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THE THERAPEUTIC EFFECT OF CAKES SUPPLEMENTED WITH *ß-GLUCAN* ON HYPERCHOLESTEROLEMIA RATS

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ABSTRACT

It is widely accepted that the mixed linkage *ß-glucan* of cereals that classified as a soluble dietary fiber, can reduce serum concentration of LDL cholesterol and may reduce the risk of cardiovascular disease .So this study was designed to investigate the therapeutic effects of Cake supplemented with 3% and 6% *ß-glucan*. ß-glucan was extracted by wet separation method, added to the wheat dough with 3% and 6%, Frinographe and extensograph were used to evaluate the rheological characterizes of dough then cakes wos made and evaluated with sensory evaluation. These products were tested on hypercholesterolemic rats, and results indicated that the products with 3% of *ß-glucan* were more acceptable than products with 6% in taste, Oder, volume, texture and colour, Also these treatment improved levels of TC, TG, LDL-c, HDL-c and VLDL-c, than other treated groups. The 3% *ß-glucan* treatment resulted in significant decreases ($p< 0.05$) in serum AST & ALT as compared to hypocholesterolemic group.

KEYWORDS: *ß-glucan*, Oat, Hypercholesterolemia, Rheological Properties, Viscosity.

INTRODUCTION

Hypercholesterolemia is the presence of high levels of cholesterol in blood .It is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular disease.[1]

Nature has been a source of medicinal treatments for thousands of years, functional foods continue to play an essential role in the primary health care of 80 % of the world's of developed and developing countries.[2]

Beta glucan (ß-glucan) is one of the most important members of the dietary fiber**.** It is found at a high level in the cell wall of fungi, yeast, rye, oat, barley and bacteria**.** It can promote health in a number of important ways, many of the health benefits associated with *ß-glucan* have been attributed to the presence of the mixed linkage 1-3, 1-4- *ß-D-glucan*. It has been studied for It's hypocholesterolemic effects; these mechanisms include: reducing the intestinal absorption of cholesterol and bile acids by binding to glucan; shifting the liver from cholesterol syntheses to bile acid production; and fermentation by intestinal bacteria to short-chain fatty acids, which are absorbed and inhibit hepatic cholesterol syntheses. Several studies have also shown that it can blunt the glycemic and insulin response.[3]

Beta-glucan can be used as a functional ingredient in some products i.e. (cake, biscuits, crackers and patties). Thus products supplemented with *ß-glucan* can produce new approaches for the treatment of CVD and diabetes**.** Although, many studies have been investigated the role of *ß-glucan* in the prevention of many disease the relationship with hypercholesterolemia in human being is still not understanding.[4]

MATERIAL AND METHODS

Material: Oat grains (*Avena sativa*) were obtained from Oat Research Dept., Crops., Institute, Agric., Res., Center, Giza, Egypt, during the 2016 harvesting period. The collected samples was transported to the laboratory and stored immediately on the refrigurator at zero °C until using in prepartion of *ß-glucan*.

Methods

***ß-glucan* preparation:** ß-glucan extracted from Oat meal according to the methods of.[5] with some modifications. The method involved the solubilization of 25gm of whole Oat meal in 1.5 L boiling deionized Milli-Q water at pH 10. Magnetic continuous stirring used for 30 min to help in extraction followed by centerfugation at 13000xg for 10 min to make starch residue, then the solution treated with thermostable œ-amylase (added at arate of 1% w/w,of available starch in the barely) and hold at 90°c for 1hour, followed by protein denaturation and subsequent alcohol –assisted precipation of ß-glucan, Purified ß-glucan concentrate was and then dried at 50°c finally it is determined according the method of.[6]

Preparation of cake

Ingredients of cake: Mix shortening (30gm), sugar (60 gm) using beater for 5 min until creamy staple; add 2 eggs and (0.5gm) Lemon juice to the shortening –sugar mix at medium

speed of beater until doubled volume. Mix (100gm) wheat flour supplemented with (3% *ß-glucan* or 6% in the other treatment) interchange with (50gm) full cream milk. Add (0.5 gm) baking powder then pour in cupcake and bake at 180 ^{o}C for 20 min.

Rheological characteristic: The rheological assessment of different dough samples was carried out using Farinograph and Extensograph. Mixed meals of wheat flour supplemented with *ß-glucan* were examined for the rheological properties using of farinograph and extensograph tests according to the methods of.[7]

Sensory evaluation: Sensory analysis was conducted by 10 untrained panelists recruited among student, faculty of home economics and staff members from the Menoufia University, whose ages ranged from 20 to 45 years. They were asked to express their opinion of cake regarding its color, taste, appearance, texture, crispness, flavor. Mouth feel and over all acceptability. And they were asked to use the control cake as the basic for determining acceptance by first assigning score to it and then evaluating each test cakes in comparison to the control, flavor, color and mouth feel were judged on a scale of 10, texture and crispiness were scored from 15 while taste and appearance were scored from 20 with total score values of 100. All data were recorded on a questionnaire to indicate the degree of likeness for the sample of each treatment (3% and 6%), using 9-points hedonic scale according to.[8]

Animals: Twenty white male albino rats, (Sprague Dawley strain) 6 weeks age, weighting (140gm ± 10) were used. The animals were derived from Research Institute of Ophthalmology, Medical Analysis Department, and Giza, Egypt. Rats were kept in cylindrical wire cages with wire bottoms. The diet was introduced in special food cups to avoid scattering of food. Water was provided to the rats by glass tube projection through the wire cage. Food and water provided ad – labium and checked daily.

Basal diet: The basic diet was prepared according to the following formula as mentioned by.[9]

Experimental design: All biological experimental were done at Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. Rats (n = 20 rats) were housed individually in wire cages in a room maintained at 25 $\pm$ 2^{o} C and kept under normal healthy conditions. All rats (16 rats) were fed basal diet for one – week before starting the experiment for acclimatization. After one week period, the rats were stratified by weight and were

divided into 4 groups (4rats each), all groups were fed for 28 days on experimental diet as follows.

Group (1): This group was fed on standard diet only as a negative control (healthy rats).**Group (2)**: This group was fed basal diet +1% cholesterol as a positive control. **Group (3)**: This group was fed basal diet containing 10% cake supplemented with 3% *ß-glucan* +1% cholesterol.**Group (4)**: This group was fed basal diet containing 10% cake supplemented with 6 % *ß-glucan* + 1% cholesterol.

Blood sampling: In all experimental groups, blood samples were collected after 12 hours fasting at the end of each experiment, using the reto orbital method by means of micro capacity glass haparinized tubes. Blood samples were collected into dry clean centrifuge tubes and left to clot in water both (37^{o} C.) for half an hour. The blood was centrifuged for 10 minutes at 3000 pm to separate the serum. Serum was carefully aspirated into clean cuvette tube and stored frozen at – 20^{o} C for analysis as described by.[10]

Organs weight and fixation

The organs (liver, heart, kidney, spleen and lungs) were removed, cleaned and weighted.

Biological evaluation

Determination of lipid profile: Total cholesterol, Serum tricylglyceride, Total lipids High density lipoprotein (HDL) in the plasma were determined according to the method of[11,12,13 and 14] by a quantitative enzymatic colorimetric method using standard kit. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activites were measured according to the methods described by.[15,16], respectively. Low density lipoprotein cholesterol (LDL-cholesterol) was calculated according to the method of.[17] using the following formulae

VLDL = TG / 5

LDL = TC - (VLDL + HDL)

Statistical Analysis: Repeated –measures one –way ANOVA was used to examine the effect of treatments over time on plasma cholesterol concentrations, liver functions, kidney functions and body weight, using Sigma Stat soft ware 002E.

RESULTS AND DISCUSSION

Rheological characteristics of dough: Effects of *ß-glucan* addition on farinographic characteristic of wheat dough are shown in table (1).

Table. (1). The effect of *ß-glucan* addition on farinographic characteristic of wheat dough.

Test results		Samples No.		
		3% ß-glucan	6% ß-glucan	Standard
Farinograph	W.A%	85.80	58.70	57.80
	A (min)	0.5	0.5	1.0
	B (min)	1.0	1.0	1.5
	C (min)	2	1	3
	D.S (B.U)	160	200	90
Extensograph	E (min)	65	55	115
	S (B.U.)	220	210	250
	P.N	3.39	3.82	2.17
	Energy cm^2 (area under curve)	24	16	55

Test methods[6]: The main consideration in Oat products is the effect of fiber on the rheological properties of the dough, because of high water –holding capacity of most fibers. The formula absorption generally needs to be increased with increasing level s of fiber.[18] He showed that the soft wheat class are mainly used for manufacturing cookies , cakes which required low protein concentration and low water absorption, compared with hard wheat class are mainly used for bread baking.

Sensory evaluation of cakes supplemented with different levels of *ß-glucan*: Sensory evaluation of cakes supplemented with different levels of *ß-glucan* was tabulated at table (2) from such data it could noticed that cake supplemented with 3% *ß-glucan* indicated the highest sensory evaluation *ß-glucan* these results agreed with.[19]

Table. (2): Sensory evaluation of cakec supplemented with different levels of β-glucan.

Properties	Supplementation		
	Standard Cake	Cake3% β-glucan	Cake6% β-glucan
Appearance20	20±0.00	19±0.17	19±0.22
Taste20	19±0.22	19±0.52	18±0.62
Texture15	14±0.16	14±0.36	13±0.16
Crispiners15	14±0.21	14±0.51	14±0.31
Color10	9±0.41	9±0.31	9±0.33
Flavor10	10±0.00	9±0.15	9±0.19
Montn Feel 10	9±0.20	9±0.30	9±0.18
Over all acceptability100	95±1.23	94±1.71	91±1.86

Biological evaluation

The effect of cakes supplemented with different levels of *ß-glucan* consumption on serum lipid fractions profile of rats feeding on diet containing cholesterol was tabulated in Table (3). From such data it could be noticed that the mean value of serum (TC), (TG), (HDLc), (LDLc) and (VLDLc) of cake group with 3% ß-glucan were (_5.6541 170.66±0.8516, 224.50±1.3612, 34.001±1.1643, 91.89 ±0.9169and 44.84±0.3621 mg/dl) respectively. They very high significantly decreased (P<0.0001) when compared with the mean value of positive control group (181.24±1.2562, 247.40±0.5732, 37.71±0.4872, 93.00±0.62 and 48.55±0.1123) respectively with % change (-5.6541, -9.1326, -7.2834, -28559 and -9.29) respectively.

Finally the mean value of serum (TC), (HDLc) and (LDLc) of cake group with 6% ß-glucan were (170.99±1.00, 34.407±0.5721 and 90.74±0.956) respectively. They very high significantly decreased (P<0.0001) when compared with the mean value of positive control group (181.24±1.2562, 37.71±0.4872 and 93.75±1.25) respectively with % change (-5.96,-8.27 and -2.4193) respectively.

While the mean value of (T.G) and (VLDLc) were very high significantly increased when compared with the mean value of positive control group (273.00±1.413 and 54.21±0.5111 mg/dl) respectively with % change (+9.39 and +9.39) respectively.\These results agreed with]20] .Shown that effect of reduced and high molecular weight Oat ß-glucan on lipid fractions of hypercholesterolemic Syrian golden hamsters and found that consumption of concentrated Oat ß-glucan lowers plasma total cholesterol (TC) and non –HDLc) concentration. (Kandutsch, et al., 2009), they evaluated the LDLc –lowering effect of a concentrated Oat ß-glucan extracts at both 3 and 5 gm doses and reported that the mean LDLc levels fell by 15%in the 5gm group and 9% in 3 gm group while HDLc levels were un changed by treatment.

Table. (3). Effect supplementation with different levels of β-glucan on serum lipid fractions of rats.

Groups	Total cholesterol(mg/dl) Mean±SD	% Change	Triglycerides (TG)(mg/dl) Mean±SD	% Change	HDLc(mg/dl) Mean±SD	% Change	LDLc (mg/dl) Mean±SD	% Change	VLDLc (mg/dl) Mean±SD	S% Change
Control(-)	69.50±0.9212	……..	103.04±0.500	…….	31.70±0.9673	…….	19.240±0.5122	……..	20.19±0.1000	……..
Control(+)	181.24±1.2562	+165.65	247.40±0.5732	_2.6271	37.71±0.4872	+18.9867	93.00±0.62	+374.1213	48.55±0.1123	+141.8794
Cake with 3% β-glucan	170.66±0.8516	_5.6541	224.50±1.3612	_9.1326	34.001±1.1643	_7.2834	91.89 ±0.9169	-28559	44.84±0.3621	-9.2918
Cake with 6% β-glucan	170.99±1.00	_5.9689	273.00±1.4131	+ 9.3112	34.407±0.5721	_8.2773	90.74±0.9564	_2.4192	54.21±0.5111	+9.3939
Sig.	0.000***		0.000***		0.000***		0.000***		0.000***	

Table. (4): Statistical analysis (LSD) OF Serum Lipid profiles of rats were fed diet supplemented different level of β-glucan.

Groups	Total cholesterol	Triglycerides	HDLc	LDLc	VLDLc
V-vs.V+	-112.0000*	-144.6500*	-6.0000*	-74.7500*	-28.9400*
V-vs.C3%	-102.2500*	-121.7500*	-3.2500*	-71.0000*	-24.35000*
V-vs.C6%	-102.2500*	-168.2500*	-2.7500*	-71.5000*	-33.6500*
V+vs.C3%	10.7500*	22.0000*	2.7500*	2.7500*	4.5000*
V+vs.C6%	10.7500*	--22.5000*	3.2500*	3.2500*	-4.6000*
C6% vs.C3%	0.0000	46.5000*	-0.5000	0.5000	9.3000*

The mean difference is significant at the 0.5 level

v- (Control-)

v+ (Control+)

C3 % (Cake supplemented with 3% β-glucan)

C6% (Cake supplemented with 6% β-glucan)

The effect of supplementation with different levels of *ß-glucan* on kidney function

The effect of cakes supplemented with different levels of *ß-glucan* consumption on kidney functions of rats were fed diet containing cholesterol was tabulated in Table (5). *ß-glucan*, cake group with 3% *ß*-glucan and cake group with 6% *ß*-glucan (0.6250±1.281, and 0.7152±2.877mg/dl) respectively were very high increased significantly ($p<0.0001$) when compared with the mean value of positive group (0.52±1.73 mg/dl) with percentage of change +17.14 and + 36.19 mg/dl) respectively.

Table. (5): effect of supplementation with different levels of ß-glucan on creatinine of rates.

Groups	Creatinine Mg/dl	%Change
Control(-) Mean±SD	0.6374±5.00	……
Control(+) Mean±SD	0.5251±1.732	-17.6471
Cake with 3% β-glucan Mean±SD	0.6250±1.281	17.14373
Cake with 6% β-glucan **Mean±SD**	0.7152±2.877	36.1914
Sig.	0.000***	

Table. (6): Statistical analysis (LSD) OF creatinine of rats feeding with diet supplemented different level of β-glucan.

Groups	Creatinine
V-vs.V+	-0.1124*
V-vs.C3%	-102.2500*
V-vs.C6%	-102.2500*
V+vs.C3%	-9.0000*
V+vs.C6%	-0.1900*
C6% vs.C3%	-1.0000*

The mean difference is significant at the 0.5 level

v- (Control-)

v+ (Control+)

C3 % (Cake supplemented with 3% β-glucan)

C6% (Cake supplemented with 6% β-glucan)

The effect of supplementation with different levels of *ß-glucan* on liver function of rats

The effect of products supplemented with different levels of ß-glucan on liver functions of rats were fed diet containing cholesterol was tabulated in Table (7). Serum glutamic oxaloacetic transaminase of Cake group 3% ß-glucan (86.260±0.9881) was significant decreased from the mean value of control positive group (117.8251±0.550u/L) with percentage of change (--26.7514) while the value (122.7154±0.577) was very high significant increasing when compared with the mean value of positive group (117.8251±0.550 u/L) with percentage of change (+3.3865). The same attends were observed for the serum glutamic pyruvic transaminases (SGPT). The mean value of SGPT of cake group 3% ß-glucan (48.3500±0.8954 u/L) was decreased significantly from the mean value of control positive group (57.550±0.5884 u/L) with % change (-16.0869), while the value (45.3500±1.500) was decreased significantly for cake group with 6% ß-glucan from the mean value of control positive group (57.550±0.5884u/L) with % change (-22.3242). These results are in agreement with.[21]

Diagnostic value of plasma aminotransferase (SGOT and SGPT)

Aminotransferases are normally intracellular enzymes. Thus, the presence of elevated levels of amino transferase in the plasma indicates damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lyses, resulting release of intracellular enzymes into the blood. Two amino transferases were found in plasma are of particular diagnostic value SGOT and SGPT. These enzymes are elevated in nearly all liver

diseases, but are particularly high in conditions that the causes extensive cell necrosis, such as severe viral hepatitis and prolongated circulatory collapse. Serial enzyme measurments are often useful in determining the course of liver damage.[22] Also, amino transferases may be elevated in non hepatic disease, such as myocardial infraction and muscle disorders; however, these disorders can usually be distinguished clinically from liver disease.[23]

Table. (7): The effect of supplementation with different levels of β-glucan on liver functions of rats.

Groups	SGOT mean±SD U/L	Change%	SGPT mean±SD U/L	%Change
Control(-)	37.637±0.5000	……	25.5000±1.000	……
Control(+)	117.8251±0.550	+221.9211	57.550±0.5884	+125.5801
Cake with 3% β-glucan Mean±SD	86.260±0.9881	-26.7514	48.3500±0.8954	-16.0869
Cake with 6% β-glucan Mean±SD	122.7154±0.577	+3.3865	45.3500±1.500	-22.3242
Sig.	0.000***		0.000***	

Table. (8): Statistical analysis (LSD) OF Liver functions of rats were fed diet supplemented different level of β-glucan.

Groups	SGOT	SGPT
V-vs.V+	-81.0000*	-32.0000*
V-vs.C3%	-47.500*	-22.700*
V-vs.C6%	-84.000*	-19.75O*
V+vs.C3%	30.500*	9.2000*
V+vs.C6%	-4.000*	12.2500*
C6% vs.C3%	-34.500*	3.000*

The effect of supplementation with different levels of *ß-glucan* on the percentage of body weight gain of rats

The effect of cakes supplemented with different levels of ß-glucan on body weight gain (%) of rats feeding on diet containing cholesterol was tabulated in Table (9) The mean value of body weight gain (%) studied groups (cake group with 3% ß-glucan and cake group with 6% ß-glucan) (75.20±20.9169 and 73.74±2.9564%) respectively were significantly increased (P <0.01), when compared with the mean value of control positive group (55.89±17.6275) with % change +35.8951 and+30.1127) respectively.

Table. (9): The effect of diet supplementation with different levels of β-glucan on the body weight gain of rats.

Groups	Initial weight Mean ±SD (gm)	% Change	Final weight Mean ±SD (gm)	% Change	Body weight gain (gm) Mean ±SD	% Change	Bod weight gain% Mean±SD (gm)	% Change
Control(-)	140.00 ±0000	……..	213.74±33.5900	…….	71.70±33.9673	……	51.95±23.9122	……..
Control(+)	140.00 ±0000	0	217.40±25.5732	+2.7271	77.71±25.4872	+8.3867	55.89±17.6275	+9.7213
Cake with 3% β-glucan	130.00±0.0000	-7.1337	298.50±26.3612	+36.1265	99.001±2.1643	+25.6655	75.20±20.9169	+35.8951
Cake with 6% β-glucan	130.00±0.0000	-7.1337	226.00±2.4131	3.3112+	94.407±23.5721	+19.5691	73.74±2.9564	+30.1127
Sig.	0.000***		0.27(NS)		1.27(NS)		0.06*	

Table. (10): Statistical analysis (LSD) OF animals weight of rats were fed diet supplemented different level of β-glucan.

Groups	Initial weight	Final weight	Body weight gain(gm)	Body weight gain (%)
V-vs. V+	0.0000*	-6.0000	-6.0000	-4.288
V-vs.C3%	10.0000*	-17.000	-27.000	-24.7766
V-vs.C6%	10.0000*	-13.3000	-23.3000	-21.8891
V+vs.C3%	10.0000*	-11.000	-21.000	-20.5578
V+vs.C6%	10.0000*	-7.0544	-17.3000	-17.6784
C6% vs.C3%	0.0000	3.9900	3.9900	3.0998

The mean difference is significant at the 0.5 level

v- (Control-)

v+ (Control+)

C3 % (Cake supplemented with 3% β-glucan)

C6 % (Cake supplemented with 6% β-glucan)

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