ROLE OF CELL WALL PECTIC POLYSACCHARIDES OF PRICKLY PEAR PEEL ON SOME BIOLOGICAL ACTIVITIES OF EXPERIMENTAL RATS

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ABSTRACT

The present study aims to explore the role of cell wall pectic polysaccharides of prickly pear peel on some biological activities of rats. This was achieved by altering the structure of cell wall pectic polysaccharides either by physical means (fiber-pectin) or by enzymatically degradation (EPP). Data showed that prickly pear peel represented 40.5 % of the fruit weight. It contained 16.61% alcohol insoluble solids, 12.20% total soluble solids, 7.80 % reducing sugars and 2.27% non-reducing sugars.

Biological results ascertained that the rats fed on various fractions of prickly pear peel fiber, such as fiber – pectin (FP), alcohol insoluble solids (AIS), and enzymatically treated prickly pear peel (EPP), showed a reduction in the body weight gain, serum glucose, cholesterol and triglycerides. Histopathological experiment showed that feeding rats FP and AIS attained an improvement in liver tissues than the rats fed on hyperlipidemic diet. Statistical analysis ascertained that no significant differences in relative weight of liver between of different groups, while there were significant differences existed between groups, concerning the daily feed intake, relative weight of kidney, glucose serum, cholesterol and triglycerides.

INTRODUCTION

Prickly pear fruits (*Opuntia ficus indica*) also named cactus, is considered one of the important tropical fruit grown worldwide in sandy soil, (Abou-Zaid, 1995). Prickly pear (*Opuntia spp.*) belongs to Coctaceae family and is native to the arid, and semi arid regions (Benson, 1982). Due to the capability of fruitful prickly pear to prolonged drough, it is considered as a potential alternative crop for drier regions (Szarek *et al.*, 1973).

In Egypt, about 27299 tons were harvested from 2747 feddans cultivated with prickly pear shrubs (Anon, 2001). Prickly pear fruits are a source of valuable nutrients, such as pigments, vitamins, sugars, and are eaten fresh or preserved in the form of jams, syrups, or candies (Sawaya, *et al.*,1983a and Hoffman, 1980).

Laban (1998) reported that the prickly pear fruit contain 38 - 40% peel, 14.2 - 17.1% seeds, and 43.4 - 47.3% juice. He added that the peel contains 85.7% moisture, 9.6% total sugars, 8.5% reducing sugars and 14.60%, alcohol insoluble solids.

(Sawaya *et al.*, 1983) reported that the total fiber substances of cactus contained 70.3% pectin, whereas hemicellulose, cellulose and lignin contents were 15.5%, 14.2% and 0.01%, respectively.

Dietary fiber is generally defined as lignin as plus plant polysaccharides that cannot be digested by human enzymes. The major

components of dietary fiber are cellulose, hemicellulose, pectin and lignin (Maurice, 1994).

The high water holding capacity of cactus fiber is attributed to intermolecular repulsion of negatively charged side chains with concomitant expansion and induced rigidity of molecules (Sawaya *et al.*, 1983). Stintzing *et al.*, (2001) discussed its functional components of cactus pear fruit, and their nutritional importance and their significance in plant physiology.

Dietary fiber associated with blood sugar regulation in humans suffering from diabetes mellitus type II, the non-insulin dependent diabetes mellitus (Terjo – Gonzalez, 1996).

Studies carried out by Terjo – Gonzalez, (1996) indicated that prickly pear pectin consumption modified low density lipoprotein (LDL) composition. The relative percentage of triglycerides was increased, whereas the level of free and estrified hepatic cholesterol were lowered. Moreover, an increase in LDL density was observed.

Luz – Fernandez *et al.* (1990) and Luz – Fernandez *et al.* (1992) observed that the levels of LDL and hepatic cholesterol were reduced at the same time, there was a rise in LDL density and in hepatic apolipoprotein receptives .They ascribed the increase in hepatic cholesterol demand to an enhanced excertion of bile acids and in terruption of enterohepatic circulation followed by decreased plasma LDL levels.

Dietary fiber has important therapeutic implication for certain conditions such as diabetes and hyperlipidemia, and may have preventive implications for others, such as hypertension, coronary disease, and intestinal disorders (Anderson, 1983; Anderson, *et al.*, 1984 and Jenkins *et al.*, 1986).

Cerda (1988) claims that the daily intake of 15 gm pectin causes the cholesterol level in human blood to decrease. Baig and Cerda (1980) stated that pectin lowered serum cholesterol levels by forming insoluble complexes with the serum low density lipoproteins (LDL) which transport circulating cholesterol. Iwasaki, *et al.*, (1978) reported that the possible use of pectin in treatment of diabetes. They also observed that a significant reduction of urinary glucose excretion when 15 gm / day of pectin was incorporated in the diet.

The present work aimed to study the role of cell wall pectic substances of prickly pear peel on some biological activities of albino rats.

MATERIALS AND METHODS

Materials :

Prickly pear fruits (*Opuntia ficus indica*) were obtained from Zagazig local market, Sharkia Governorate, Egypt. The fruits were at the ripe stage. Pectinex Ultra SPL and Cellubrix enzymes were kindly provided by Novo Nordisk, Ferment AG (Dittingen, Switzerland).

Methods :

Extraction of prickly pear juice and peel :

The fruits were carefully washed by tap water, manually crushed and extracted by manual hydraulic pressing. The juice was strained through two layers of cheese cloth.

Preparation of fiber-pectin :

Prickly pear peel were extracted by acidified ethanol. The pH during extraction was adjusted to pH 1.8 using hydrochloric acid. The ratio of acidified ethanol to peels was 2 : 1 w/w. The mixture was heated at 60 °C for 2 hrs, then directly cooled by tap water. The mixture was incubated at 42 °C for 24 hrs and filtered through Buchner Funnel. Successive washing with ethanol 70% and 96% was carried out to the residue, then finally washed by acetone. The residue was dried at room temperature and the product is called fiber – pectin (Siliha, 1993).

Preparation of alcohol insoluble solids :

Prickly pear peel was mixed with ethanol 96% (1:3) w/w, heated to 60 °C for 30 min with stirring. The mixture was filtered through Buchner Funnel. The residue was washed with ethanol until the filterate was colorless, giving a negative reaction with phenolsulphuric acid test (Dubois *et al.* 1956). The residue was suspended in acetone, and the residue was dried overnight to remove acetone, weighed and expressed as AIS %.

Enzymatic treatment of prickly pear peel :

Prickly pear peel was treated with Pectinex Ultra SPL and cellubrix L combination to degrade pectic substances to low molecular weight compounds. 1000 gm peel were mixed with 0.25 gm enzymes preparate (1 : 1). The enzymatic reaction was carried at 45 °C for 90 min. The enzymes were inactivated at 90 °C for 5 min in a water bath and directly cooled at room temperature with tap water.

Analytical Methods :

Total soluble solids (TSS), moisture content, pH value, total sugars, reducing and non reducing sugars were determined according to methods described in the AOAC, (1990).

Biological experiment :

Forty male albino rats (*Sprague dowely*), weighting 95 - 110 gm, were obtained from National Research Center (Cairo). Rats were housed in wire cages under the normal conditions, and were fed on a basal diet for a week as adaptation period. The rats were divided into eight groups (n = 5). The first group was used as a negative control, fed on basal diet, while the other groups were fed on hyperlipidemic diet (1% cholesterol + 10% animal fat) for four weeks. After hyperlipidemic period, seven groups were feeding according to the following scheme.

- Group 1: Rats fed on basal diet (Negative control).
- Group 2: Rats fed on hyperlipidemic diet, (Positive control).
- Group 3 : Rats fed on hyperlipidemic diet supplemented with 2.5% fiberpectin (FP)
- Group 4: Rats fed on hyperlipidemic diet supplemented with 5% (FP)
- Group 5 : Rats fed on hyperlipidemic diet supplemented with 2.5% alcohol insoluble solids (AIS).

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Group 6: Rats fed on hyperlipidemic diet supplemented with 5% (AIS).

Group 7: Rats fed on hyperlipidemic diet supplemented with 2.5 % enzymatically treated prickly pear peel (EPP).

Group 8 : Rats fed on hyperlipidemic diet supplemented with 5% (EPP).

Experimental diet :

The composition of basal diet was casein 14%, corn oil 10%, salt mixture 4%, vitamin mixtures 1%, cellulose 5%, and starch 66%. The composition of salt and vitamin mixtures were applied according to Compbell, (1961). At the end of experiment, rats were anethetized with diethyl ether. Blood samples were collected from portal vien and plasma was separated and kept at -18 °C until analysis.

Biological analysis methods :

Total cholesterol was determined according to Allian *et al.*, (1974). Triglycerides were measured according to Jacobs and Vandenmark, (1960). Serum glucose was determined by the method of Trinder, (1969).

Histopathological examination :

Specimens from the liver of rats from all groups were fixed in 10% (v/v) neutral buffered formalin, dehydrated in ethyl alcohol and cleared in xylol. Tissue section 4 - 6 u thick was stained with hoematoxylin and erosin stain (H and E x 200) according to Carlton *et al.*, (1967).

Statistical analysis

Statistical analysis of biological data were analyzed by the methods of SAS user's guide (SAS, 1999).

RESULTS AND DISCUSSION

Some chemical constituents of prickly pear peel :

Some chemical constituents of extracted peel are listed in Table (1). Alcohol insoluble solids content of prickly pear peel was accounted for 16.61%, while total soluble solids content was 12.20%. The sugars determined in prickly pear peel consisted of 7.80 % reducing sugars and 2.27% non-reducing sugars. These results are in agreement with those obtained by Siliha (1989).

Constituents	%
Peel yield	40.5
Moisture content	83.6
Alcohol Insoluble Solids (AIS)	16.61
Total Soluble Solids (TSS)	12.2
Total Sugars	10.07
Reducing Sugars	7.8
Non-reducing sugars	2.27

Table (1) : Some chemical constituents of prickly pear peel

Biological effects of prickly pear peel fiber :

It is well known that dietary fiber play an important role in many biological activities of human body. Since dietary fibers comprise different polysaccharides and lignin. It is of a great interest to elucidate the role of its individual component on the liver and kidney weights as well as the levels of serum glucose, triglycerides and cholesterol.

In so doing pectin polysaccharides were added to albino rats diet in its native form (AIS), which is the cell wall material consisting of pectin mainly in addition to cellulose and hemicellulose, in solublized form (FP), which is form of cell wall preparation in which the insoluble pectin is solublized by acid hydrolysis in alcoholic medium. The solublized pectin has the same functional preparation like the commercial pectin preparation except that cellulose, hemicellulose and other cell wall components are kept in this preparation, and degraded to low molecular weight (enzymatically treated peel). The aim of this treatment was to prepare cell wall preparation in which the pectic polysubstances are degraded into low molecular weight. The degradation of pectin includes both insoluble and soluble pectin. In such preparation, the functional properties of the pectin molecules was considered as a hydrocolloid. Moreover, this enzymatic treatment partly altered the physical structure of the cellulose fibrils.

Body weight gain :

Table (2) and Fig. (1) show the effect of diets containing different types and concentrations of dietary fiber on body weight gain. Rats fed on basal diet (negative control) had 15% less body weight than those fed on hyperlipidemic diet. Addition of dietary fiber preparations resulted in marked reduction in body weight. The extent of reduction depended on the type of dietary fiber preparation and on its concentration. In the mean time, the daily feed intake did not dramatically differ. Fiber pectin reduced body weight by 41.67% and 48.33% when added to the diet at 2.5% and 5% respectively.

 Table (2):
 Effect of dietary fiber preparations on body weight gain, daily feed intake, relative weight of liver and /or kidney in rats after

4 weeks feedir	າg.			
Parameters	Body weight gain (gm) Mean ± S.D	Daily feed intake (gm) Mean ± S.D	Relative weight of liver (%) Mean ± S.D	Relative weight of Kidney (%) Mean ± S.D
1: Basal diet (Negative control)	20.4 ± 6.19	11.64 ± 0.56	2.78 ± 0.46	0.69 ± 0.27
2:Hyperlipidemic(Positive control)	24.0 ± 8.22	11.17 ± 0.30	3.15 ± 0.42	0.76 ± 0.52
3: Hyperlipidemic+ 2.5 % FP	14.0 ± 5.48	9.78 ± 0.40	$\textbf{3.18} \pm \textbf{0.11}$	0.74 ± 0.27
4: Hyperlipidemic+ 5 % FP	12.4 ± 5.59	10.53 ± 0.54	3.07 ± 0.16	0.71 ± 0.58
5: Hyperlipidemic+2.5 % AIS	9.2 ± 4.32	11.11 ± 0.19	$3.11{\pm}~0.28$	0.69 ± 0.33
6: Hyperlipidemic+5 % AIS	18.6 ± 5.46	11.41 ± 0.40	3.19 ± 0.18	0.60 ± 0.36
7: Hyperlipidemic+ 2.5 % EPP	11.8 ± 5.76	10.23 ± 0.44	3.32 ± 0.30	0.66 ± 0.27
8: Hyperlipidemic+ 5% EPP	16.4 ± 8.2	9.66 ± 0.45	$\textbf{3.10} \pm \textbf{0.98}$	0.66 ± 0.43
F. Value	3.09*	15.25**	1.57 N	7.83 **
n = 5 * (p < 0.05) *	* (p < 0.01)	N (Non sig.)	S.D. (Sta	indard Deviation)

EPP : Enzymatically treated prickly pear peel.

On the other hand, AIS showed the highest level of reduction in body weight being 61.67% when 2.5 % AIS was added.

The addition of 5% AIS resulted in 22.5% reduction. Similarly, enzymatic degradation of cell wall pectin gave higher weight reduction resulted in a reduction of (50.8%) when 2.5% was added while adding 5% of the preparation resulted in a reduction of (31.6%). The reduction of weight resulting from the effect of fiber-pectin on the decreasing of fat absorption. In general, incorporation watermelon fiber- pectin tend to reduce body weight gain, fed consumption and fed efficiency. This may be attributed to the viscosity of fiber-pectin which forming a viscous gel and delay gastric emptying Laban, (2001).

Daily feed intake :

Data also tabulated in (Table 2) shows that there were decreasing in daily feed intake of the rats fed on 2.5 %, (FP) and 5% (EPP), they were 9.78 \pm 0.4 gm and 9.66 \pm 0.45 gm respectively. Similar results were obtained by (Baker, 1994).

Relative weight of liver and kidney :

The percent relative weight of rat liver is given in Table (2) and Fig. (1).

Negative control had the lowest liver weight, while there was no significant differences occurred between positive control and the rats fed on diets containing dietary fiber preparations. This clearly indicates that the addition of dietary fiber preparations to hyperlipidemic diets had no influence on the increase in liver weight.

Table (2) and Fig. (1) also show the effect of dietary fiber preparation on kidney weight. This indicates that negative control and diet containing AIS and enzymatic treatment, cell wall had the lowest kidney weight compared to kidney weight of rats fed on hyperlipidemic diet. The results revealed that addition of fiber-pectin to hyperlipidemic diet did not alter the kidney weight.

Serum glucose :

The normal serum glucose in albino rats fed on basal diet (negative control) was found to be 67.08 ± 5.74 mg/100 ml (Table 3 and Fig. 2). When rats were fed on diet rich in lipids (positive control), it was observed that the glucose concentration became 119.1 ± 12.6 mg/100 ml.

Compared to positive control, the rats fed on diets containing dietary fiber preparation had lower serum glucose, which demonstrates the beneficial health effect of diets rich in dietary fiber on serum glucose.

Table (3) and Fig. (2) show that the effect of different preparations on serum glucose. It was found that the diet containing enzymatically degraded pectin was significantly different than the other dietary fiber preparations. This effect suggests that the high molecular weight pectin, either soluble or insoluble, plays the major role in decreasing serum glucose concentration.

High fiber diets, probably exerted their effect on glycemic control by improving insulin sensitivity (Anderson and Akanji, 1993). The effect of watermelon fiber-pectin on blood glucose levels of diabetic rats was observed after 8 weeks feeding. It is evident that the highest reduction was occurred in the group fed with basal diet and 15% fiber – pectin (Laban, 2001).

F 1

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F2

Parameters	Glucose mg / 100ml	Cholesterol Mg / dl	Triglycerides mg / dl
Groups	Mean ± S.D	Mean ± S.D	Mean ± S.D
1: Basal diet (Negative control)	67.08 ± 5.74	70.6 ± 5.8	72.69 ± 8.88
2: Hyperlipidemic (Positive control)	119.1 ± 12.6	143.36 ± 9.22	129.65 ± 10.93
3: Hyperlipidemic+ 2.5 % FP	81.73 ± 8.2	95.92 ± 7.95	95.42 ± 6.27
4: Hyperlipidemic+ 5 % FP	80.0 ± 6.30	83.25 ± 6.62	96.83 ± 14.11
5: Hyperlipidemic+2.5 % AIS	81.40 ± 11.30	91.16 ± 6.07	96.43 ± 7.14
6: Hyperlipidemic+5 % AIS	78.11 ± 11.54	64.21 ± 31.44	95.66 ± 9.58
7: Hyperlipidemic+ 2.5 % EPP	79.6 ± 10.9	83.08 ± 7.88	89.12 ± 7.40
8: Hyperlipidemic+ 5% EPP	92.6 ± 17.7	94.57 ± 4.29	101.66 ± 4.57
F. Value	7.34**	16.72**	14.55 **

 Table (3): Effect of dietary fiber preparations on serum glucose, cholesterol and triglycerides in rats after 4 weeks feeding.

n = 5 S.D. (Standard Deviation). ** (p < 0.01)

FP : Fiber-pectin AIS : Alcohol insoluble solids

EPP : Enzymatically treated prickly pear peel.

Cholesterol

Table (3) and Fig. (2) show that the plasma of rats fed on hyperlipidemic diet had highest concentrations of cholesterol as high as 143.36 \pm 9.22 mg / dl of positive control which was higher than that found in plasma of rats fed on a diet void of lipids was 50.7%. This indicates that lipid rich diet is responsible for increasing plasma cholesterol. Addition of dietary fiber preparations to hyperlipidemic diets resulted in significant decline in the concentration of plasma cholesterol. Fiber-pectin added in a concentration of 5% caused greater reduction in plasma cholesterol (83.25 \pm 6.62 mg / dl) than that resulted by addition of 2.5 % fiber pectin (95.92 \pm 7.95 mg / dl).

The same trend was observed when 2.5 % AIS (91.16 \pm 6.07) and 5% AIS (64.21 \pm 31.44 mg / dl) were added to the hyperlipidemic diet. However, opposite effect was noticed in case of enzymatically degraded pectin. These results suggest that high molecular pectin either in the soluble form (as the case with fiber-pectin) or insoluble form (as the case with AIS), is responsible for the reduction of plasma cholesterol.

Cassidy and Calvert (1993) found that the increased intake of dietary fiber in human is generally associated with the increase in fecal volume and increases in the lipid content of feces. Cerda (1988) claims that the daily intake of 15 gm pectin causes a decrease in cholesterol level in human blood. Pectin has been shown to lower blood cholesterol levels and the (LDL) cholesterol fractions without changing levels of (HDL), cholesterol and or triglycerides (Behall and Resier, 1986). When prickly pear pectin was used in rats feeding, the plasma cholesterol was markedly reduced by binding bile acid and reduce hepatic cholesterol (Fernandez *et al.*, 1994).

Statistical analysis showed high significant differences between groups. The content of a vailable free carboxyl groups of pectin will determine the magnitude of its impairing effect on cholesterol absorption. This elucidates that pectin impairing action would be due to the biochemical interaction between the free carboxyl groups of cholesterol forming some complexes which resist the absorption in the small intestine (EI-Shewey *et al.*, 1998).

Complete degradation of pectin and partial degradation of cellulose

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(as in the case of enzymatically degraded pectin) caused lower effect on the reduction of plasma cholesterol. Because of the enzymatic treatment partly altered the physical structure of the cellulose fibrils.

Triglycerides

Due to feeding rats on hyperlipidemic diet, the amount of triglycerides was found to be $(129.65 \pm 10.93 \text{ mg} / \text{dl})$, while on feeding other groups of rats on diet void of lipid, the concentrations of triglycerides dropped by 43.93%. Dietary fiber added to the diet rich-lipid resulted in significant reduction in triglycerides content in the blood. Both fiber-pectin and AIS caused the same effect on triglycerides content, added either as 2.5 % or 5%. While the situation differed in case of enzymatically degraded pectin. Addition of 2.5 % of enzymatically degraded pectin gave the lowest level of triglycerides while addition of 5% gave the highest level of triglycerides.

These results coincide with those obtained for serum glucose and plasma cholesterol indicating that enzymatically degraded cell wall polysaccharides had lower effect on reducing the level of these components, in the blood compared to fiber-pectin and AIS. These results are of prime importance not only from the nutritional point of view, but also for the fruit and vegetable juices industry. It clearly shows that the use of commercial enzymes (pectinase and cellulase) in the extraction of juices or fruit pulp in order to reduce viscosity or to increase the yield, the resultant products will not acquire the full benefit from their dietary fiber.

Histopathological examination :

Fig. (3) shows histopathological scanning of rats liver in eight groups after 4 weeks feeding using microscopical examination. Photomicrographs showed that the liver tissue of negative control had the normal histological structure of hepatic lobules from central vien and concentrically arranged hepatocytes (Fig. 3 A). However, liver of rats from group (3) showed Kupffer cell activation and hepatocellular vacuoletions associated with sporadic necrosis of heptocytes (Fig. 3 B).

Liver sections from rats in group (4) reveal kupffer cell activation, hyperplasia of epithelial lining bile ducts, formation of newly formed bile ducts as well as portal infiltration with mononuclar inflammatory cells (Fig. 3 C).

Histopathologically, liver of rats from group (5 and 6) shows more or less similar histopathological changes, which confined as congestion of central veins as well as hepatoportal blood vessels, appearance of newly formed bile ductules (Fig. 3 - D and E), together with collagen fiber deposition in the portal traids of some examined sections (Fig. 3 - E). Liver of rats from groups (7 and 8) shows similar histopathological changes confined as Kupffer cell activation, hydropic degenration of heptocytes and portal haemorrhages (Fig. 3 - F and G).

F 3

-F3

Examined liver sections from group (2) revealed that congestion of central veins and hepatic sinusoids together with vacular degeneration of hepatocytes with signt ring appearance (fatty changes) (Fig. 3 - H). It is clear that the rats fed on fiber-pectin and alcohol insoluble solids showed the improvement of liver tissues than the rats fed on hyperlipidemic diet (positive control). These results are in accordance with those of Girgis *et al.*, (2004).

From obtained results, it can be concluded that the fractions of prickly pear peel fiber namely (FP, AIS and EPP), played a vital role in some biological activities of rats such as glucose, cholesterol and triglycerides. Finally, this fiber could be used as a therapeutic nutrition for human.

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دور السكريات العديدة فى الجدار الخلوى لقشور ثمار التين الشوكى على بعض الأنشطة البيولوجية لفنران التجارب السيد شريف عبد الوهاب' – هانى حلمى محمد' و حسن صليحة" (١) معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – الجيزة – مصر (٢) قسم الاقتصاد المنزلى – كلية التربية النوعية – جامعة الزقازيق – مصر (٣) قسم علوم الأغذية – كلية الزراعة – جامعة الزقازيق - مصر

وتهدف الدراسة الحالية إلى أهمية معرفة دور الجدار الخلوى فى قشور ثمار التين الشوكى على بعض الأنشطة الحيوية لفئران التجارب, وذلك عن طريق تغيير التركيب فى السكريات العديدة البكتينية بالجدار الخلوى, وذلك باستخدام طرق مختلفة لإنتاج البكتين الغنى بالألياف أو عن طريق التكسير الانزيمى. أظهرت نتائج التحليل الطبيعى والكيماوى أن قشور ثمار التين الشوكى تمثل ٥,٠٤% من وزن الثمار الطازجة. كما أنها تحتوى على ١٦,٦١% من المواد غير الذائبة فى الكحول, ١٢,٢٠ من المواد الصلبة الذائبة الكلية, ٧,٨٠ من السكريات المختزلة و ٢,٢٧ من السكريات غير المختزلة .

أظهرت النتائج البيولوجية حدوث انخفاض في وزن الجسم , جلوكوز الدم , الكوليسترول , والجليسريدات الثلاثية وذلك نتيجة تغذية فئران التجارب على وجبات بها بكتين مرتبط بالألياف , مواد صلبة غير ذائبة في الكحول , وقشور معاملة انزيمياً .

وأظهرت اختبارات الفحص لقطاعات تشريحية على أنسجة كبد فئران التجارب, أنه حدث تحسن واضح لخلايا كبد الفئران التى تغذت على البكتين المرتبط بالألياف والمواد البكتينية الصلبة الغير ذائبة في الكحول بمقارنتها بالفئران التي تغذت على وجبات مرتفعة في محتواها من الدهون

أثبت التحليل الإحصائي أنه لا توجد فروق معنوية بين المجموعات وذلك بالنسبة للوزن النسبي للكبد , بينما كانت هناك فروق معنوية بين المجموعات وذلك لوزن الجسم . كما أن هناك فروقا معنوية مرتفعة بين المجموعات وذلك للوجبة المأخوذة يوميا , للوزن النسبي للكلي , جلوكوز الدم , الكوليسترول , والجليسريدات الثلاثية .