

## Production of Functional Biscuits for Lowering Blood Lipids

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**Abstract:** Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but, also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers. In this regard, functional foods play an outstanding role. Therefore this study was designed to examine the effect of addition of some plant sources (e.g. barley, mustard, defatted mustard, flaxseed meal and flaxseed oil) as a source of active healthy components to prepare functional prebiotic biscuits for lowering blood lipids. Functional biscuits were formulated by replacing wheat flour in biscuit formula by different plant meals at 5, 10, 15, 20, 25 and 30% levels or shortening by flaxseed oil at 25, 50, 75 and 100% levels. Data of sensory evaluation revealed that biscuit samples with 10% defatted mustard meal (DMM), 15% mustard and flaxseed meals (MM and FM), 30% barley meal (BM) and 100% flaxseed oil (FO) were significantly acceptable as control. Biscuits made with 10% DMM and 15% MM contained 1.37 and 1.25 times more protein than the control. Biscuits supplemented with 30% BM and 15% FM contained 2.84 and 3.31 times more of total dietary fibers, respectively than the control.  $\beta$ -glucan content of BM-biscuits was 1.82%. Flaxseed oil-biscuits were particularly rich in alpha linolenic acid (42.76%) and it contained a less amount of linoleic acid (13.52%). The biological evaluation of hypercholesterolemic rats fed on diets supplemented with different functional prebiotic biscuits for 8 weeks significantly ( $p \leq 0.05$ ) reduced serum total cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins and ratios of total cholesterol/high density lipoproteins cholesterol, low density lipoproteins/high density lipoproteins cholesterol, atherogenic index and increased high density lipoproteins. Histopathological examination revealed that feeding of diets containing flaxseed oil, flaxseed and barley meals to the hypercholesterolemic rats lowered the degree of lesions of heart and liver tissues. Thus, it could be concluded that flaxseed oil, flaxseed meal or barley meal based bakery products could be developed as a useful therapy for hyperlipidemia in developing countries like Egypt.

**Key words:** Functional prebiotic biscuits • Plant sources • Sensory evaluation • Total cholesterol • Triglycerides • Low density lipoproteins • High density lipoproteins • Histopathological examination

### INTRODUCTION

Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia, is a major risk factor for the development of cardiovascular diseases. The search for new drugs able to reduce and/ or to regulate serum cholesterol and triacylglycerol levels has gained importance over the years, resulting in numerous reports on significant activities of natural agents [1]. Lipids in the

blood includes cholesterol low density lipoproteins and high density lipoproteins (LDL- and HDL-cholesterol) and triglycerides. People with diabetes and impaired glucose tolerance (IGT) are at a high risk of having too much LDL-cholesterol and triglycerides levels in their blood, as well as too little HDL-cholesterol, than in general population, putting them at a high risk of developing heart disease and circulation problems. High levels of LDL-cholesterol and triglycerides are usually treated with

a combination of healthy eating and increasing physical activity. If high levels persist, the doctor may prescribe medication [2].

Flaxseed and its components (flax meal, oil, lignan and secoisolariciresinol diglucoside) may improve cardiovascular health because of their numerous attributes. These components reduce the development of hypercholesterolemic atherosclerosis by 46%, 69%, 73% and 34%, respectively, in the rabbit model [3]. Bloedon *et al.* [4] reported that flaxseed is a rich source of alpha linolenic acid (ALA), fiber and lignan, making it a potentially attractive functional food for modulating cardiovascular risk. They study the effects of flaxseed on markers of cardiovascular risk in hypercholesterolemic adults. Compared to wheat, flaxseed significantly reduced LDL-c at 5 weeks (-13%,  $p < 0.005$ ), but not at 10 weeks (-7%,  $p = 0.07$ ) and reduced lipoprotein by a net of 14% ( $p = 0.02$ ). Eating barley-containing foods improved several cardiovascular risk factors. For example, Core [5] found that increasing whole grain foods in a healthy diet of men could reduce blood pressure. The diet with higher soluble fiber also had the greatest effect on reducing total and low-density lipoprotein (LDL) cholesterol levels, the so-called bad cholesterol. On average, total cholesterol was lowered 14 percent in men with previously high cholesterol levels after consuming the fiber-rich diets with low soluble fiber, 15 percent in those following diets with mid soluble fiber and 21 percent in those following diets with high soluble fiber. Barley appears to be more effective in lowering blood cholesterol than other whole grains like wheat and rice because of its high beta-glucan content [6-8]. Other study found that moderately hypercholesterolemic subjects who consumed 3 or 6 grams of beta-glucan from barley demonstrated decreased levels of total cholesterol. Whole-grain barley and dry milled barley products must contain at least 0.75 grams of soluble fiber per serving in order to make this health claim [9]. A research meta-analysis determined that each 10 grams increasing in total dietary fiber intake produced a 14% reduction in all coronary events and a 27% decrease in the risk of coronary death [10]. Ötles and Cagindi [11] reviewed that wheat, buckwheat, oat, barley, flaxseed, psyllium, brown rice, soy and products are notified the most common cereal based functional foods and nutraceuticals. These cereals and their beneficial effects improve the health, prevent and reduce the risk factor for several diseases. Other study showed that functional foods, such as soy protein and flaxseed, help

reduce the risk of cardiovascular disease and can easily be included in the diet. For example, flaxseed can be used to replace eggs in baking or cooking, which will add valuable  $\alpha$ -linolenic acid and soluble fiber that are important to consume since they have been found to reduce total cholesterol levels [12].

The aim of this study was to prepare and evaluate some grains and seeds meal and flaxseed oil as a source of active healthy nutrients, which has beneficial affects for lowering plasma cholesterol level, in the manufacture of some functional foods such as biscuits.

## MATERIALS AND METHODS

**Plant Material and Biscuits Preparation:** French soft wheat flour *Triticum aestivum* (72% extract) was obtained from Flourland mill Company, 6<sup>th</sup> October City, Giza, Egypt. Hull-less barley grain (*Hordium vulgare* L.) Giza 130, season 2010, was obtained from Barley Research Section, Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. Flaxseed (*Linum usitatissimum* L.) variety "Sakha 1" season 2010 was purchased from Fiber Crop Research Center, Agriculture Research Center, Giza, Egypt and Mustard seed (*Brassica alba*) season 2010, was obtained from Medical and Aromatic Plants Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. Flaxseed oil was prepared from flaxseed (*Linum usitatissimum* L.) variety "Sakha 1" season 2010 at Extract and Press Oil Unit, National Research Center, Dokki, Egypt. The seeds were cleaned manually to remove dust particles, damaged seeds, seeds of other crops and other impurities such as weeds and metals, then milled by a laboratory mill to get full fat barley, flaxseed and mustard meals. The resulted meals were packed in polyethylene bags and stored at  $-18^{\circ}\text{C} \pm 2$  until use. Defatted mustard meal was obtained using a solvent (n-hexan) extraction in Soxhlet apparatus for 18hr, then drying and grinding the cake, while flaxseed oil was extracted by hydrolyic cold-press.

Blends of wheat flour with barley, flaxseed, mustard and defatted mustard meals were prepared by substituting 5, 10, 15, 20, 25 and 30% of wheat flour by the above mentioned meals and were used for biscuits production, while biscuits with flaxseed oil were prepared by substituting 25, 50, 75 and 100% of shortening used in

biscuit formula with flaxseed oil. Biscuits were prepared according to the method of Sai Manohar and Haridas Rao [13], Wheat flour 300 g, Sugar 90 g, Shortening 60g, Ammonium bicarbonate 3 g, Sodium chloride 3 g, Sodium bicarbonate 1.2g and Baking powder 0.9 g. Sugar and fat were creamed at room temperature for 3-4 min in a mixer, sodium and ammonium bicarbonate and sodium chloride were dissolved in (60-67 ml water), added to the above cream and mixed for 5 min to obtain a homogenous cream. Wheat flour sieved twice with baking powder and added to above cream and mixed for 3 min. Biscuit dough was sheeted to a thickness of 3.5 mm, sheets were cut using circular shape (45 mm diameter), placed on a tray and baked at 160°C for 15 min, then allowed to cool for 1h. The biscuits were packaged in polyethylene bags and stored in air-tight containers at room temperature for further analysis.

**Chemicals:** Kits of total cholesterol, HDL-cholesterol, triglycerides and glucose were obtained from Stanbio Laboratory, 1261 North Main Street, Boerne, Texas 78006, USA. LDL-cholesterol was obtained from Quimica Clinica Aplicada S. A., A 7 Km 1081- P.O. Box 20- E 43870 Amposta, Spain. The chemical used including the solvents, were of analytical grade.

**Animals:** Forty eight female Wister albino rats weighing about (110±20 g) were obtained from Animal house, National Research Center, Cairo, Egypt. The animals were housed in aerated wire cages at 20-22°C ±1, 60 % relative humidity and 12 hours light and 12 hours dark conditions. All rats feed a commercial diet for a week as adaptation period, before starting the experiment for acclimatization. After this week, the rats were divided into 2 main groups and fed with their respective diet for 4 weeks:

- The first group (n= 6 rats) was fed on basal diet only, as a control negative.
- The second group (n= 42 rats) was fed for one month on hypercholesterolemic diet (basal diet containing (1% cholesterol, 0.2% bile salt and 0.2% choline chloride) to induce hypercholesterolemia [14].

After this period the second groups (hypercholesterolemic rats) were divided into 7 subgroups of six animals each as follows:

**Subgroup1:** Fed on hypercholesterolemic diet as a positive control group.

**Subgroup2:** Fed on hypercholesterolemic diet containing control biscuit.

**Subgroup3:** Fed on hypercholesterolemic diet containing barley biscuit.

**Subgroup4:** Fed on hypercholesterolemic diet containing mustard biscuit.

**Subgroup5:** Fed on hypercholesterolemic diet containing defatted mustard biscuit.

**Subgroup6:** Fed on hypercholesterolemic diet containing flaxseed biscuit.

**Subgroup7:** Fed on hypercholesterolemic diet containing flaxseed oil biscuit.

The experiment continued for 8 weeks.

**Samples Collection:** At the end of the experiment period, rats were fasted overnight, anesthetized with ether and blood samples were withdrawn from orbital plexus venous by using fine capillary glass tubes, placed in centrifuge tubes without anticoagulant and allowed to clot. Serum prepared by centrifugation (3000 rpm for 10 min) for measurement of some serum biochemical parameters.

**Chemical Analysis:** Moisture, crude protein, crude fat, crude fiber, ash and total dietary fibers were determined according to the methods described by the AOAC [15]. Soluble and insoluble dietary fibers were determined according to Asp *et al.* [16].  $\beta$ -glucan was determined according to the method described by Carr *et al.* [17]. The fatty acid methyl esters were analyzed by gas chromatography [18].

**Sensory Evaluation:** Biscuits produced were evaluated for their appearance, texture, color, flavor, mouthfeel and overall acceptability by 15 staff member according to the method described by Sudha *et al.* [19].

**Biochemical Analysis:** Biological Analysis: Serum glucose was determined by the method of Trinder [20], serum total cholesterol was assayed by the method of Allain *et al.* [21], serum HDL-cholesterol was determined according to Lopes-Virella *et al.* [22], serum triglycerides was determined as described by Wahlefeld [23].

**Histopathological Examination:** The animals were sacrificed and organs (heart and liver) were excised immediately and thoroughly washed with ice-cold

physiological saline then specimens from heart and liver tissues were fixed immediately in 10% neutral buffered formalin, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 6  $\mu\text{m}$  in thickness were prepared and stained with haematoxylin and eosin [24] and examined microscopically.

**Statistical Analysis:** Data were expressed as the mean values and standard error for three replicates and statistically analyzed by performing analysis of variance technique using the Statistical Analysis System [25]. Differences among means were compared using Duncan's Multiple Rang Test [26] at significant level 95% ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

**Proximate Chemicals Composition of Wheat Flour and Different Plant Sources:** Data in Table 1 showed that the highest value of protein was recorded for defatted mustard meal (48.01%) followed by mustard meal (30.55%) and flaxseed meal (23.76%), while barley meal and wheat flour had lower crude protein values being 11.18 and 9.12%, respectively. The fat content of flaxseed and mustard meals were found to be higher (43.21 and 32.09%, respectively) than that of barley, defatted mustard meals and wheat flour. It was observed that flaxseed, defatted mustard and mustard meals contained the highest crude fiber (10.09, 9.67 and 8.16%, respectively) and ash levels (4.10, 5.17 and 4.58% respectively), while the lowest values of crude fiber (0.19 and 1.29%) and ash (0.57 and 1.94%) were observed in wheat flour and barley meal, respectively. Moisture content and nitrogen free extract (NFE) were higher in wheat flour and barley meal compared with other different tested samples. The energy value (Kcal/100g) was ranged from 350.44 for defatted mustard meal to 559.29 for flaxseed meal. These results are approached with that obtained by Mentés *et al.* [27], Yalçın *et al.* [28], Lipilina and Ganji [29] and Khattab and Arntfield [30]. The dietary fiber (IDF, SDF and TDF) contents of the whole meals of different plant sources are shown in Fig. 1. Results in Fig. 1 indicated that flaxseed meal had higher content of TDF, SDF and IDF (28.51, 13.48 and 14.96 respectively) followed by defatted mustard and mustard seed meals and the less value for barley meal. The TDF, SDF and IDF contents of barley meal are in agreement with those previously reported by Yalçın *et al.* [28], Helm and Francisco [31], Flax Council of Canada [32] and Pennington Biomedical Research Center [33].

The total, soluble and insoluble  $\beta$ -glucan contents of barley meal are given in Fig. 2, whole barley meal contained 4.33, 2.98 and 1.53 of total, soluble and insoluble  $\beta$ -glucan respectively. Levels of  $\beta$ -glucan can vary dramatically between varieties, but usually range from 2 to 6% dry weight [34, 35]. Helm and Francisco [31] found that the range of total  $\beta$ -glucan for different hull-less barley varieties was from 3.70 to 5.77%. Fig. 3 show the fatty acid composition of the oil extracted from barley, mustard and flaxseed meals. According to the results shown in this Fig. nine fatty acids were identified in the barley oil. The fatty acids composition of the barley oil was characterized by linoleic, palmitic and oleic acids as a major fatty acids. Barley oil contained about 25.13% saturated fatty acids with palmitic being the principle saturated fatty acids (18.48%), followed by caprylic and stearic acid. The unsaturated fatty acids level was about (74.87%) of the total fatty acids, which linoleic acid comprising (44.97%) of the total amount followed by oleic (18.08%), lauroleic (6.13%) and linolenic (5.18%). Two minor fatty acids, lauric (C12:0) and myristic (C14:0) were identified in the barley oil and made up (1.53%) of the total fatty acids. Similar results are obtained by Osman *et al.* [36] and Qian *et al.* [37]. Results in same Fig. showed that the ratio of n-6 to n-3 fatty acids in barley oil was 8.36, these means that barley oil can be used as a good source of linoleic acid. The relatively high linoleic acid content makes the barley oil nutritional valuable for its effects on cardiovascular disease and cancer prevention [38]. As could be seen in Fig. 3 mustard seed oil was found to contain 95.70% of unsaturated fatty acids content, 67.12% of monounsaturated fatty acids and 28.58% of polyunsaturated fatty acids. In contrast, mustard oil contained relatively low amount of saturated fatty acid (4.30%). Similar results were obtained by Samman *et al.* [39] and Siemens [40]. Result in Fig. 3 showed that flaxseed oil contained low level of saturated fatty acids (11.35%) and a moderate amount of monounsaturated fatty acids (21.92%). Roughly 66.73% of the fatty acids in flaxseed oil were polyunsaturated fatty acids. Flaxseed oil was particularly rich in alpha-linolenic acid (50.51%) and contained a less amount of linoleic acid, an omega-6 fatty acid (15.61%). These results are in agreement with EL-Beltagi *et al.* [41] who found that fatty acid analysis of the flaxseed of the cultivars grown in Egypt showed 46-50% linolenic acid.

**Sensory Evaluation of Functional Biscuits:** The influence of substitution of wheat flour in biscuit formula with different levels of whole seeds and grains meal (i.e. 5, 10, 15, 20, 25 and 30%) on the sensory attributes

Table 1: Phesico-chemical characteristics of whole seeds and grains meal (% w/w, fresh weight)

Samples	Chemical composition#					Total carbohydrates (NFE)**	Value of energy Kcal/100g***
	Moisture	Protein*	Fat	Ash	Crude fiber		
Wheat flour	9.35± 0.09b	9.12± 0.20 d	2.18± 0.02c	0.57± 0.02e	0.19± 0.02d	87.94± 0.23a	407.86±0.08c
Barley meal	10.74± 0.06a	11.18± 0.22d	2.22± 0.06c	1.94± 0.01d	1.29± 0.11c	83.36± 0.04b	398.14±0.07d
Mustard meal	4.17± 0.02e	30.55± 1.22b	32.09± 0.70b	4.58± 0.02b	8.16± 0.49b	24.62± 1.23d	509.49±4.40b
Defatted mustard meal	6.34± 0.12c	48.01± 0.90a	1.96± 0.04c	5.17± 0.01a	9.67± 0.06a	35.19± 0.87c	350.44±0.72e
Flaxseed meal	4.68± 0.03d	23.76± 0.10c	43.21± 0.08a	4.10± 0.02c	10.09± 0.35a	18.84± 0.36e	559.29±1.14a

# Means of three replicates ± standard error, Means within the same column followed by the same letter(s) are not significantly different at P ≥ 0.05  
 \* Wheat flour (NX5.70); Barley, Flaxseed, Mustard and defatted mustard (NX6.25) \*\* NFE: Nitrogen Free Extract was calculated by difference.  
 \*\*\* (Protein X 4.0 + Fat X 9.0 + Carbohydrate X 4.0)

Table 2: Sensory evaluation of biscuits made from wheat flour blends with different meals.

Biscuits samples	Suppl. %	Appearance of surface (10)	Texture(20)	Color(10)	Color of crumb (10)	Flavor (20)	Taste (20)	Mouthfeel (10)	Overall acceptability(100)
Wheat flour									
biscuit (Control)	0	7.07±0.40ef	16.47±0.36cdefg	6.93±0.40de	7.53±0.35cd	16.00±0.59efg	16.07±0.53defg	7.53±0.36cd	77.60±2.31fghi
Barley biscuit	5	8.40±0.31abcd	16.73±0.52bcdef	8.53±0.29abc	8.80±0.20ab	17.67±0.37abcd	17.73±0.47abcd	8.47±0.24abc	86.33±1.98abcde
	10	8.80±0.24ab	17.13±0.54bcdef	8.80±0.30ab	8.73±0.28ab	17.73±0.38abcd	18.00±0.46abc	8.33±0.25abc	87.40±1.99abcd
	15	8.80±0.34ab	17.20±0.53abcde	8.93±0.27a	8.60±0.29ab	17.87±0.38abcd	18.20±0.43abc	8.53±0.19abc	88.27±1.86abcd
	20	9.00±0.34a	17.73±0.44abcd	8.86±0.26ab	8.60±0.29ab	18.13±0.32abc	18.20±0.45abc	8.60±0.21abc	89.13±1.64ab
	25	8.93±0.23a	18.07±0.41ab	8.93±0.25a	8.80±0.33ab	18.13±0.32abc	18.53±0.35ab	8.73±0.18ab	90.13±1.52a
	30	8.67±0.27ab	18.47±0.41a	8.80±0.33ab	8.33±0.39abc	18.40±0.36ab	18.73±0.40a	8.80±0.20ab	90.20±1.72a
Mustard biscuit									
	5	7.47±0.40df	16.80±0.38bcdef	7.80±0.31bcde	8.20±0.33abc	16.73±0.48bcdefg	16.87±0.27bcdefg	7.80±0.24bcd	81.67±1.61defg
	10	7.93±0.25abcde	16.67±0.41bcdef	8.20±0.24abc	8.73±0.25ab	17.07±0.43abcdef	17.00±0.41abcdef	8.20±0.24abc	83.80±1.48abcdef
	15	8.60±0.19abc	17.27±0.32abcde	8.93±0.27a	9.13±0.22a	17.60±0.24abcde	17.13±0.35abcdef	8.53±0.22abc	87.20±0.90abcd
	20	8.53±0.24abc	16.53±0.39bcdefg	8.80±0.22ab	8.93±0.25ab	16.40±0.40defg	15.13±0.56gh	7.73±0.34bcd	82.07±1.35cdefg
	25	8.27±0.40abcd	15.73±0.59efg	8.27±0.38abc	8.67±0.33ab	15.53±0.62gh	13.13±0.64ij	7.13±0.43d	76.33±1.75ghij
	30	8.00±0.51abcde	15.07±0.73g	7.80±0.50bcde	8.27±0.46abc	14.00±0.86h	11.80±0.94jk	6.80±0.54d	71.73±2.68ij
Defatted									
mustard biscuit									
	5	8.27±0.30abcd	17.07±0.49abcdef	8.60±0.25abc	8.67±0.29ab	17.60±0.51abcde	17.47±0.55abcdef	8.33±0.27abc	86.00±2.26abcde
	10	8.87±0.22ab	17.60±0.45abcd	8.93±0.18a	8.93±0.18ab	17.87±0.46abcd	17.67±0.44abcde	8.53±0.32abc	88.40±1.86abc
	15	7.93±0.25abcde	17.27±0.45abcde	8.40±0.19abc	8.27±0.23abc	16.87±0.58bcdef	15.67±0.48fg	8.27±0.32abc	82.67±2.11bcdefg
	20	7.20±0.37e	16.73±0.41bcdef	7.60±0.38cde	7.33±0.33cd	16.20±0.53defg	13.73±0.81hi	7.60±0.43cd	76.40±2.51ghij
	25	7.07±0.42ef	16.53±0.41bcdefg	7.00±0.43de	6.80±0.38d	15.53±0.62gh	12.20±0.85ijk	7.07±0.47d	72.20±2.99ij
	30	7.00±0.39ef	16.27±0.06defg	6.87±0.40e	6.60±0.35de	15.67±0.67fg	11.33±0.83k	7.07±0.43d	70.80±2.67j
Flaxseed biscuit									
	5	7.80±0.17bcde	16.87±0.35bcdef	8.13±0.19abc	8.13±0.22abc	18.20±0.28abc	18.07±0.30abc	8.27±0.25abc	85.47±1.20abcde
	10	7.60±0.19cde	17.60±0.29abcde	7.93±0.18abcd	7.93±0.22bc	18.40±0.27ab	18.33±0.33ab	9.07±0.18a	86.87±1.19abcd
	15	7.80±0.14cde	17.87±0.26abc	8.33±0.21abc	8.13±0.24abc	18.60±0.21a	18.67±0.29ab	9.27±0.15a	88.67±0.88abc
	20	6.93±0.28ef	16.40±0.54cdefg	6.80±0.20e	6.67±0.21de	17.47±0.42abcde	17.33±0.39abcdef	8.27±0.21abc	79.87±1.60efgh
	25	6.00±0.31g	15.60±0.65fg	5.67±0.30f	5.67±0.32f	16.60±0.65cdefg	16.40±0.59cdefg	7.53±0.29cd	73.47±2.37hij
	30	6.07±0.40fg	15.73±0.53efg	5.87±0.46f	5.87±0.43ef	16.93±0.62abcdef	15.87±0.65efg	6.80±0.48d	73.13±2.71ij

\*Different letters are significantly different (p ≤ 0.05) in columns for each group.

Table 3: Sensory evaluation of flaxseed oil biscuit\*

Biscuit samples	Flax oil Suppl. level %	Appearance of surface (10)	Texture(20)	Color(10)	Color of crumb (10)	Flavor(20)	Taste(20)	Mouthfeel(10)	Overall acceptability(100)
Wheat flour biscuit 100%									
shortening(control)	0	7.07±0.40b	16.47±0.36b	6.93±0.40b	7.53±0.35b	16.00±0.59b	16.07±0.53b	7.53±0.36b	77.60±2.31b
Flaxseed oil biscuit	25	8.13±0.26a	18.07±0.25a	8.53±0.26a	8.40±0.27a	17.67±0.35a	17.93±0.27a	8.53±0.24a	87.27±1.43a
	50	8.60±0.24a	18.00±0.24a	8.87±0.22a	8.87±0.22a	17.93±0.32a	18.07±0.25a	8.47±0.26a	88.40±1.30a
	75	8.73±0.21a	17.93±0.24a	9.07±0.18a	8.93±0.15a	18.07±0.32a	18.33±0.27a	8.73±0.23a	89.80±1.38a
	100	8.73±0.23a	18.13±0.56a	8.80±0.30a	8.87±0.26a	18.07±0.41a	18.40±0.34a	9.00±0.20a	90.00±1.94a

\*Different letters are significantly different (p ≤ 0.05) in columns for each group.

Table 4: Chemical composition of biscuit prepared from different composite meals (Dry weight basis %)

Biscuit samples	Chemical composition*					Total carbohydrate (NFE)**	Value of energy Kcal/100g***
	Moisture	Protein	Fat	Ash	Fiber		
Control biscuit(100% wheat flour)	3.71±0.18d	8.37±0.05 e	14.01±0.06bc	1.32±0.02 a	0.21± 0.02 d	76.09±0.00 a	463.93±0.30ab
Barley biscuit (30% barley meal)	4.23±0.11 b	9.30±0.38cd	14.62±0.17ab	1.49±0.01 c	0.43±0.02 c	74.15±0.27bc	465.38±0.82ab
Mustard biscuit (15% mustard meal)	3.90±0.29 c	10.48±0.48 b	14.96±0.06 a	1.61±0.01 b	0.83±0.00 a	72.15±0.45 e	465.16±0.44ab
Defatted mustard biscuit (10% defatted mustard meal)	3.92±0.29c	11.44±0.23 a	13.71±0.20 c	1.68±0.02 a	0.70±0.10ab	72.46±0.41de	458.99±1.14 c
Flaxseed biscuit (15% flaxseed meal)	4.56±0.05 a	10.01±0.05bc	14.42± 0.28ab	1.68±0.02 a	0.66±0.03 b	73.23±0.26cd	462.74±1.18 b
Flaxseed oil biscuit (100% flaxseed oil)	2.54±0.22 c	8.97±0.43 de	14.54±0.19ab	1.44±0.01 d	0.17±0.01 d	74.88±0.51b	466.26±0.76 a

\* Means within the same column followed by the same letter(s) are not significantly different at p ≥ 0.05 level. Means of three replicates± standard error  
 \*\* NFE: Nitrogen Free Extract was calculated by difference. \*\*\* (Protein X 4.0 + Fat X 9.0 + Carbohydrate X 4.0)

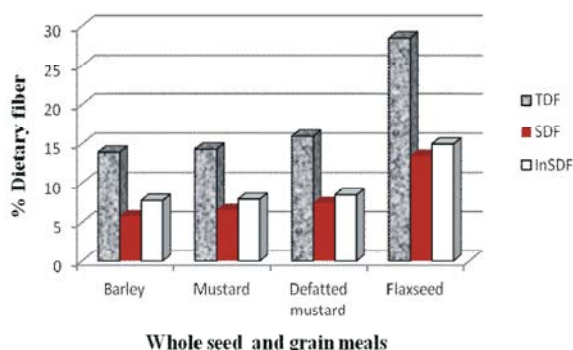


Fig. 1: Dietary fiber fractions (% dry basis) of whole seeds and grains meal

TDF : Total dietary fiber  
SDF: Soluble dietary fiber  
IDF: Insoluble dietary fiber

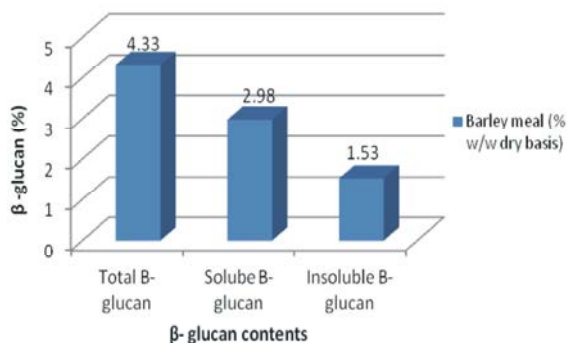


Fig. 2: beta-glucan fractions (% w/w dry basis) of hull-less barley meal

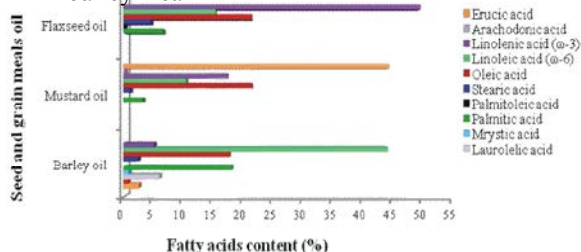


Fig. 3: Fatty acid composition of the oils extracted from barley, mustard and flaxseed meals

of the prepared biscuits was studied. Results of sensory evaluation in terms of appearance of surface, texture, color, crumb color, flavor, taste, mouthfeel and overall acceptability of biscuits samples supplemented with different levels of plant sources are presented in Table 2. It was observed that significant differences ( $p \leq 0.05$ ) were found between biscuits samples supplemented with 5, 10, 15, 20, 25 and 30% of barley meal

and the control sample for appearance of surface, color, crumb color, flavor, mouthfeel and overall acceptability. The results showed that as the barley meal ratio increased, all of sensory attributes scores increased, but this increasing was not significant. Biscuits containing 30% barley meal showed mean maximum overall acceptability of 90.2 compared to 77.6 for the control sample. In general, fortification of biscuits with barley meal (5 to 30%) significantly improved sensory quality attributes than control sample. Supplementation of wheat flour with 30% of hull-less barley meal had acceptable sensory attributes than control biscuits. Furthermore, it was observed that biscuits containing 10 and 15 % of defatted mustard, mustard and flaxseed meals recorded higher scores in most sensory attributes compared to the control sample.

The influence of replacing of shortening in biscuit formula with different levels of flaxseed oil (25, 50, 75 and 100%) on their sensory evaluation was studied (Table 3). No significant differences ( $p \geq 0.05$ ) in all sensory attributes were noticed between the biscuits containing different levels of flaxseed oils. However, a significant enhancement was observed in different sensory attributes of biscuits when flaxseed oil was used at different levels compared to the control sample. These results are in harmony with those of Zaghoul *et al.* [42] who found that the replacement of the palm oil used in making biscuits with oils from Borage, linseed and sesame seeds at (25, 50, 75 and 100% replacement level) up to 50% level did not negatively affect any of the studied sensory characteristics compared with the control sample, when the palm oil was completely replaced with any of the studied three oils, the produced biscuit was still highly accepted as judged by panelists. From the overall acceptability rating, it was concluded that defatted mustard meal, mustard meal, flaxseed meal and barley meal could be incorporated up to 10, 15 and 30% level, respectively, in the formulation of biscuits without affecting their sensory quality. Sensory evaluation of biscuits also revealed that flaxseed oil could substitute the shortening used in making biscuits up to 100% level without any significant difference compared with the control biscuit samples (Table 3).

**Chemical Composition of Biscuits:** The proximate chemical composition of biscuit samples prepared from different composite meal samples or flaxseed oil are given in Table 4. Results showed that the addition of 30% barley, 15% mustard, 10% defatted mustard and 15% flaxseed meals to the wheat flour by substitution resulted

in a considerable improvement in protein, ash and crude fiber of biscuit samples. Consistent with the normal characteristics of biscuits, their moisture content was generally below 5%. The low level of moisture is an important property of biscuits, which contributes to their keeping quality and crisp texture. Moisture content of biscuit samples in present study was ranged from 2.54 to 4.56%, the relatively higher moisture content in barley, mustard; defatted mustard and flaxseed biscuits could be due to protein and fiber contents of various plant meals which give high-water absorption, moisture-binding capacity as well as lubricity. These results are in agreement with those obtained by Hooda and Jood [43], Bashir *et al.* [44] and Tyagi *et al.* [45]. The typical initial moisture content of biscuit dough ranges from about 11 - 30%, comprising both water added at the dough mixing stage and water naturally occurring in the ingredients. Thermal processing reduces the final moisture content to 1- 5% in the final product [46].

It could be seen from the results in Table 4, the protein, ash, fat and crude fiber contents were increased in obtained functional biscuit samples by the substitution wheat flour with different tested plant meals. While the fat content of defatted mustard biscuits was decreased by 2.1% as compared to control sample and might due to the incorporation of defatted mustard meal as well as the replacement of 100% of shorting in with an equivalent percentage of flaxseed oil in the recipe of biscuit resulted in decrease in crude fiber content (0.17%) compared to the control sample (0.21%), since flaxseed oil did not consider source of fiber. These results are consistent with those obtained by Tyagi *et al.* [45], Zaib-un-Nisa [47], Shahzadi *et al.* [48], Olaoye *et al.* [49] and Vitali *et al.* [50]. Energy values of different biscuit samples ranged from 458.99 kcal/100g dry matter in defatted mustard fortified biscuit to 466.26 kcal/100g dry matter in flaxseed oil biscuit. The energy content of 100g of these prepared biscuits is quite high and constitutes more than one third of daily energy requirements (1000-1500 kcal/day) for a school age child as recommended by FDA [51].

Furthermore, all the dietary fiber constituents increased significantly ( $p \leq 0.05$ ) in biscuits containing barley, mustard, defatted mustard and flaxseed meals as shown in Fig. 4. In comparison to control sample, significant increase of total dietary fiber content was achieved by addition of 30% barley meal (183.85%), 15% mustard meal (69.57%), 10% defatted mustard meal (144.09%), 15% flaxseed meal (230.75%). Whereas, the use of flaxseed in the recipe of biscuit making, did not have impact on total dietary fiber of flaxseed oil biscuits.

These results are in agreement with those obtained by Vitali *et al.* [50] and Gambus *et al.* [52]. The same trend was also observed for the  $\beta$ -glucan content in barley biscuit samples (Fig. 5). The total  $\beta$ -glucan content of biscuit samples enriched with barley meal was 1.82%. The majority of  $\beta$ -glucan in tested sample was soluble (approximately 51.1%), whereas the insoluble fraction was constitute 48.9%. These results are in line with those of Škrbic *et al.* [53]. In addition, the unsaturated fatty acid level of barley meal biscuits was about 59.16% of the total fatty acids, while oleic acid comprising 32.37% of the total amount followed by linoleic acid (19.03%) and linolenic acid (1.80%) as compared to control biscuits (Fig. 6). The fatty acid profile of biscuits made from flaxseed meal or flaxseed oil characterized by high linolenic acid (11.41 and 42.47%, respectively). The linolenic acid is the most important polyunsaturated fatty acid and has major concern on health from nutritional point of view. Thus, fatty acid profile of wheat flour could be improved by its supplementation either with barley, mustard, defatted mustard, flaxseed meals or flaxseed oil. Similar results were obtained by Gambus *et al.* [52], Daun *et al.* [54] and Hussain [55].

#### **Nutritional Value of Functional Prebiotic Biscuits:**

Biscuits are a popular foodstuff consumed by a wide range of populations due to their varied taste, long shelf life and relatively low cost. Because of competition in the market and increased demand for healthy, natural and functional products, attempts are being made to improve biscuits' nutritive and functionality by modifying their nutritive composition. In the present study effort have been made by substitution either of wheat flour in the basic recipe either with barley, mustard, defatted mustard and flaxseed meal or with flaxseed oil to develop nutritionally rich functional biscuits. Results in Table 5 showed that the addition of 30% barley, 15% mustard, 10% defatted mustard and 15% flaxseed meals to the wheat flour by substitution resulted in a considerable improvement in protein, total dietary fibers,  $\beta$ -glucan,  $\alpha$ -linolenic acid and linoleic acid. Dietary Reference Intake for  $\beta$ -glucan have not been set but the Food and Drug Administration [51] recommended a daily intake of 3g barley  $\beta$ -glucan for recognized health benefits but only as a part of a diet low in saturated fats and cholesterol. A 100g portion of barley enriched biscuits could meet 60.67% of the level proposed by the FDA (Table 5). Škrbic *et al.* [53] found that a 300 g portion of white or whole grain bread made with barley flour increased the  $\beta$ -glucan in the final product and could meet 70% or 75% of the level proposed by the FDA, respectively.

Table 5: Contribution of macronutrients intakes to the relevant DRIs consuming an average portion (100g) of enriched biscuits [53].

Macronutrients	Gender	DRIs(g/day)	Contribution to DRIs (%)*					
			Wheat biscuits	Barley biscuits	Mustard biscuits	Defatted mustard biscuits	Flaxseed biscuits	Flaxseed oil
Proteins	Male	56	14.95	16.61	18.71	20.43	17.88	16.02
	Female	96	18.19	20.22	22.78	24.87	21.76	19.50
Carbohydrates	Adult	130	58.53	57.04	55.50	55.74	56.33	57.60
Energy (Kcal/ day)	Male	2900	15.99	16.05	16.04	15.83	15.96	16.08
	Female	2200	21.09	21.15	2.14	20.86	21.03	21.19
β-glucan	Adult	3**	-	60.67	-	-	-	-
Total dietary fibers	Male	38	8.47	24.05	14.37	20.68	28.03	9.29
	Female	25	12.88	36.56	21.84	31.44	42.60	14.12
α-linolenic acid (n-3)	Male	1.6	2.50	16.25	30.63	-	103.13	385.63
	Female	1.1	3.64	23.64	44.55	-	150.00	560.91
Linoleic acid (n-6)	Male	17	7.41	16.65	8.94	-	8.18	11.59
	Female	12	10.50	23.58	12.67	-	11.58	16.42

\* Dietary Reference Intakes (DRIs) for adults (males/females of 19-70 years of age) set by the Food and Nutrition Board of the National Research Council.

\*\* Value recommended by the (FDA, [50] for recognized health benefits).

Table 6: Serum lipid profile# of hypercholesterolemic rats fed on diets supplemented with different functional prebiotic biscuits for 8 weeks.

Parameter	Healthy group Control*(-ve)	Hypercholesterolemic group-Control*(+ve)	Hypercholesterolemic sub-groups***						Normal range
			Biscuit (100% Wheat flour)	Barley biscuit	Mustard biscuit	Defatted mustard biscuit	Flaxseed biscuit	Flaxseed oil biscuit	
Cholesterol (mg/dl)	158.41±0.47h	456.66±0.58a	235.50±0.19b	180.39±0.28f	186.37±0.13d	191.14±0.22c	183.92±0.18e	170.48±0.15g	< 200
Triglycerides (mg/dl)	121.66±0.63c	173.84±0.31a	140.84±0.25b	103.65±0.24e	103.89±0.65e	115.01±0.84d	102.67±0.62e	96.79±0.18f	> 60
LDL- c (mg/dl)	69.60±0.38h	199.46±0.36a	105.21±0.56b	86.68±0.44e	88.76±0.40d	93.97±0.38c	84.08±0.81f	74.79±0.52g	< 100
VLDL-c (mg/dl)	24.33±0.13c	34.77±0.06a	28.17±0.05b	20.73±0.05e	20.78±0.13e	23.00±0.17d	20.53±0.12e	19.34±0.04f	< 150
HDL- c (mg/dl)	68.35±0.65a	38.95±0.33g	41.43±0.33f	64.18±0.86c	56.12±0.75d	50.36±0.24e	64.37±0.39c	66.09±0.85b	> 45
Glucose (mg/dl)	77.74±0.21cd	96.84±0.36a	92.86±0.24ab	79.63±0.55cd	71.13±0.68d	74.21±0.47cd	81.88±0.74bcd	83.76±0.44bc	70-105
Total protein (g/dl)	6.93±0.19b	8.96±0.20a	7.50±0.04b	6.98±0.10b	7.03±0.21b	7.11±0.15b	7.16±0.22b	7.03±0.25b	6-8.2
TC/HDL-c	2.32±0.01g	11.72±0.01a	5.68±0.01b	2.81±0.01e	3.32±0.01d	3.79±0.00c	2.85±0.00e	2.57±0.01f	--
LDL/HDL-c	1.02±0.01g	5.12±0.00a	2.54±0.00b	1.35±0.00e	1.58±0.01d	1.87±0.00c	1.31±0.01f	1.13±0.00h	--
HTR%	43.15±0.37a	8.53±0.11h	17.59±0.17g	35.58±0.51c	30.11±0.41e	26.34±0.13f	34.99±0.23d	38.77±0.52b	--
Atherogenic index (AI)	1.32±0.01h	10.72±0.01a	4.68±0.01b	1.81±0.01e	2.32±0.01f	2.79±0.00c	1.86±0.00d	1.58±0.01g	--

#Means within the same row followed by the same letter(s) are not significantly different at (p > 0.05) level. Means of 6 replicates± standard error HTR% = (HDL-c / TC) X 100

AI = (TC - HDL-c) / HDL-c \*Control (-ve) fed on basal diet \*\*Control (+ve) fed on high-fat high-cholesterol diet VLDL-c= TG/5 [58] \*\*\*Hypercholesterolemic sub-groups: fed on cholesterol enriched diets supplemented with different functional prebiotic biscuits

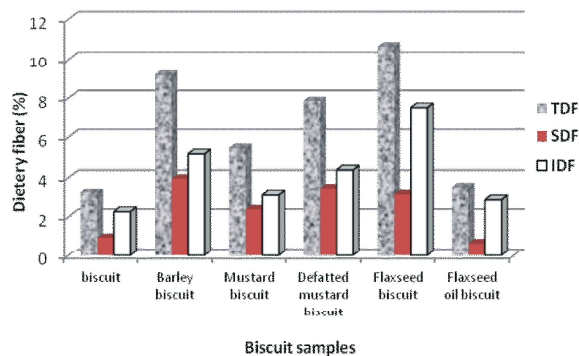


Fig. 4: Dietary fiber fractions (% dry basis) of biscuits prepared from wheat flour blends with different meals

TDF : Total dietary fiber  
SDF: Soluble dietary fiber  
IDF: Insoluble dietary fiber

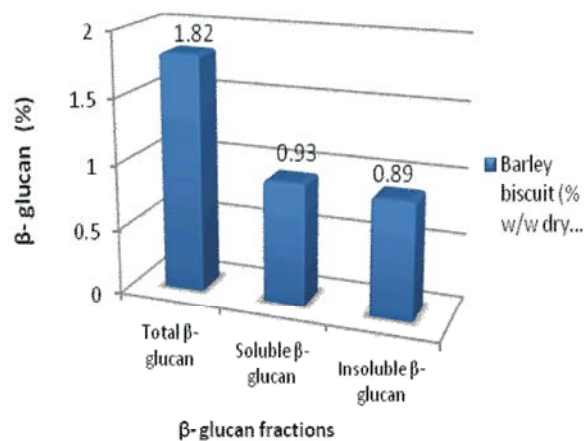


Fig. 5: β-glucan fractions of 30% barley-biscuits (% w/w dry basis)



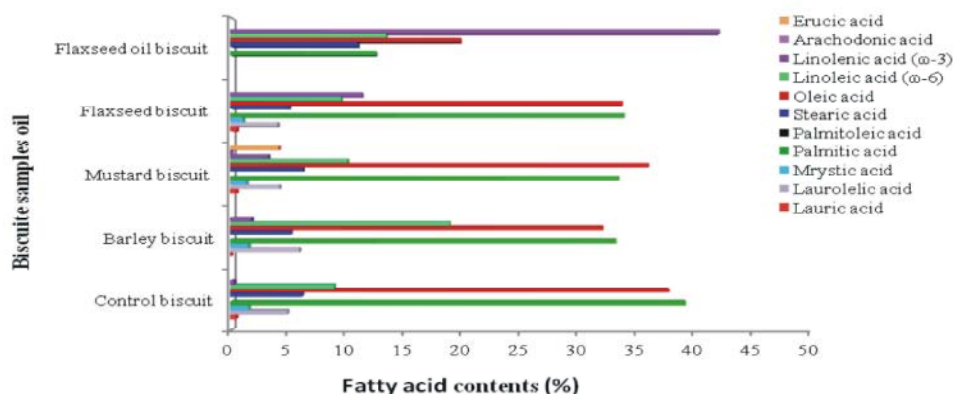


Fig. 6: Fatty acid composition of oils extracted from biscuits supplemented with barley, mustard, flaxseed meals and flaxseed oil

From the results above, it could be concluded that using different plant meal sources such as barley, flaxseed, mustard or defatted mustard as well as flaxseed oil in biscuit production, resulted in improving the nutritional and functional properties of the final product. These results are in accordance with those obtained by Lipilina and Ganji [29] and Škrbic *et al.* [53] and. Hence, development and consumption of such therapeutic bakery products would help to raise the nutritional status of the population.

**Biological Evaluation of the Functional Prebiotic Biscuits in Hypercholesterolemic Rats:** In the present study the effect of short-term consumption of high fat high cholesterol diet supplemented with 30% barley, 15% mustard, 10% defatted mustard, 15% flaxseed-enriched biscuits and biscuits prepared from 100% flaxseed oil compared with biscuits prepared from wheat flour (100% as control) on lipid profile was studied.

**Serum Lipid Profile:** The effect of feeding cholesterol-enriched diet (1% cholesterol, 0.2% choline chloride, 0.2 % bile salts) supplemented with different biscuit samples, to the hypercholesterolemic rats for 8 weeks on the serum lipid profile is presented in Table 6. Data revealed that reduction in total serum cholesterol level was higher in the group of rats fed on cholesterol-enriched diet with flaxseed oil biscuits (62.67%) followed by barley biscuits (60.49%), flaxseed biscuits (59.72%), mustard biscuits (59.19%) and defatted mustard biscuits (58.14%). While the lower reduction was obtained in the group of rats fed with wheat biscuits (48.43%) as compared with the group of hypercholesterolemic rats (positive control). This substantial reduction of serum cholesterol due to intake of diets based on barley,

flaxseed oil, flaxseed, mustard or defatted mustard might be attributed to the combined effect of fatty acids, dietary fibers and plant proteins present in barley, flaxseed or mustard meal. These results are in agreement with Gambus *et al.* [52] and Tzang *et al.* [56]. Compared with hypercholesterolemic diet, the cholesterol enriched diet supplemented either with barley, mustard, defatted mustard, flaxseed, flaxseed oil biscuits or biscuits prepared from wheat flour, resulted in a significant decrease ( $p \leq 0.05$ ) in serum triglycerides level. The more pronounced lowering effect of biscuit diets was observed in the group of rats fed on cholesterol-enriched diet supplemented with biscuits prepared from flaxseed oil followed by flaxseed meal, barley meal, mustard meal and defatted mustard meal, while the reduction in serum triglycerides level in hypercholesterolemic rats fed on diet with wheat biscuits was the lowest (18.98%) as compared with other examined groups, these results are in agreement with Gombus *et al.* [52] and Mohamed *et al.* [57].

The effect of feeding cholesterol-enriched diet supplemented with different biscuits samples to the hypercholesterolemic rats for 8 weeks, on low density lipoproteins cholesterol (LDL-c) and very density lipoproteins cholesterol (VLDL-c) and High density lipoproteins cholesterol (HDL-c) are given in Table (6). The results showed that flaxseed oil biscuits diet exhibited maximum decrease (62.5%) in serum LDL-c level followed by the flaxseed biscuits diet (57.85%). The diet prepared from cholesterol enriched diet supplemented with defatted mustard biscuits or wheat biscuits showed less reduction (52.89% and 47.25%, respectively) as compared with other biscuit diets. The results further substantiated that serum LDL-c reduction was more prominent in the rats groups fed on cholesterol enriched diets supplemented with

different functional prebiotic biscuits as compared to cholesterol enriched diet supplemented with wheat biscuits, similar results were also obtained by Queenan *et al.* [59]. The rats group fed on flaxseed oil biscuits diet showed the lowest content of serum VLDL-c level among rats groups fed on different diets as compared to high fat high cholesterol diet (Table 6). It could be seen from the same Table that serum HDL-c concentration increased significantly ( $p \leq 0.05$ ) due to the incorporation of either barley, mustard, defatted mustard, flaxseed or flaxseed oil in diets. It is evident from the results that rats group fed on cholesterol enriched diet supplemented with wheat biscuits possessed the lowest concentration (41.43 mg/dl) of serum HDL-c followed by the group of rats fed on cholesterol enriched diet supplemented with defatted mustard biscuits. Thus increase in HDL-cholesterol content of rats fed on biscuits enriched with flaxseed oil, flaxseed, barley, mustard or defatted mustard meal might be due to the effect of soluble fiber, protein, lignans combined by omega fatty acids [60-62].

Total cholesterol/ High density lipoproteins-cholesterol (TC/HDL-c), Low density lipoproteins-cholesterol/ High density lipoproteins-cholesterol (LDL/HDL-c) and High density lipoproteins-cholesterol/ Total cholesterol ratios (HTR %) are predictors of coronary risk [63]. The results presented in Table (6) also indicated that the rats groups fed on biscuits diets supplemented with flaxseed oil, flaxseed and barley meal had a significant lower ratios of TC/HDL-c and LDL/HDL-c when compared to high fat high cholesterol diet group (2.57, 2.85, 2.81; 1.13, 1.31, 1.35 vs. 11.72 and 5.12, respectively). While the rats groups fed on biscuits diets supplemented with mustard and defatted mustard meal showed TC/HDL-c ratios that characterized with low coronary risk (3.32 and 3.79, respectively). Meanwhile, feeding of wheat biscuits diet to the rats resulted in a TC/HDL-c ratio which approach to the ratio that characterized with middle coronary risk (5.68). The group of rats fed on flaxseed oil biscuits diet exhibited maximum increase in HTR (%) followed by the groups of rats fed on barley biscuits diet and flaxseed biscuits diet as could be seen in Table 6. Whereas, the group of rats fed on wheat biscuits diet showed lowest increase in the HTR (17.59%). It could be concluded that, the effect of plant meal biscuits diets on TC/HDL-c and LDL/HDL-c ratios and the HTR (%) in descending order was flaxseed oil biscuit diet > flaxseed biscuit diet = barley biscuits diet > mustard biscuit diet > defatted mustard diet > wheat biscuits diet. It could be seen from the obtained results that adding plant meal enriched biscuits to the diet could produce

significant reduction in LDL/HDL-c ratios and thus improve beneficial lipoprotein ratios to reduce the risk of heart disease in hypercholesterolemic individuals. These results are in harmony with those obtained by Kalra and Jood [60], Morise *et al.* [64] and Vijaimohan *et al.* [65].

Atherogenic index (AI) indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidney. The higher the AI, the higher is the risk of above organs for oxidative damage [66]. Atherogenic lipoprotein profile of plasma is an important risk factor for coronary artery disease (CAD). It is characterized by high ratio of low-density lipoprotein cholesterol (LDL-c) to high-density lipoprotein cholesterol (HDL-c) and increased level of triglycerides (TGs) [67]. Predominance in plasma of small dense LDL and small VLDL particles is associated with an increased risk of CAD while large HDL particles are associated with decreased risk [68, 69]. The results revealed that the rats group fed on flaxseed oil biscuits diet showed a maximum lower atherogenic index value (1.58) followed by the rats group fed on barley biscuits diet (1.81) and the rats of group fed on flaxseed biscuits diet (1.86) as compared to the group of rats fed on high fat high cholesterol diet (10.72). These results are in accordance with those obtained by Makni *et al.* [1].

It could be concluded from the present study that the reduction in serum total cholesterol, triglycerides, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol might be due to synergistic effect of dietary fiber and omega-3, omega-6 and omega-9 fatty acids present in full fat flaxseed, barley and mustard meal-enriched biscuits, while in the case of defatted mustard meal biscuits it might be due to the synergistic effect of dietary fiber, protein and /or other components, which could help to reduce the risk of cardiovascular disease among the masses who will consume barley, mustard, defatted mustard, flaxseed or flaxseed oil biscuits. Feeding of cholesterol enriched diets supplemented with different plant sources biscuits to the hypercholesterolemic rats, resulted in a significant lower in the serum glucose concentration ranging from 71.13 to 83.76 mg/dl as compared to the group of rats (96.84 mg/dl) fed on high fat high cholesterol diet (positive control). In contrast, feeding of cholesterol enriched diet supplemented with wheat biscuits to the hypercholesterolemic rats did not affect the serum glucose concentration, whereas, no significant difference was apparent in the serum glucose concentration between the positive control group (96.84 mg/dl) and the group of rats fed on wheat biscuits

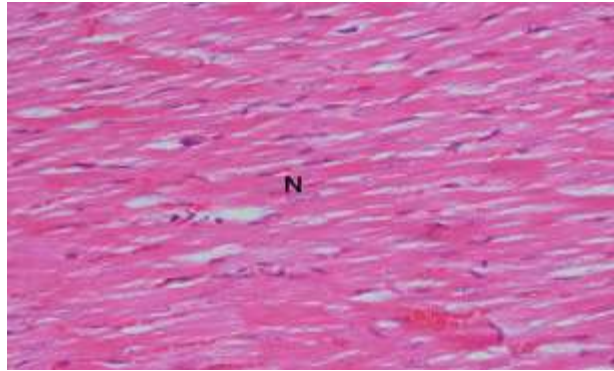


Fig. 7: (A-H) Histopathological changes of heart tissue. Photomicrographs of heart tissue sections from normal, hypercholesterolemic and treated rats. (A): Section of heart tissue from a control rat showing the normal structure of the cardiac muscle fibers, each fiber appeared cylindrical, elongated fiber with oval large vesicular nucleus (N). Little amount of connective tissue was present between the muscle fibers contained the blood capillaries. (Hx. & E. X 100).

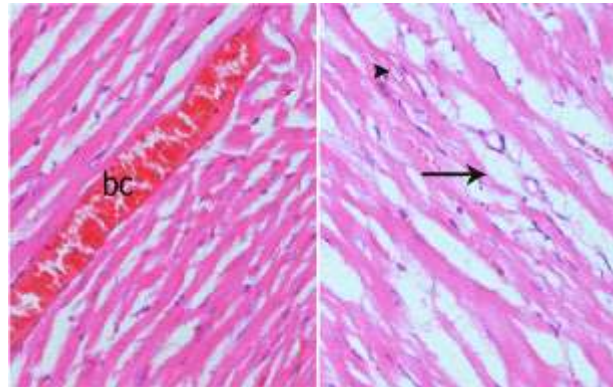


Fig. 7: (B). Section of heart tissue from rats feeding of high fat high cholesterol diet to the rats led to severe damage to the heart tissue, on the left side of the figure marked dilatation and congestion of blood capillaries (bc) with atrophy (thinning) of the muscle fibers. On the right side of the figure, deposition of fat is observed in the form of fatty infiltration in between the muscle fibers (arrow). The nuclei of the muscle fibers become more flattened (arrow head). (Hx. & E. X 100).

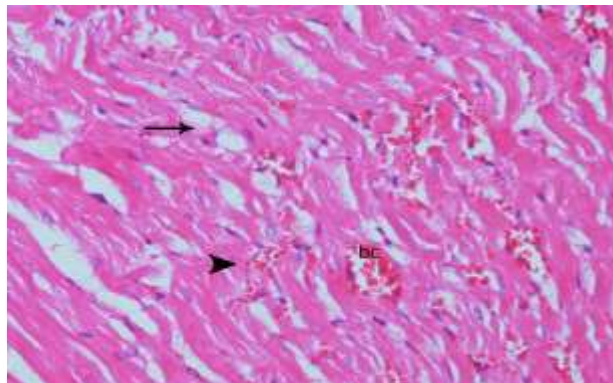


Fig. 7: (C). Section of heart tissue from rats feeding on diet based on biscuits prepared from wheat showed a slight decrease in blood vessels dilatation and congestion (bc) and in fatty infiltration (arrow). Beside small areas of hemorrhage could be seen in between the muscle fibers (arrow head). Many of the cardiac muscle fibers appeared wavy devoting a decrease in their contractile efficiency. (Hx. & E. X 100)

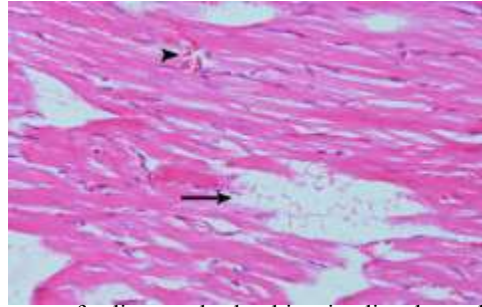


Fig. 7: (D). Section of heart tissue from rats feeding on barley biscuits diet showed that fatty infiltration (arrow) was present as well as small foci of hemorrhage (arrow head).(Hx. & E. X 100)

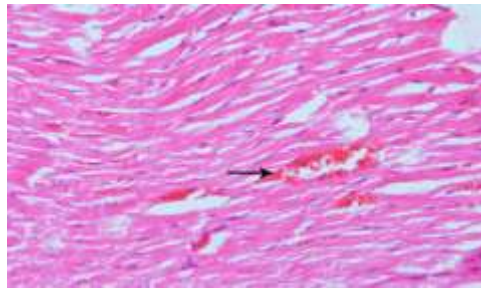


Fig. 7: (E). Section of heart tissue from rats feeding on diets based on biscuits prepared from mustard meal showed that the fatty infiltration is greatly reduced, while the blood capillaries are still dilated and congested (arrow). (Hx. & E. X 100)

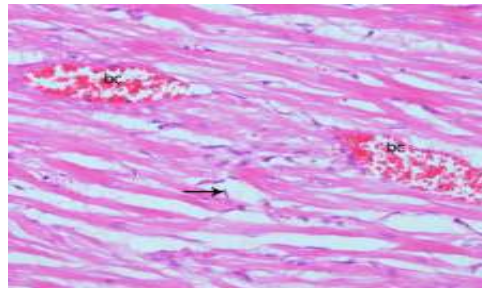


Fig. 7: (F). Section of heart tissue from rats feeding on diets based on biscuits prepared from defatted mustard meal showed a poor treating/ protective effect as blood capillaries was still dilated and congested (bc) also fatty infiltration was only markedly observed (arrow) between the muscle fibers in the group of rats fed defatted mustard biscuits diet. (Hx. & E. X 100)

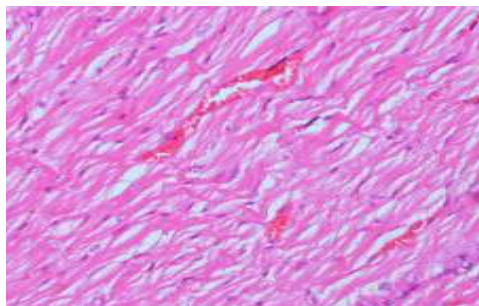


Fig. 7: (G). Section of heart tissue from rats feeding on diet based on biscuits prepared from flaxseed meal revealed a good improving effect as the fatty infiltration was not observed and the dilatation with congestion in blood capillaries were greatly reduced. However, waviness of cardiac muscle fibers was still noticeably present.(Hx. & E. X 100).

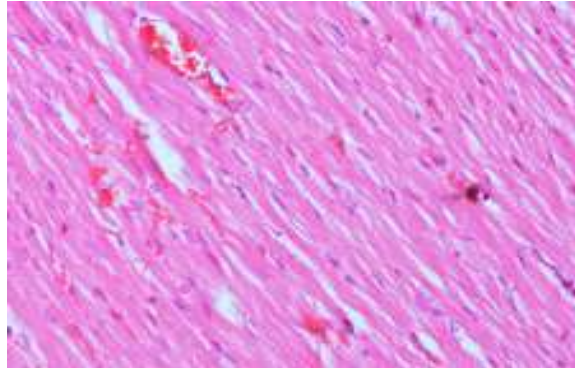


Fig. 7: (H). Section of heart tissue from rats feeding on diet based on biscuits prepared from flaxseed oil. Whereas, flaxseed oil biscuits diet showed a very good ameliorating effect as there was great diminution in blood capillaries dilatation and congestion, as well as no fatty infiltration was observed and the cardiac muscle fibers appeared quit normal. (Hx. & E. X 100).

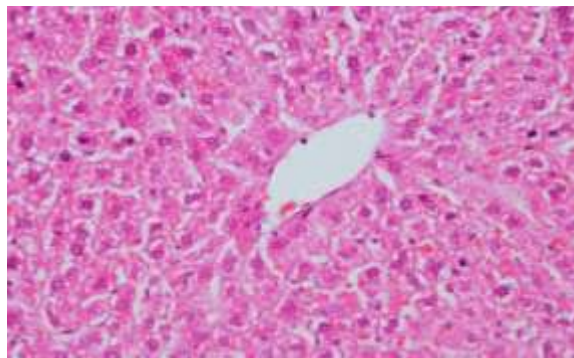


Fig. 8: (A-H) Histopathological changes of liver tissue. Photomicrographs of liver tissue sections from normal, hypercholesterolemic and treated rats. (A): Section of liver tissue from a control rat showing normal appearance of liver tissue, where the hepatic cells are arranged in cords radiating from the central vein forming the classical lobule. Hepatocytes are polygonal in shape with large vesicular rounded central nucleus. The cards of hepatocytes that one of thickness of 1-2 cells and separated from each other by equal-sized blood sinusoids. The lumens of these sinusoids certain blood cells and nuclei of kupffer cells. (Hx. & E. X 200).

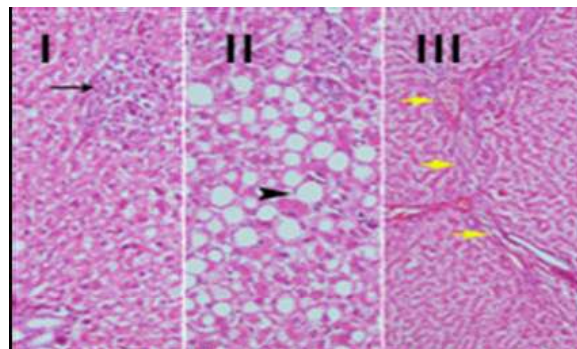


Fig. 8: (B) Section of liver tissue of rats feeding of high fat high cholesterol diet to the rats resulted in significant morphological changes in the liver tissue where same samples showed focal monocellular infiltration (black arrow) accompanied with vacuolar degeneration in most of the cells (BI). Other samples showed massive number of fat vacuolar (arrow head) in the cytoplasm of hepatocytes with diffuse monocellular infiltration in between the cells (BII). Others showed portal vein dilatation and congestion with fibrosis extending between the cells together with dilatation of the blood sinusoids between the hepatocytes (yellow arrows), (BIII). (Hx. & E. X 100 & 50).

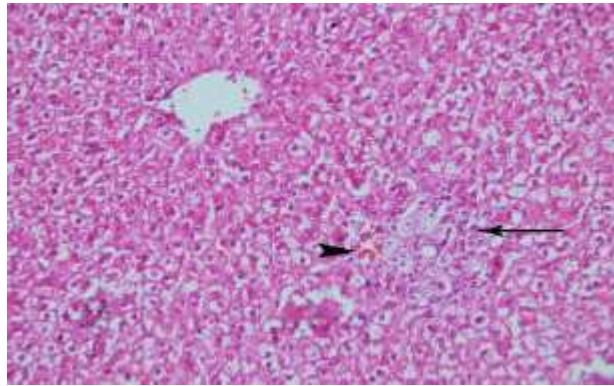


Fig. 8: (C) Section of liver tissue of rats feeding of cholesterol enriched diets supplemented with wheat biscuits to the rats showed also maximal alterations in the liver tissue as compared with other groups fed different biscuit samples. Liver section showed focal area of degeneration (arrow) and hemorrhage (arrow head). Most of the liver was still suffering from vacuolar degeneration denoting a poor ameliorating effect of the diet prepared from wheat biscuits. (Hx. & E. X 100).

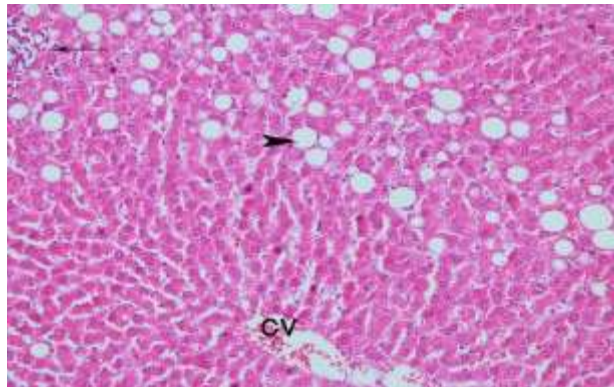


Fig. 8: (D) Section of liver tissue of rats feeding of diet based on biscuits prepared from barley meal showed a well improving effect, whereas the congestion of the blood vessels (CV) was decreased and the fatty degeneration was also restricted to the periphery of the lobules (arrow head). Although small focal areas of monocellular infiltration (arrow) were present. (Hx. & E. X 100).

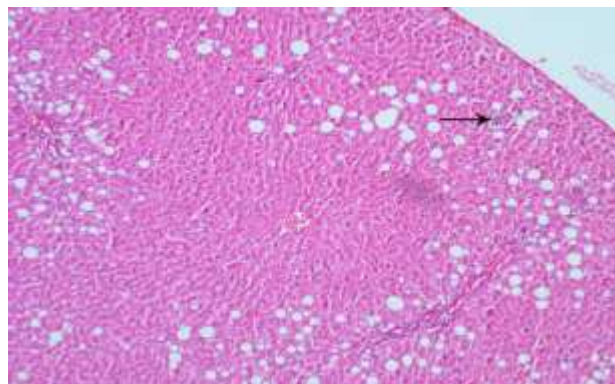


Fig. 8: (E) Section of liver tissue of rats feeding of cholesterol enriched diet supplemented with mustard biscuits to the rats resulted in a mild ameliorating effect as fatty degeneration was noticed at the periphery of the lobules with focal areas of degeneration (arrow). In addition, fibrosis with mild dilatation and congestion of blood vessels at the portal areas were noticed (Hx. & E. X 50).

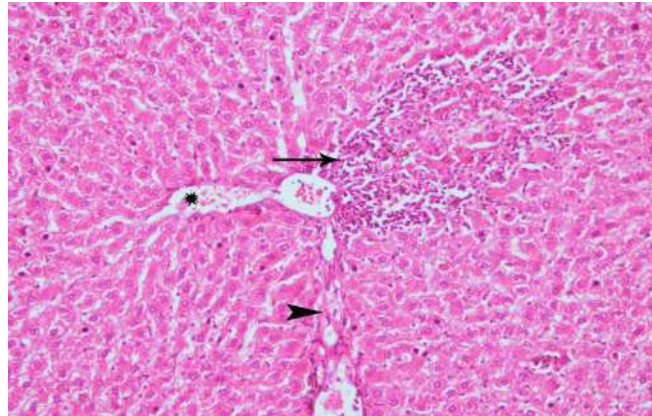


Fig. 8: (F) Section of liver tissue of rats feeding of cholesterol enriched diet supplemented with defatted mustard biscuits to the rats resulted in a good improving effect of the diet. Most of the hepatocytes appear normal except a focal area of degenerating cells that is surrounded with local infiltration (arrow). A very fine fibrosis between the cells (arrow head) and mild dilatation of blood sinusoids (\*) are observed. (Hx. & E. X 100).

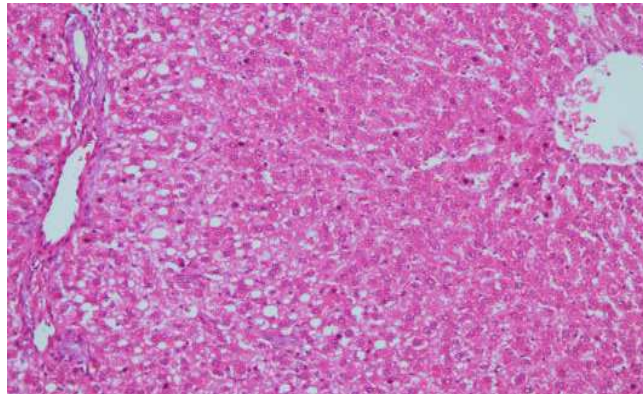


Fig. 8: (G) Section of liver tissue of rats feeding of diet based on biscuits prepared from flaxseed meal showed a good ameliorating effect of the flaxseed biscuit as a mild degree of fatty degeneration is still noticed at the periphery of the lobules. Dilatation of portal vein with fibrosis around it is observed at the portal area with dilatation of central vein. Many hepatocytes show pyknotic (deeply stained) (Hx. & E. X 50)

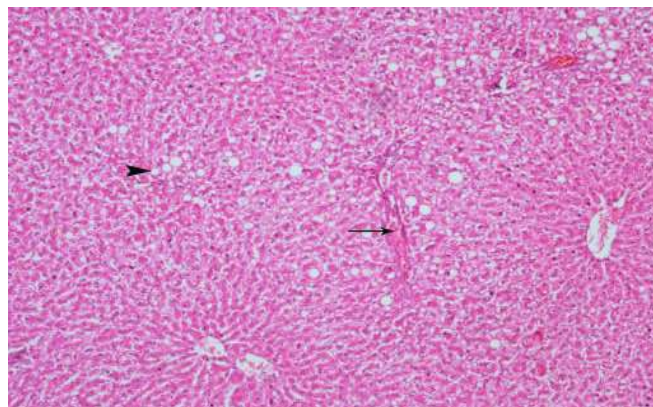


Fig. 8: (H) Section of liver tissue of rats feeding of diet based on biscuits prepared from flaxseed oil. The liver tissue showed a very good improving effect as the liver tissue regained its normal architecture except for a very slight fatty degeneration (arrow head) and fibrosis (arrow) at the periphery of the lobules was observed. (Hx. & E. X 50)

(92.86 mg/dl) [12,70,71]. It could be concluded from the results of the present study that diets prepared from flaxseed oil, flaxseed, barley, mustard or defatted mustard biscuits showed more hypoglycemic response as compared to diet prepared from wheat biscuits. The superior hypoglycemic response of diets based on plant source supplemented biscuits might be attributed to the more soluble dietary fiber content of diets. Therefore, it could be suggested that biscuits supplemented with flaxseed oil, flaxseed, barley, mustard and defatted mustard meal were able to regulate the blood glucose levels and control the glycemic index of normal individuals and diabetic patients.

#### **Histopathological Examination of Heart and Liver Tissues of Treated and Untreated Rats:**

The histopathological investigations of heart and liver tissues are illustrated in Figs. 7-8. The results of histopathological examination are in a good agreement with those of the serum lipid parameters. It could be concluded that using flaxseed oil gave the best results in ameliorating the damaging effects of increasing fat and cholesterol in diet on heart and liver tissues followed by flaxseed meal and barley meal, while the worst results were observed by using wheat biscuits diet followed by mustard biscuits and defatted mustard diet. These results are in accordance with those of Vijaimohan *et al.* [65] who reported that rats fed high fat diet caused significant morphological changes in the liver tissue indicating fatty liver and portal inflammation with large hepatocytes, whereas, rats fed high fat diet supplemented with flaxseed oil showed minimal alterations with reduced fat deposition. They also mentioned that the results of histopathological examination confirmed the protective effect of flaxseed oil against the histopathological changes in the group of rat fed high fat diet. In addition, rats fed high fat high cholesterol diet resulted in severe damage to the liver and heart tissue, whereas, feeding of diets contained 10% total dietary fiber either from wheat, corn or barley bran to the hypercholesterolemic rats lowered the degree of lesions in the liver and heart of hypercholesterolemic rats [72]. Moreover, rats fed high fat diet (10% soybean oil + 5% lard), with 1 % cholesterol in the diet resulted in liver steatosis. While, animals fed high fat diets (10% from peanut, trout, flaxseed or chicken skin + 5% lard + 1% cholesterol) resulted in a variation in the intensity of liver steatosis. The flaxseed diet was the most efficient diet in protecting the liver parenchyma (more than 75% of hepatocytes affected) and peanut and chicken skin diet (more than 50% of hepatocytes affected), [1, 73]. The results in the present study showed that defatted mustard

meal, mustard meal, flaxseed meal, barley meal and flaxseed oil could be incorporated or substituted up to 10, 15, 30 and 100%, respectively, in the formulation of biscuits without affecting their sensory quality and improving the nutritional and functional properties of the final product. In addition, feeding of hypercholesterolemic rats on diets supplemented with different functional biscuits resulted in reduced serum total cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins, atherogenic index and increased high density lipoproteins. Histopathological examination of heart and liver tissues showed that functional biscuits particularly flaxseed oil-enriched biscuits lowered the degree of lesions in the group of rats fed high fat high cholesterol diet. Thus, it could be concluded that flaxseed oil, flaxseed or barley meal based bakery products could help to cure and control the cardio vascular diseases in developing countries like Egypt.

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