contain 6 rats as the negative control group G1(-Ve) and fed on basal diet which prepared according to **Ain (1993)** along the period of experimental. The 2nd group (30 rats) were fed on high cholesterol diet which contain (5% animal fat + 1% cholesterol + 0.25% colic acid) for two weeks to induce hypercholesterdimia for rats. Then, hypercholesterolemic rats were divided into 5 groups (6 rats for each) as follows:

Group 1: (-Ve) the negative control group fed on basal diet.

Group 2: (+Ve) the positive control group (hypercholesterolemic rats) which fed on basal diet only.

Group 3: hypercholesterdemic group fed on rusk containing 30% sunflower oil (control rusk).

Group 4: hypercholesterdemic group fed on rusk containing 30% sesame oil.

Group5: hypercholesterdemic group fed on rusk containing 30% fenugreek oil.

Group6 (G6): hypercholesterdimia group fed on rusk containing mixture of sunflower, sesame and fenugreek oils at ratio 1: 1: 1 W:W:W.

The rusk products were added by 5% of basal diet.

Blood sampling and biochemical analysis

The rats were killed after overnight fasting. Blood samples were collected from the eye plexuses under diethyl ether anesthesia after 3, and 6 weeks from the start. The samples were collected in tubes 5 ml syringe by cardiac puncture and were centrifuged at 3000 rpm for 15 min to separate serum. The triglyceride was analyzed according to **Young (2001)**, total cholesterol was analyzed according to **Burtis (1999)**, HDL-Cholesterol was measured by enzymatic colorimetric method using Randox kits (**Tietz, 1995**). LDL-Cholesterol and VLDL-cholesterol were calculated by using the method of **Friedewald *et al.* (1972)** as follows: $VLDL-C = \text{Triglycerides}/5$.

$$LDL-C = \text{Total cholesterol} - (\text{HDL-C} + \text{VLDL-C})$$

The liver enzymes activities, alanine amino transferase (ALT), aspartate amino transferase (AST) and total protein were determined using kits (**Young, 2001**). Kidney functions was determined as serum creatinine and blood urea nitrogen by enzymatic colorimetric methods using commercial kits sigma-Aldrich (**Young, 2001**).

Histopathological Examination

Liver of the rat was taken immediately after sacrificing the rats and immersed in 10% buffered neutral formalin solution, the fixed specimens then trimmed, washed and dehydrated in bedded, in paraffin cut into sections of 4-6 microns thickness and stained with haematoxylin and Cosin stain according to **Suvarna *et al.* (2013)**. Histopathological studies were monitored by microscopic examination of

paraffin embedded slices of liver from rats. All sections are examined at 400X magnification using a light microscope in Faculty of Veterinary, Zagazig University, Egypt.

Statistical Analysis

The obtained results were statistically tested by analysis of multi variance ANOVA and discriminative test. ANOVA functions and Roy test both with 0.05 significance level were used as Unitarian statistical procedures to assess significant differences among means (**Steel and Torrie, 1980**).

RESULTS AND DISCUSSION

Fatty acids Composition of Plant Oils (%)

Fatty acids composition of sunflower, sesame and fenugreek oils are shown in Table 1. Results indicated that sunflower oil had the highest total saturated fatty acids 18.14% of total fatty acid. Whereas, fenugreek oil and sesame oil had the highest total unsaturated fatty acid amounted to be 86.19 and 85.05%, respectively. Sunflower oil had a high concentration of linoleic acid (54%), moderate amount of oleic acid (26.19%) and low amount of stearic acid (8.42%), palmitic acid (9.16%), arachidic acid (0.33%) and behenic acid (0.23%). Meanwhile, linolenic acid (0.62%), vaccenic acid (0.60%), gadolic acid (0.23%), alpha octadecatetraenoic (0.11%), and eicosaenoic acid (0.11%) were found in very low concentration. **Salas *et al.* (2011)** reported that sunflower oil contain, palmitic acid (5.0%), stearic acid (6.5%), oleic acid (36.6%), linoleic acid (51.0%), arachidic acid (0.3%) and behenic acid (0.6%). **Arshad and Amjad (2012)** showed that, sunflower oils have a negligible amount of saturated fatty acid (SFA), but more amount of linoleic acid and oleic acid. **Budryn *et al.* (2014)** revealed that, fatty acids composition for sunflower oil were 5.71% palmitic acid, 3.34% stearic acid, 24.06% oleic acid, 64.97% linoleic acid, 0.09% linolenic acid, 0.22% arachidic acid, 0.17% eicosaenoic acid and 0.75% behenic acid. **Orsavova *et al.* (2015)** found that, sunflower oil contain palmitic acid 6.2%, stearic acid 2.8%, oleic acid 28.0%, linoleic acid 62.2%, linolenic acid 0.16%, arachidic acid 0.21% and gadolic acid 0.18%.

Table 1. Composition of fatty acids in plant oils(%)

Fatty acid	Sunflower oil	Sesame oil	Fenugreek oil
Palmitic acid C16:0	9.16	9.11	8.54
Palmitolic acid C16:1	ND*	0.15	0.12
Stearic acid C18:0	8.42	5.15	4.48
Vaccinic acid C18:1	0.60	0.94	0.94
Oleic acid C18:1 ω 9	26.19	42.92	37.11
Linoleic acid C18:2 ω 6	54.00	39.70	45.40
Linolenic acid C18:3 ω 3	0.62	0.95	2.39
Alpha octadecatetra enoic acid C18:4 ω3	0.11	ND*	ND*
Arachidic acid C20:0	0.33	0.69	0.64
Eicosa enoic acid C20:1 ω11	0.11	ND*	ND*
Gadolic acid C20:1 ω9	0.23	0.39	0.23
Behenic acid C22:0	0.23	ND*	0.15
Saturated fatty acids	18.14	14.95	13.81
Unsaturated fatty acids	81.86	85.05	86.19

ND*: Not detected

Also, fatty acids content for sesame oil are cleared in the same Table which the oleic acid (42.92%) was the major fatty acid found in sesame oil followed by linoleic acid (39.70%), palmitic acid (9.11%), stearic acid (5.15%) and arachidic acid (0.69%). Meanwhile, linolenic acid (0.95%), vaccinic acid (0.94%), gadolic acid (0.39%) and palmitolic acid (0.15%) were found in very small concentrations. **Ahmad *et al.* (2006)** indicated that, myristic acid (0.61%), palmitic acid (12.3%), stearic acid (3.9%), oleic acid (43.2%), linoleic acid (42.6%), linolenic acid (0.27%) and arachidic acid (0.23%) were detected in sesame oil. Moreover, **Sowmya *et al.* (2009)** reported that, sesame oil was contained palmitic acid (10.8%), stearic acid (5.7%), oleic acid (43.0%) and linoleic acid (40.1%). Also, **Orsavova *et al.* (2015)** estimated that, sesame oil contain palmitic acid (9.7%), palmitolic acid (0.11%), stearic acid (6.5%), oleic acid (41.5%), linoleic acid (40.9%), linolenic acid (0.21%), arachidic acid (0.63%) and gadolic acid (0.32%).

Concerning fenugreek oil, it is characterised by a high concentration of linoleic acid (45.4%), moderate level of oleic acid (37.11%), low levels of palmitic acid, stearic acid, arachidic acid and behenic acid to be 8.54%, 4.48%, 0.64% and 0.15%, respectively. While, linolenic acid (2.39%), vaccinic acid (0.94%), gadolic acid (0.23%) and palmitolic acid (0.12%) were found in a small concentrations. Fatty acids concentration of fenugreek oil were affected by genetic factors and environmental conditions during fruit development and maturity (**Egan *et al.*, 1981**). **Ziwar (2010)** found that Fenugreek oil contain high amount of unsaturated fatty acid (83%), linoleic acid recorded the highest percentage (47%) followed by oleic acid (36%). While, total saturated fatty acids was 17% which contain palmitic acid (11%) and steric acid (6%). Also, **Ali *et al.* (2012)** reported that fenugreek oil contained high amount of linoleic acid (42.5%). While, linolenic acid, oleic acid and palmitic acid contents were found to be 18, 20 and 10.5%, respectively. Also, the oil contained small amounts of stearic acid (6.5%) and arachidic acid (2%).

Chemical Composition of Rusk containing Different Ratio of Plant Oils

Table 2 shows the proximate chemical composition of rusk prepared from plant oils (sunflower oil, fenugreek oil and sesame oil). It is observed that, rusk containing a mixture of sunflower, sesame and fenugreek oils (T3) had the highest content of moisture (2.58%) followed by control sample (C) 2.49% and (T1) 2.31%. Also, rusk containing a mixture of sunflower oil, sesame oil and fenugreek oil (T3) had the highest content of protein to be 9.6% followed by T2, T1 and control rusk product to be 8.6, 8.4 and 7.9%, respectively. Moreover, T3 and T2 had the highest content of crude fat and ash to be (26 and 28%) and (1.30 and 0.81%) for fat and ash, respectively. While, control rusk (C) and rusk product containing 30% sesame oil (T1) had the lowest content of crude fat and ash (22, 0.55%), respectively. On the other hand, control rusk (C), had high content of carbohydrates 67.06%, followed by T1 66.65%. While, T2 and T3 had the lowest content to be 60.45, and 60.52%, respectively.

It could be concluded that, rusk product containing a mixture of plant oils (T3) had the highest content of moisture, crude protein, fat and ash. While, it had the lowest content of carbohydrate. Meanwhile, the control rusk had the highest content of carbohydrate and the lowest contents of crude protein, crude fat and ash.

Ingale and Shrivastava (2011) indicated that sunflower seed contained 4.61% moisture, 36.85% fat, 25.08% crude protein, 27.76% carbohydrate and 4.82% ash. **Zebib *et al.* (2015)** revealed that the chemical composition of sesame (Egyptian varieties) contained 2.96% moisture, 52.7% crude oil, 26.23% crude proteins, 9.77% carbohydrate, and 5.83% ash. Also, **Mijena (2017)** found that the chemical composition of hulled sesame seeds contained 2.27% moisture, 52.22% crude fat, 27.0% crude proteins, 15.11% carbohydrate, and 3.4% ash. However, some differences in the composition may be due to environmental stress, climatic conditions, geographical, cultivation and harvesting practices. **Naidu *et al.* (2011)** found that sesame (Egyptian varieties) contain 11.44%

moisture, 6.71% fat, 27.57% crude proteins, and 3.9% ash. The obtained results agree with **Gupta, *et al.* (2011)**. **Shalaby *et al.* (2016)** showed that rusk products contain 28.05% fat, 0.55% ash and 11.30% crude proteins. But **Almasodi (2018)** found that control rusk products contained 3.80% moisture, 3.52% fat, 11.30% crude proteins and 83.81% carbohydrate and 0.55% ash.

Sensory Evaluation of Rusk containing Plant Oils

The sensory evaluation of food products play an important role in food choices. Hedonic testing is often used to determine consumers attitude towards the food by measuring a degree of acceptance of a new product or improving the existing food product (**Meilgard *et al.*, 1991; Poster, 1991**).

Sensory evaluation of rusk which contain different types and levels of oils are presented in Table 3. The results revealed that, there were significant differences ($p \leq 0.05$) in all sensory properties (appearance, colour, flavour, taste, texture and overall acceptability) of rusk samples compared with the control rusk sample (C). There were insignificant differences for colour score between control sample (C) and others rusk samples. On the other hand, there were a significant differences ($p \leq 0.05$) between control rusk sample and others samples for flavour, taste and texture which containing different ratio and types of tested oils caused a significant decrease. Generally, it could be concluded that, the rusk produced by particular replacement of sunflower oil with mixture of sunflower, sesame and fenugreek oils (T3) gave rusk more acceptable appearance, colour, flavour, taste, texture and acceptability (8.11, 7.91, 7.66, 7.54, 8.0 and 7.66), respectively rather than the rusk produced by added other ratio and type of oils. Decreasing the fat content or substituting fat with different components has a huge influence on the texture characteristics of biscuits (**Zoulias *et al.* 2002; Rodriguez-Garcia *et al.*, 2012**). Also, mixing different types of oils can moderate the properties of each oil and give the product more acceptability (**Abdulkarim *et al.*, 2010; Bakhtiary, 2014**). **Sowmya *et al.* (2009)** found that, cake produced

Table 2. Chemical composition of rusk containing different plant oils

Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)
C	2.49	7.9	22	0.55	67.06
T1	2.31	8.4	22	0.64	66.65
T2	2.14	8.6	28	0.81	60.45
T3	2.58	9.6	26	1.30	60.52

C : control rusk 30% sunflower oil T1: Rusk containing 30% sesame oil T2 : Rusk containing 30% fenugreek oil T3 : Rusk containing a mixture of sunflower, sesame and fenugreek oils at ratio 1:1:1

Table 3. Sensory evaluation of rusk containing different plant oils

Treatment	Appearance (9)	Colour (9)	Flavour (9)	Taste (9)	Texture (9)	Acceptability (9)
C	8.44 ^a ± 0.59	7.83 ^a ± 0.59	8.33 ^a ± 1.07	8.11 ^a ± 1.00	8.50 ^a ± 0.76	8.16 ^a ± 0.68
T1	7.61 ^{bc} ± 1.07	7.72 ^a ± 0.87	7.55 ^b ± 0.85	7.78 ^{ab} ± 0.72	8.11 ^a ± 1.02	7.77 ^{ab} ± 0.80
T2	7.91 ^{abc} ± 0.86	7.83 ^a ± 0.68	7.71 ^b ± 0.57	7.33 ^{bc} ± 0.84	8.09 ^a ± 0.89	7.71 ^{abc} ± 0.57
T3	8.11 ^{ab} ± 0.83	7.91 ^a ± 0.73	7.66 ^b ± 0.97	7.54 ^{abc} ± 0.93	8.00 ^{ab} ± 0.90	7.66 ^{abc} ± 1.02
LSD	0.60	0.51	0.59	0.58	0.62	0.55

Values with different letters in the same column or row are significantly different at (P < 0.05)

C : Control rusk 30% sunflower oil T1: Rusk containing 30% sesame oil

T2 : Rusk containing 30% fenugreek T3 : Rusk containing a mixture of sunflower, sesame and fenugreek oils at ratio 1:1:1

by replacement fat with 50% sesame oil gave cake better characteristics than the control cake with 100% fat. **Kaur et al. (2012)** found that, replaced oil with refined rice bran oil up to 50% level in the preparation of bread caused a significant improvement in baking quality of the product.

Results presented in Table 4 show that triglyceride, total cholesterol levels of the positive control group were significantly higher than that of the other experimental groups through the course of the experimental periods compared to the negative control group at zero time. Feeding hypercholesterolemic rats on rusk containing plant oils caused a significant (P ≤ 0.05) decrease of triglycerides and total cholesterol compared to the positive control group at 3 weeks. By increasing the period of feeding hypercholesterolemic rats fed on rusk products caused a high significant (P < 0.05) reduction in serum triglyceride and total cholesterol levels especially for G6 which fed on rusk containing mixture of sunflower, sesame

and fenugreek oils compared to the positive control group for triglyceride and total cholesterol. Rats fed on high cholesterol diet had high level of LDL (104.67 mg/dl), VLDL (27.94 mg/dl) and low level of HDL 35.29 mg/dl compared to the negative control group at zero time. Feeding hypercholesterolemic rats on rusk containing plant oils caused a significant (P ≤ 0.05) improvement in serum HDL levels for treated groups compared to the positive control group. Meanwhile, feeding hypercholesterolemic rats for 6 weeks caused a significant (P < 0.05) increase in HDL- cholesterol levels to be 52.94, 54.60, 54.03 and 57.08 mg/dl for G3, G4, G5 and G6, respectively compared to the positive control group (34.51 mg/dl) and a significant decrease in the levels of LDL and VLDL. While, feeding hypercholesterolemic rats on different rusk products caused a significant decrease in LDL/HDL ratio compared to the positive control group. Moreover, G6 had the lowest LDL/HDL ratio (0.85) and there were no significant differences between G4, G5 and G6 at the end of the experimental period.

Table 4. Effect of rusk containing plant oils on serum lipid profile of hypercholesterolemic rats

Feeding period (week)	Group	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	LDL/HDL Ratio (mg/dl)
Zero time	G1(-Ve)	105.60 ^f ± 3.00	141.85 ^{cd} ± 3.92	43.50 ^e ± 2.64	77.22 ^c ± 1.88	21.12 ^f ± 0.60	1.77 ^c ± 0.09
	G2(+Ve)	139.70 ^b ± 6.70	167.91 ^{ab} ± 3.27	35.29 ^f ± 0.67	104.67 ^{ab} ± 5.05	27.94 ^b ± 1.34	2.96 ^{ab} ± 0.18
3	G1	118.27 ^{de} ± 8.97	135.17 ^{efgh} ± 9.21	44.93 ^e ± 3.96	72.58 ^{cd} ± 7.64	23.65 ^{de} ± 1.79	1.63 ^{cd} ± 0.32
	G2	142.87 ^b ± 4.10	165.31 ^b ± 5.36	35.49 ^f ± 1.38	101.24 ^b ± 5.52	28.57 ^{ab} ± 0.82	2.86 ^b ± 0.24
	G3	120.67 ^{cde} ± 4.93	141.42 ^{cde} ± 1.56	49.44 ^{cd} ± 0.67	67.85 ^{de} ± 2.19	24.13 ^{cde} ± 0.98	1.37 ^{de} ± 0.06
	G4	128.20 ^c ± 1.90	138.49 ^{cdef} ± 2.46	51.01 ^{bcd} ± 1.93	61.90 ^{ef} ± 1.71	25.64 ^c ± 0.38	1.21 ^{ef} ± 0.05
	G5	127.47 ^c ± 3.05	136.02 ^{defg} ± 3.48	50.57 ^{bcd} ± 0.19	59.95 ^{fg} ± 4.15	25.49 ^c ± 0.61	1.18 ^{ef} ± 0.08
	G6	127.30 ^c ± 2.70	132.41 ^{fgh} ± 2.63	52.34 ^{abc} ± 1.79	54.81 ^{ghi} ± 2.51	25.46 ^{cd} ± 0.54	1.04 ^{fg} ± 0.08
6	G1	121.70 ^{cde} ± 5.90	143.08 ^c ± 2.61	47.00 ^{de} ± 6.31	68.40 ^d ± 1.61	24.34 ^{cde} ± 1.18	1.47 ^{de} ± 0.19
	G2	151.50 ^a ± 2.80	173.85 ^a ± 3.64	34.51 ^f ± 0.50	109.04 ^a ± 3.62	30.30 ^a ± 0.56	3.16 ^a ± 0.12
	G3	119.20 ^{de} ± 4.50	136.37 ^{defg} ± 1.68	52.94 ^{abc} ± 2.84	59.50 ^{fgh} ± 5.19	23.84 ^{cde} ± 0.90	1.12 ^{fg} ± 1.16
	G4	124.67 ^{cd} ± 5.13	134.37 ^{fgh} ± 5.04	54.60 ^{ab} ± 1.39	54.80 ^{ghi} ± 4.87	24.93 ^{cde} ± 1.02	1.00 ^{fg} ± 0.06
	G5	117.33 ^{de} ± 4.60	130.85 ^{gh} ± 1.12	54.03 ^{ab} ± 2.67	53.35 ^{hi} ± 2.92	23.46 ^e ± 0.92	0.99 ^{fg} ± 0.10
	G6	116.83 ^e ± 3.15	129.64 ^b ± 0.44	57.08 ^a ± 1.57	49.20 ⁱ ± 1.60	23.36 ^e ± 0.63	0.85 ^g ± 0.05
LSD		7.16	6.14	2.21	6.02	1.82	0.29

Values with different letters in the same column or row are significantly different ($P < 0.05$)

G1(-Ve): negative control rat fed on basal diet. G2(+Ve): positive control rat fed on high cholesterol diet G3: hypercholesterolemic rats fed on 5% rusk containing 30% sunflower oil G4: hypercholesterolemic rats fed on 5% rusk containing 30% sesame oil G5: hypercholesterolemic rats fed on 5% rusk containing 30% fenugreek oil G6: hypercholesterolemic rats fed on 5% rusk containing a mixture of sunflower, sesame and fenugreek oils at ratio 1:1:1

The obtained results are in line with **Hamden *et al.* (2011)** who indicated that administration of omega-3 with fenugreek terpenes caused a significant decrease in TG, TC, LDL and a significant increase in serum High-density lipoprotein (HDL) for diabetes rats which improve blood lipid profile. **Boulbaroud *et al.* (2012)** studied the effect of feeding ovariectomized (OVX) female wister rats on basal diet containing 10% sesame oil caused a significant ($P \leq 0.05$) decrease in TG, TC, LDL and a significant increase in serum high-density lipoprotein (HDL) compared with the positive control. Also, **Taha *et al.* (2014)** stated that treatment hyperlipidemia rats (i.p injection of Triton WR1339 at does 200mg/Kg /three times /week) by 5 and 10 % of sesame oil (SSO) for 4 weeks and found that adding 5% SSO has more significant effect than adding 10% SSO on lipid profile. Moreover, **Al-Ahdab (2015)** studied the

effect of sesame oil, *Nigella sativa* L oil and mixture of both at does (5 mg/kg b.wt.) for 6 weeks on lipid profile of hypercholesterolemic rats. Revealed that oral administration of sesame oil, *Nigella sativa* L oil and their mixture caused a significant decreased in serum levels of TC, TG, low density lipoproteins cholesterol (LDL-c), very low density lipoproteins cholesterol (VLDL-c) and a significant increase in serum high-density lipoprotein (HDL) compared with hypercholesterolemic rats.

The obtained results are matched with **RM *et al.* (2016)** who studied the effects of vegetable oils such as sunflower oil, coconut oil, palm oil, olive oil and vanaspati on lipid profile. Vegetable oils caused increase in total cholesterol except sunflower oil. caused a significant decrease in serum TG, LDL, VLDL increase in serum High-density lipoprotein (HDL) Even though HDL level was observed in coconut oil treated groups.

Also, **Duavy *et al.* (2017)** studied the effect of dietary supplementation with olive and sunflower oils by 12% on lipid profile of rats fed high cholesterol diet, 1%. Sunflower oils caused a significant decrease in serum triglycerides (TG), total cholesterol (TC) low-density lipoprotein (LDL) and a significant increase in serum high-density lipoprotein (HDL). Meanwhile, group treated with olive oil caused a better improvement when compared with other treated groups by sunflower oil.

El-Masry *et al.* (2018) reported that fenugreek seed powder has hypolipidemic effect when added to diet by 5% with improving atherogenic index and caused a significant decrease in TG, TC, LDL, VLDL and a significant increase in serum High-density lipoprotein (HDL) for diabetes rats which improve blood lipid profile.

Effect of Rusk Containing Plant Oils on Liver Functions

Liver play an essential role in regulating plasma lipid level through LDL clearance and HDL recruitment, while lipid uptake must affect the hepatic fat composition and thus burden the liver function (**Friis-liby *et al.*, 2004**). As shown in Table 5, the effect of administering rusk on serum levels of total protein, ALT and AST enzymes activities. Hypercholesterolemic rats (G2) was characterized by a significant increase in total protein to be 7.10 g/dl compared with normal group (G1) 6.77 g/dl at zero time. There wasn't a significant differences between G4 and G6 compared with G2 after three weeks of feeding on rusk products. While, after six weeks of feeding there was a significant increase in total protein for G3, G4, G5 and G6 compared with hypercholesterolemic rats (G2). Also, G2 rats were characterized by a significant increase in ALT and AST enzymes activity to be 52.33 U/L and 46.66 U/L compared to the negative control group to be 34.00 U/L at zero time. While, feeding hypercholesterolemic rats on rusk caused a significant decrease in ALT and AST enzymes activities for all treated groups G3, G4, G5 and G6 (25.00, 21.00, 18.33 and 18.33 U/L) and (19.33, 19.00, 19.33 and 18.00 U/L) respectively compared to the positive control group (60.33 U/L) after 6 weeks of

feeding. So, treatment hypercholesterolemic rats with rusk containing plant oils, caused a significant decrease in ALT and AST enzymes activities levels compared with the positive control group after 6 weeks of feeding.

Taha *et al.* (2014) showed that sesame oil caused a significant improvement in liver function which ALT and AST enzymes activity significantly decreased compared to the positive control group. **Al-Ahdab (2015)** found that sesame oil caused a significant decrease in ALT and AST levels compared with positive control group.

These results are in line with **Periasamy *et al.* (2014)** who found that treated rats by sesame oil (1 and 2 ml/kg) from 22nd to 28th day caused significant decrease in levels of serum AST and ALT. **Mbarki *et al.* (2017)** indicated that, feeding rats on basal diets supplemented with fenugreek seeds caused significant decrease in ALT, AST and total protein compared with control.

Effect of Rusk Containing Plant Oils on Kidney Functions

Results presented in Table 6 show that hypercholesterolemia rats group (+Ve) was accompanied by a significant increase in kidney function parameters compared to the negative control group and treated groups. Meanwhile, feeding hypercholesterolemic rats on rusk containing plant oils caused a significant ($P \leq 0.05$) decrease in urea levels to be 32.08, 30.49, 31.34 and 29.52 mg/dl for G3, G4, G5 and G6, respectively compared to the positive control group 42.22 mg/dl. Also, there was a significant decrease in creatinine levels for G3, G4, G5 and G6 to be 0.92, 0.84, 0.88 and 0.81 mg/dl compared to the positive control group (1.34 mg/dl) at 3 weeks of feeding. By increasing the period of feeding hypercholesterolemic rats fed on rusk products caused a high significant ($P < 0.01$) reduction in serum urea and creatinine levels especially for G6 which fed on rusk containing sunflower, sesame and fenugreek oils to be 28.33 mg/dl and 0.77 mg/dl, respectively compared to the positive control group (41.98 mg/d) and (1.30 mg/dl) for urea and creatinine levels, respectively.

Table 5. Effect of rusk containing plant oils on the liver functions of hypercholesterolemic rats

Feeding period (week)	Group	Total protein (g/dl)	ALT (U/L)	AST (U/L)
Zero time	G1(-Ve)	6.77 ^{de} ± 0.08	34.00 ^{cde} ± 5.00	27.00 ^c ± 4.00
	G2(+Ve)	7.10 ^a ± 0.01	52.33 ^b ± 4.50	46.66 ^{ab} ± 5.50
3	G1	6.78 ^{cde} ± 0.19	31.00 ^{cdef} ± 7.21	24.33 ^{cd} ± 2.30
	G2	7.00 ^{ab} ± 0.11	55.33 ^{ab} ± 2.88	45.33 ^b ± 12.09
	G3	6.94 ^{abcd} ± 0.05	35.66 ^c ± 5.77	24.33 ^{cd} ± 2.30
	G4	7.06 ^a ± 0.14	25.00 ^{fgh} ± 4.00	23.00 ^{cd} ± 4.00
	G5	6.80 ^{cde} ± 0.08	27.66 ^{defg} ± 2.30	25.66 ^{cd} ± 2.30
	G6	7.01 ^{ab} ± 0.01	27.66 ^{defg} ± 2.30	23.00 ^{cd} ± 0.00
6	G1	6.76 ^{de} ± 0.06	26.33 ^{efg} ± 2.30	27.00 ^c ± 4.00
	G2	6.72 ^e ± 0.06	60.33 ^a ± 7.63	54.33 ^a ± 4.04
	G3	6.93 ^{abcd} ± 0.11	25.00 ^{fgh} ± 4.00	19.33 ^{cd} ± 3.51
	G4	6.96 ^{abc} ± 0.20	21.00 ^{gh} ± 4.00	19.00 ^{cd} ± 0.00
	G5	6.86 ^{bcde} ± 0.05	18.33 ^h ± 2.30	19.33 ^{cd} ± 3.51
	G6	7.04 ^a ± 0.06	18.33 ^h ± 2.30	18.00 ^d ± 1.73
LSD		0.18	7.79	8.65

Values with different letters in the same column or row are significantly different (P< 0.05)

G1(-Ve): negative control rat fed on basal diet . G2(+Ve): positive control rat fed on high cholesterol diet G3: hypercholesterolemic rats fed on 5% rusk containing 30% sunflower oil G4: hypercholesterolemic rats fed on 5% rusk containing 30% sesame oil G5: hypercholesterolemic rats fed on 5% rusk containing 30% fenugreek oil G6:hypercholesterolemic rats fed on 5% rusk containing a mixture of sunflower, sesame and fenugreeks oils at ratio 1:1:1

Table 6. Effect of rusk products fortified with plant oils on the kidney functions of hypercholesterolemic rats

Feeding period (week)	Group	Urea (mg/dl)	Creatinine (mg/dl)
Zero time	G1(-Ve)	33.25 ^{cde} ± 2.10	1.03 ^{bc} ± 0.05
	G2(+Ve)	39.40 ^b ± 0.74	1.41 ^a ± 0.09
3	G1	34.03 ^{cd} ± 1.13	1.00 ^{bcd} ± 0.11
	G2	42.22 ^a ± 1.32	1.34 ^a ± 0.12
	G3	32.08 ^{def} ± 1.41	0.92 ^{cde} ± 0.05
	G4	30.49 ^{fgh} ± 0.66	0.84 ^{ef} ± 0.04
	G5	31.34 ^{efg} ± 0.39	0.88 ^{def} ± 0.09
	G6	29.52 ^{gh} ± 0.45	0.81 ^{ef} ± 0.03
6	G1	35.60 ^c ± 0.44	1.06 ^b ± 0.13
	G2	41.98 ^{ab} ± 2.63	1.30 ^a ± 0.10
	G3	29.93 ^{fgh} ± 0.32	0.88 ^{def} ± 0.06
	G4	29.17 ^{gh} ± 0.93	0.80 ^{ef} ± 0.01
	G5	29.75 ^{fgh} ± 0.87	0.80 ^{ef} ± 0.04
	G6	28.33 ^h ± 0.57	0.77 ^f ± 0.05
LSD		2.32	0.12

Values with different letters in the same column or row are significantly different (P< 0.05)

G1(-Ve): negative control rat fed on basal diet. G2(+Ve): positive control rat fed on high cholesterol diet G3: hypercholesterolemic rats fed on 5% rusk containing 30% sunflower oil G4: hypercholesterolemic rats fed on 5% rusk containing 30% sesame oil G5: hypercholesterolemic rats fed on 5% rusk containing 30% fenugreek oil G6:hypercholesterolemic rats fed on 5% containing a mixture of sunflower, sesame and fenugreeks oils at ratio 1:1:1.

Hamden *et al.* (2010) stated that fenugreek oil caused a significant decrease in urea and creatinine levels in treated groups compared with the positive control group. Also, **Periasamy *et al.* (2010)** and **Mbarki *et al.* (2017)** showed that sesame oil may enhance the ability of the kidney to remove these waste products from the blood by decreasing the levels of urea and creatinine.

Histopathological examination

Histopathological of the rat's liver tissues are shown in Photos 1, 2, 3, 4, 5 and 6.

It is observed in Photo 2 that liver sections showed moderate congestion of the portal blood vessels with mild biliary proliferation, lymphocytosis and round cells infiltration. The latter were seen aggregated interstitially most of the hepatocytes were apparently normal however a few cells showed cloudy swelling, hydropic degeneration and fatty changes. Photo 3 shows that liver sections showed congestion of portal blood vessels, biliary proliferation and focal interstitial round cells aggregations. Photo. 4 shows that liver sections showed mild congestion of the portal blood vessels, dilatation of the lymphatic and mild biliary proliferation. Moderate number of hepatocytes showed hydropic degeneration. Photo 5 shows that liver sections had congestion of hepatic blood vessels with apoptotic changes of some hepatocytes.

Examined sections from liver showed mild congestion of the portal blood vessels, biliary proliferation and round cells infiltration which aggregated interstitially in addition to hydropic degeneration of some hepatocytes (Photo 6). Consumption of sesame oil containing sesamol, sesamin and other lignans had anti-inflammatory effects (**Chavali and Forse, 1999; Chavali *et al.*, 2001**). Which, sesamol attenuated the recruitment of inflammatory cells, mast cells, CD68 (+) Kupffer cells, and neutrophils in liver injury (**Periasamy *et al.*, 2011**). Sesamol can reduce the recruitment of inflammatory cells by production of cytokine. In addition, sesamol can improve activation during systemic inflammation by reducing the transcription of pro-inflammatory cytokines that induces inflammation (**Hsu *et al.*, 2006; Hsu *et al.*, 2013**). Sesame oil significantly decreasing levels of leptin. Leptin have an adipocyte-derived cytokine, the main regulator of hepatic triglyceride content (**Fishman *et al.*, 2007**). So, these results are in agreement with **Periasamy *et al.* (2014)** who revealed that sesame oil caused a significant protection against fibrotic collagen. Also, sesame oil had protects against steatohepatic fibrosis by decreasing inflammatory cytokines, oxidative stress, , leptin and TGF- β 1. Also, sesame oil decreased proinflammatory cytokines that might attenuate the hepatic inflammation, steatosis, and eventually fibrosis.

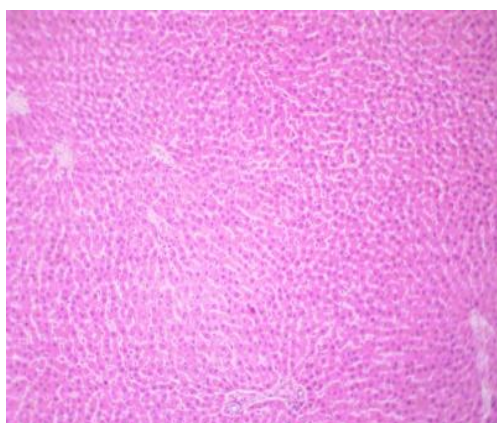


Photo. 1. G1(-Ve): Liver tissue of negative control group H and E (X 400)

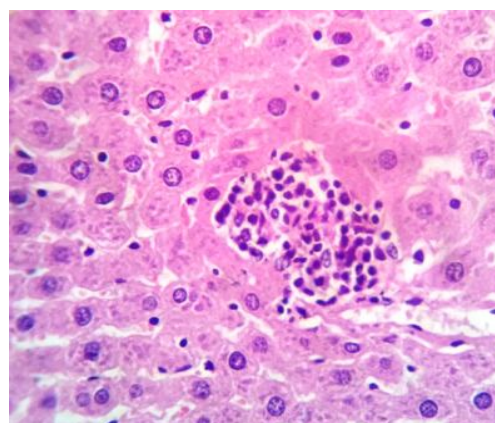


Photo.2. G2 (+Ve): Liver tissue of positive control group H and E (X 400)

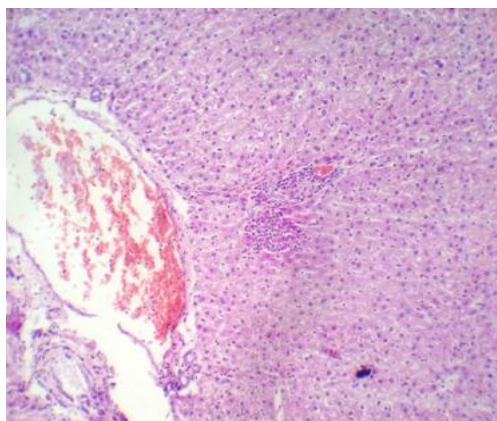


Photo 3. G3: Liver tissue of hypercholesterolemic rats fed on rusk containing 30% sunflower oil H and E (X 400)

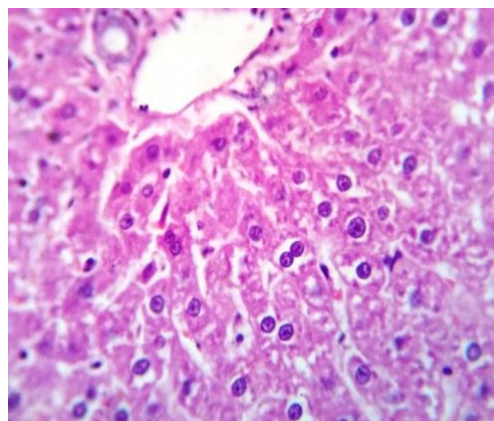


Photo 4. G4: Liver tissue of hypercholesterolemic rats fed on rusk containing 30% sesame oil H and E (X 400)

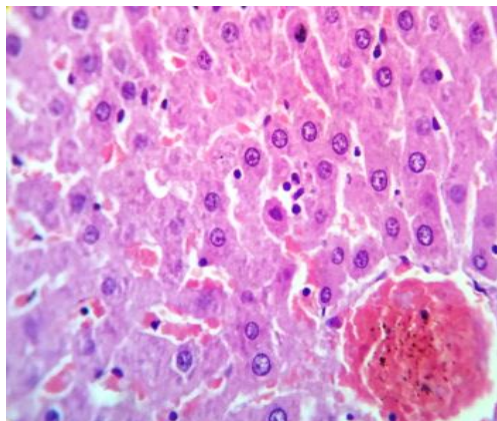


Photo 5. G4: Liver tissue of hypercholesterolemic rats fed on rusk containing 30% fenugreek oil H and E (X 400)

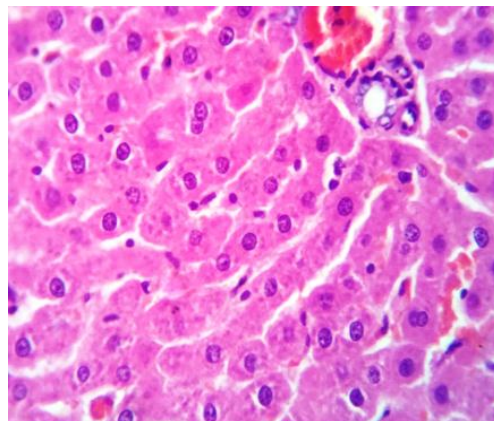


Photo 6. G6: Liver tissue of hypercholesterolemic rats fed on rusk which containing a mixture of sunflower, sesame, and fenugreek oils 1:1:1 H and E (X 400)

Mbarki *et al.* (2017) found that, feeding rats on diets supplemented by fenugreek seeds is significantly effective in protecting the liver.

Conclusion

The present study has demonstrated the potency of using plant oils as sunflower, sesame and fenugreek oils and their mixture as functional ingredients in bakery products (rusk) which caused significant improvement in lipid profile, liver and kidney functions for hypercholesterolemic rats. These findings provide a basis for the use of these plant oils and their mixture. Further, research must be done on the future on new plants oils with the high content of different bioactive compounds and

extended their applications in human diets, industrial instead of margarine and animal fats which may have induced health hazards and side effects for the human being.

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تأثير البقسماط المحتوي على بعض الزيوت النباتية على الفئران المصابة بارتفاع مستوى الكوليسترول

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تستخدم بعض الزيوت النباتية كزيت دوار الشمس، زيت السمسم وزيت الحلبة في عمليات التصنيع الغذائي وخاصة منتجات المخازن كإضافات طبيعية وآمنة بالإضافة لما لها من الفوائد الصحية العديدة. لذا تم إجراء هذا البحث بغرض استخدام كل من زيت دوار الشمس، زيت السمسم، زيت الحلبة وخليط منهم في تصنيع أحد منتجات المخازن (البقسماط) وتأثير ذلك على التركيب الكيميائي وكذلك الخواص الحسية للبقسماط بالإضافة الى تقدير محتوى هذه الزيوت من الأحماض الدهنية سواء المشبعة وغير المشبعة ومن ثم دراسة تأثير تغذية الفئران المصابة بارتفاع نسبة الكوليسترول في الدم على البقسماط المدعم بهذه الزيوت النباتية او خليط منهم لمدة ٦ اسابيع على مستويات دهون الدم وكذلك وظائف الكبد والكلية. وأوضحت النتائج ان زيت الحلبة سجل أعلى محتوى من الأحماض الدهنية غير المشبعة (٨٦.١٩%) يليه زيت السمسم ثم زيت دوار الشمس (٨٥.٠٥ و ٨١.٨٦%) على التوالي، ولوحظ أن البقسماط المصنع من خليط الزيوت النباتية بنسبة وزنية ١:١:١ سجل أعلى محتوى لكل من الرطوبة، البروتين، الدهون والرماد بينما اقل محتوى للكربوهيدرات مقارنة بالعينة الكنترول والمعاملات الأخرى. وكذلك لوحظ ان تغذية الفئران المصابة بارتفاع نسبة الكوليسترول في الدم على البقسماط المصنع من تلك الزيوت او خليط منهما ادي الى حدوث انخفاض معنوي لكل من الجليسيريدات الثلاثية، الكوليسترول الكلي، LDL، VLDL وكذلك نسبة LDL/HDL بالدم وكذلك حدوث ارتفاع معنوي لل HDL مقارنة بالعينة الضابطة الموجبة. هذا بالإضافة الى حدوث تحسن معنوي لكل من وظائف الكبد (البروتين الكلي، ALT و AST) ووظائف الكلى (اليوريا والكرياتينين) مقارنة بالمجموعة المصابة بفرط الكوليسترول. ولذلك توصي الدراسة بتصنيع البقسماط المدعم بكل من زيت عباد الشمس، زيت السمسم وزيت الحلبة بنسبة وزنية ١:١:١ حيث ان له تأثير إيجابي على خفض مستويات الكوليسترول بالدم.

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