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Biological and histopathological effects of psyllium husk seed and dome in hypercholesterolemic rats

Samah M. Ismael
Home Economic Dept., Faculty of Specific Education,
Ain Shams Univ., Cairo, Egypt
E-mail: samahdr2@hotmail.com

ABSTRACT

The present work was conducted to study the biological effect of different ratios of Doum and Psyllium husks seed as a source of dietary fiber on rats suffering from increase serum cholesterol. The results indicated that, samples of Doum and Psyllium husks seed contain high levels of carbohydrate being 94.94% and 89.85%, respectively. The crude fibers of these plants ranged between 6.7% in Psyllium and 13.62 % in Doum. The total dietary fibers (TDF) of the studied samples were significantly different and varied between 80.9% in Psyllium husks seed and 39% in Doum. As regard to the three dietary fiber fractions results indicated that the highest ratio of hemicelluloses was in Psyllium husks seed while, celluloses and lignin were recorded adverse direction.

Two main experimental groups of rats were used. Group 1 (n=12 rats) used as a negative control group fed on basal diet. The other group (n = 60 rats) fed one week on diet containing 1% cholesterol + 0.2% cholic acid to induce high serum cholesterol, then this group was divided into five subgroups (12 rats each). The first subgroup fed on diet containing 1% cholesterol + 0.2% cholic acid (cholesterol diet), as a control positive group. Subgroups (2, 3, 4 and 5) fed on cholesterol diets containing 10 and 20% doum and Psyllium husks seed powder, respectively.

The results indicated that, animals fed on diets containing Psyllium husks seed powder at levels of 10 and 20% produced larger fecal weight then those fed on diets containing the Doum powder at the same both ratios. Also, Psyllium husks seed powder groups recorded reduce in body weight gain and serum lipid profile (total lipid, cholesterol, triglycerides, LDL-c, VLDL-c) as compared with control negative group, followed Doum powder group.

Description of histopathological alterations induced in colon, Aorta ,and heart of rats fed different rich-sources of dietary fibers of Doum and Psyllium husks seed showed the protective role of dietary fibers to prevent infection of high serum cholesterol.

Key words: Doum , *Hyphaene thebaica* , Psyllium husk seed, *Plantago ovate*, Dietary fibers, Albino rats, Lipid Profile, Hypercholesterolemic, Colon, Aorta, heart, Brain.

INTRODUCTION

Natural fibers as an alternative fiber reinforcement material have significant advantages over glass. Indeed, they are more environmentally friendly, healthier and safer. Their application in polymer composites requires the understanding of their structure and mechanical properties. Other advantages of using the cellulose fibers as reinforcement are their low densities and their nonabrasive aspect. Various natural fibers have been widely used. Others, however, remain of limited use such as in the case of palms (Sghaier *et al.,* 2009). Palm fibers were chosen due to the abundance and the high diversity of palm trees in Tunisia. Composites reinforced with natural fibers present problems of fiber-matrix adhesion because of the

gummy material that coats fibers. The fiber-matrix interaction has been changed by modifying the surface properties of the fiber (Herrera and Valadez, 2005).

The palms belong to the family Palmae (Aracaceae), among which we find the African doum palms (FAO, 2005) which grow wild in the drier parts of southern, southwestern, north, western and eastern Africa. The doum palms, *Hyphaene compressa* and *H. coriacea*, are important non-cultivated food fruit-plants (Lokuruka, 2007).

Doum (*Hyphaene thebaica*) is one of the members of family Palmae. It is common in Egypt, west India, several parts of Africa and known in Arabic language as Doum palm or gingerbread palm because the plant has the taste and the consistency of gingerbread. In Egypt, the Doum palm has been cultivated since ancient times and has long been considered as a sacred tree, symbolizing masculine strength. It was also planted in belief of Pharaohs that it protected and supplied the person with shade, water and food after death. The outer layer of the fruit is edible and can be prepared either in sliced or in a powder form, which is further dried then added to food as a flavouring agent. In Turkey and Kenya, the powder prepared from the outer covering of the fruit is added to water, milk and left to stand to make a mild alcoholic drink. In other countries, the terminal meristem is tapped for making palm wine. Roots are used in the treatment of Bilharzia, while fruit pulp is chewed to control hypertension (Bonde *et al.,* 1990). Some people in Egypt use Doum as a medicinal plant for the treatment of several diseases and hypertension. Doum palm is among the more important plant families that supplies human with dietary fibers, carbohydrates and antihypertension substances. The phytochemicals, called lignans, having apparent anticarcinogenic action in animals as mentioned by Carter (1993).

There are over 200 species in genus *Plantago*, a number of plants in this genus are commonly known as psyllium. The husks are derived from the seeds of *Plantago ovata* Forsskaol. The common name of *Plantago ovata* in India, ispaghula, comes from the words "isap" and "ghol", with the meaning of "horse ear" in Persian, which describes the shape of the Plantago ovata's seeds (Miyuki *et al.,* 2017).

Plantago ovata is an annual herb mainly cultured in North Gujarat in India. The plant is normally 12 to 18 inch in height, with numerous small white flowers. The seeds are enclosed in capsules. Generally, psyllium is cultured for its mucilage content, which is a white fibrous material with hydrophilic property. The mucilage can be obtained by mechanical milling/grinding, and is usually referred to as husk. Interest in psyllium arose due to a variety of health benefits. In world market, India is the primary country that produces and exports psyllium, while the United States is the largest importer of psyllium, and over 60% of imported psyllium husk is used in pharmaceutical products (Cheng *et al*., 2004).

High serum cholesterol level is an important risk factor of cardiovascular disease (CVD). Studies have been done to explore the effect of psyllium on patients with CVD, administered the soluble fraction from psyllium incorporated into a low-fat diet. Thus, conclusion that soluble fraction of psyllium presented a more beneficial effect than insoluble fiber for CVD secondary prevention was drawn (Sola *et al* ., 2007) .

Psyllium is thought to act as a hypocholesterolemic supplement due to its function of inhibiting fat absorption, as well as increasing bile acid excretion. Since fat absorption and bile acid excretion is meal-related, psyllium can perform the most effect when combined with meal. Psyllium is a very useful dietary fiber in

terms of lowering cholesterol with great application potential. The underlying mechanisms of the hypocholesterolemic effect of psyllium were explored for many years. It was found that psyllium does not function as a bile acid sequestrant which is the main function of cholestyramine. Many studies have shown that psyllium may also possess anticarcenogenic properties, especially for colon cancer and breast cancer. Although the underlying mechanisms of psyllium's cancer prevention capacity are still not thoroughly clear, a large body of research has been done to reveal the possible effects (Cheng *et al*., 2004; Nakamura *et al.*, 2005).

The present study conducted to evaluating the effects of different ratios of Doum and psyllium husk seed as a source of dietary fiber on the serum lipid profile and some organs (colon, aorta , heart and brain) of rats suffering from increase serum cholesterol.

MATERIALS AND METHODS

Materials:

Doum (*Hyphaene thebaica* L.):

Samples of Doum were obtained from Al-Azher district, they were additionally air dried for 1 h. It was broken to small pieces and then ground to a powder form with hammer mill.

Psyllium seed husks (*Plantago genus*)**:**

These were obtained from Al-Azher district (it is called red katona or lesan El-hamal). they were additionally air dried for 1 h., ground to a powder form with hammer mill.

Methods:

Proximate analysis

Moisture, protein, fat, crude fiber and ash content of the investigated samples were carried out according to the (AOAC, 2000). The total carbohydrates are given by difference.

Dietary fiber content

Dietary fiber (DF) content was determined by an enzyme method according to AOAC (2000). The sum of insoluble (IDF) and soluble dietary fiber (SDF) contents, determination of insoluble dietary fiber compounds cellulose, hemicellulose and lignin were estimated according to Perez *et al.* (1997).

Biological experiment:

Animals and Diets:

One hundred and twenty female albino rats weighing about 160 ±5 g, one month of age were purchased from Research Institute of Ophthalmology Giza-Egypt. The animals were acclimatized for one week as an adaptation period. Rats were then divided into 6 groups, each of 12 rats. The first control group (negative) was fed the standard diet without added cholesterol or fiber sources with the composition given in Table (1). The 2nd positive control was fed on a diet containing 1% cholesterol (C) and 0.2% cholic acid for one week. Each group of the other 4 groups was fed diet containing 1% cholesterol and 0.2% cholic acid plus 10 or 20% of powdered doum and Psyllium husks. Rats had free access to food and tap water. The investigated six diets were fed for a period of 60 days. Rats were housed one animal in each cage with a bedding of wood shavings. The animal room was temperature controlled (22±2°C) and had a 12 h light –dark cycle (light on at 5:00 -17:00 h) and relative humidity of 50%.

Diets were first prepared without fiber sources addition. Fiber sources (doum and Psyllium husks powders) were additionally air dried for 1 h. Fiber sources were ground with hammer mill and screened through screens with pore size 80µm .

Table (1). Composition of six experimental diets (%).

Ingredient	Group					
	1	**2**	**3**	**4**	**5**	**6**
Casein	20	20	20	20	20	20
Corn oil	10	10	10	10	10	10
Corn starch	60	58.8	48.8	38.8	48.8	38.8
Cellulose	5	5	5	5	5	5
Mineral mixa	4	4	4	4	4	4
Vitamin mix	1	1	1	1	1	1
Cholesterol		1	1	1	1	1
Cholic acid		0.2	0.2	0.2	0.2	0.2
Doum			10	20		
Psyllium husks					10	20
Total	100	100	100	100	100	100

Source: **(Balasinska , 1998)**

Powders of fiber sources were added to the aforementioned prepared diets (Table 1) at 10 and 20 %. The diets were fed to rats every day. Foods were introduced at a rate of 25 g/day. The average food intake per day for each rat was calculated and feces were gathered and weighed one day each week.

Feces **(**Stools) of rats of different groups were collected and weighed after 24 hrs on the 30th and 60th day.

Biological Determination

Biological evaluation of the different tested diets was carried by determination
(average of food intake (FI) and feces output), body weight gain% (BWG %) and organs weight / body weight % according to Chapman *et al.* (1959) after 30 and 60 days.

BWG % = [(Final weight - Initial weight) / (Initial weight)] X 100

Organ weight/ body weight % = (Organ weight / Final weight) X 100

Analytical methods of serum

After 30 days of the experimental, six rats were selected randomly from each group, then the animals were anesthetized with ether and sacrificed and the brain, heart, aorta and colon were taken. Colon was cut longitudinally and the feces were collected.

The followed steps were done in 6 rats after 30 and 60 days of treatment in each group according to Schermer (1967).

The treated animals were fasted for 12 h. Blood samples were withdrawn from orbital plexus venous by using fine capillary glass tubes, placed in centrifuge tubes without anticoagulant and allowed to clot. After the serum prepared by centrifugation (3000 rpm for 15 min), to obtain clear serum and frozen at -18^{o}C until analyzed. Then animals were anesthetized with ether and sacrificed and quickly dissected to take the brain, heart, aorta and colon. These organs were weighed and heart, aorta and colon then kept until histological investigations.

Biochemical analysis

Serum cholesterol and triglyceride concentrations were measured enzymatically according to Richmond (1973) and Fassati and Principe (1982), respectively.

Lipoproteins (HDL-C and LDL-C) were isolated by precipitation and centrifugation and then enzmatically determined (lopezo-Virella *et al.*, 1977; Wieland and Seidel , 1983 , respectively).

Total lipids were determined by their reaction with sulfuric, phosphoric acids and vanilline to form pink color complex (Zollner and Kirsch , 1962) .

Histopathology of colon, aorta and heart:

The tissues sample from the colon, aorta and heart and brain of rats in different groups were fixed immediately after dissection in 10% formalin saline for 24 h., then dehydrated in ascending grades of alcohol, cleaned in xyline and embedded in paraffin wax. Tissues were sectioned at a thickness of 3 micron and stained with hematoxylin and eosin stains (Banchroft *et al.,* 1996) and were examined by the light microscope for detection of any histopathological alterations.

Statistical analysis

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System (SAS , 1996) .

RESULTS AND DISCUSSION

Chemical composition of doum and psyllium husks powders:

Table (2) shows the results of chemical composition of Doum and Psyllium husks. It was clear that samples contained high levels of carbohydrate being 94.94% and 89.85% for Psyllium husks and Doum, respectively. Crude fiber of Psyllium husks was 6.7%, while it was 13.62% in Doum sample. On the other hand, the same samples had either very low levels of fat being 0.01 and 0.43 % for Psyllium husks and Doum, respectively. These results agree with those reported by Souci *et al.* (2000). On the other hand, the moisture content in the investigated plants were 6.04% in Doum and 7.0% in Psyllium husks. Ash varied between 3.66% in Psyllium husks and 6.52% in Doum. Many beneficial properties of these plants can be utilized in novel dietary fiber functional foods that can target special needs of populations.

Table (2): Chemical composition of tested samples (% dry matter)

Constituents (%)	Mean ±SDM of Samples	
	Doum	Psyllium husks
Moisture	6.04±0.14^{c}	7.00±0.12^{a}
Ash	6.52±0.18^{b}	3.66±0.07^{d}
Protein	3.20±0.07^{c}	1.40± 0.14^{d}
Fat	0.43±0.15^{c}	0.01±0.01^{d}
Crude fiber	13.62±0.06^{b}	6.70± 0.14^{d}
Carbohydrate	89.85±0.10^{b}	94.94±0.32^{a}

Data are presented as means ± SDM (n=3).
Means within a row with different letters are significantly different at P≤ 0.05.
Carbohydrates : calculated by difference

Fiber analysis of doum and psyllium husks powders:

Dietary fiber (DF) means that fraction of the edible parts of plants or their extracts, or synthetic analogues, that are resistant to the digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides (degree of polymerization >2) and lignins, and promotes one or more of the following

beneficial physiological effects; laxation, reduction in blood cholesterol, modulation of blood glucose (FSANZ, 2001; Baljit and Nirmala, 2010**).**

DF is one of the major phytochemicals present in the selected sources Doum and Psyllium husks. It can be divided into two categories insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) according to their water solubility. Water soluble fraction (SDF) consists mainly of nonstarchy polysaccharides such as beta-glucans and pentosans (arabinoxylan). The total dietary fiber (TDF) of the studied samples Doum and Psyllium husks were significantly different, varied between 80.95% Psyllium husks and 38.64% of Doum as seen in Table (3).

Table (3): Dietary fiber fractions of tested sample.

Fiber fractions %	Mean ±SDM of Samples	
	Doum	Psyllium husks
TDF	38.64±0.50 [b]	80.95±0.24 [a]
IDF	12.32±0.65 [b]	1.15 ±0.64 [d]
SDF	26.32 ±1.0 [b]	79. 80 ±0.53 [a]
Fiber fractionation		
Celluloses	13.67±0.32 [c]	5.93±0.18 [d]
hemicelluloses	6.78±0.31 [b]	44.28±0.31 [a]
Lignin	10.84±0.08 [a]	0.44±0.08 [d]

Data are presented as means ± SDM (*n=3*.
Means within a row with different letters are significantly different at P≤ 0.05.

It is well accepted that a high intake of soluble fiber is associated with favorable affects on human health (Anderson *et al.,* 2000). Psyllium husks is an excellent source of natural soluble fiber and contains more soluble fiber than Doum. Soluble Fiber is known to decrease serum cholesterol, postprandial blood glucose, and insulin levels (Edge *et al.*, 2005). Psyllium husk has been reported as a medicinally active natural polysaccharide. It has been used for the treatment of constipation, diarrhea, inflammation bowel diseases-ulcerative colitis (Ramkumar and Rao, 2005), obesity in children and adolescents (Pittler and Ernst, 2004), high cholesterol (Moreyra *et al.,* 2005) and diabetes (Anderson *et al.*, 1999) as well as colon cancer (Singh, 2007).

The insoluble DF fractions represent about 31.9 and 1.4% from TDF for Doum and Psyllium husks, respectively. Traditional insoluble fibers as required to add bulk as well as rapidly fermentable, viscous fibers to bring about cholesterol lowering. Nidhi *et al.* (2016) reported that, the seeds husk contain about 78% soluble fibers. It has a scientifically proven use for treatment of constipation, diarrhoea, inflammatory bowel disease-ulcerative colitis, obesity in children and adolescents reducing high LDL-cholesterol reducing hyperglycaemia, reducing risk of colon cancer, and weight management.

The UK recommending an average intake of 18 g non starch polysaccharides (NSP). Recommendations for dietary fibers intake in children have recently been reviewed and a new recommendation of age (years)+5 g to age (years)+10 g as an indication for fiber intake at ages between 2 and 20 years has become widely accepted. Whereas intake of 5 g/day is recommended in the weaning period. As regard to the three dietary fiber fractions celluloses, hemicelluloses and lignin, were 13.67, 6.78 and 10.84% in

Doum and 5.93, 44.28 and 0.44 % in Psyllium husks, respectively.

Biological evaluation of Doum and Psyllium husks powders on experimental rats:

Food consumption

Table (4) represents the food consumption of each group of the albino rats. Among the experimental groups, however, no substantial differences of food consumption were observed and food consumption did not significantly ($P \leq 0.05$) differed.

Animals fed on diets containing Psyllium husks at levels of 10 and 20% followed by those fed on diets containing the Doum at the same both levels produced larger fecal weight. In cantrast, a smaller fecal weight was observed for the control positive (+ve) animals in the same Table after 30 and 60 days.

Table (4): Mean food intake and weight of feces (g) of experimental rats fed on different ratios of Doum and Psyllium husks during 60 days.

Concentration	Mean ±SDM of food intake and weight of feces (g/day/rat)			
	After 30 days		After 60 days	
	Food intake	Feces	Food intake	Feces
Control (-ve)	24.1±0.8	4.2±0.5	24.8±0.6	4.0±0.7
Control (+ve)	24.5±0.7	3.6±0.6	25.0±0.2	3.9±1.1
Doum				
10 %	24.2±0.7	4.9±0.6	23.2±0.3	4.7±0.7
20 %	24.3±1.3	5.0±0.5	24.6±1.0	4.7±0.6
Psyllium husks				
10 %	24.5±1.4	5.6±0.6	24.7±0.8	5.0±0.8
20 %	25.0±1.1	5.3±0.7	24.9±1.3	5.1±0.5

Body weight gain of experimental rats fed on different concentrations of Doum and Psyllium husks:

Body weight change is often a very sensitive indicator of animal well being it integrates many other parameters and often, in particular, food consumption. The final body weights and body weight gain (%) of all rats groups after 30 and 60 days of treatment are given in Table (5). BWG% were slight significant different as compared to control (-ve) group 17.39±0.57. On the other hand, control (+ve) group fed on standard diet with cholesterol (1%) and cholic acid (2%) was 20.50±0.12 increased as compared to the other groups during the whole experiment period up to 60 days. The lowest rates of body weight gain occurred in those groups fed on diets containing 20% Psyllium husks (17.31%) and 10% (17.95%) of the same sample followed by Doum (10 and 20%) 18.13 and 19.62%, respectively.

Table (5): Mean body weight gain (g) of albino rats fed on different ratios of Doum and Psyllium husks during 60 days.

Body weight (g)	Treatments					
	Control (-ve)	Control (+ve)	Treated by			
			Doum (%)		Psyllium husks (%)	
			10	20	10	20
IBW	161±1.08[a]	161±1.87[a]	160±2.73[a]	158±2.0[b]	156±2.9[c]	156±1.0[c]
7	166±2.22[a]	164±1.93[c]	164±1.88[c]	165±2.42[b]	164±3.86[d]	162±1.6[e]
15	168±1.65[f]	171±2.92[c]	170±2.21[d]	171±1.69[c]	169±4.07[e]	163±2.3[g]
22	171±1.69[e]	174±3.05[b]	173±2.05[c]	174±1.16[b]	171±4.15[e]	166±2.1[f]
30	175±2.67[b]	177±0.67[a]	175±0.48[b]	177±1.12[a]	171±1.56[d]	170±1.12[e]
37	181±1.56[a]	179±1.43[b]	176±2.67[d]	178±1.77[c]	174±2.9[e]	174±2.52[e]
45	184±2.92[c]	189±2.21[a]	183±1.42[d]	181±0.72[f]	176±1.12[g]	177±2.76[h]
52	187±1.07[d]	192±3.07[a]	186±2.02[e]	186±0.85[e]	182±2.92[g]	180±1.3[h]
FBW	189±1.7[c]	194±1.65[a]	189±1.92[c]	189±0.74[c]	184±1.33[e]	183±0.76[f]
BWG%	17.39±0.57[a]	20.50±0.12[c]	18.13±0.30[b]	19.62±0.63[b]	17.95±0.54[a]	17.31±0.24[a]

Data are presented as means±SDM (*n*= 12 up to 30 days and n=6 up to the 60 days).
Data in a row with different superscript letters are statistically different ($P \leq 0.05$).
IBW= Initial body weight; FBW= Final body weight; BWG= Body Weight gain

Organs weight/body weight % of experimental rats treated with Doum and Psyllium husks for 30 and 60 days:

Heart and brain weights after 30 and 60 days are presented in Table (6). The heart weight ranged between 0.5 and 0.9 g after 30 days of feeding on the different diets. After 60 days the average weight for the 6 groups ranged between 0.7 and 0.9 g. Only the hearts of control negative group and rats fed on diets with added 10 and 20% Psyllium husks weighed significantly less than the Doum groups. It could be seen in Table (6) that rats fed on Doum 20% weight loss in their hearts after 60 days when compared with weight after 30 days. The rat groups 10 and 20% Doum and Psyllium husks, respectively showed decrease in brain weight after 60 days of feeding when compared to their weights after 30 days,

Table (6): Mean weight (g) of hearts and brains of albino rats fed on different diets after 30 and 60 days.

Concentration	Mean ±SDM of organs weight			
	Heart		Brain	
	30 days	60 days	30 days	60 days
Control (-ve)	0.6±0.17[bc]	0.9±0.07[a]	1.3±0.20[a]	1.5±0.07[a]
Control (+ve)	0.8±0.1[bc]	0.8±0.14[a]	1.3±0.11[a]	1.4±0.07[a]
Doum				
10 %	0.9±0.20[ab]	0.8±0.07[a]	1.2±0.26[a]	1.2±0.05[abc]
20 %	0.9±0.11[ab]	0.7±1.3[a]	1.2±0.17[a]	1.1±0.28[bc]
Psyllium husks				
10 %	0.5±0.11[c]	0.8±0.07[a]	1.1±0.20[a]	1.1±0.42[bc]
20 %	0.6±0.15[c]	0.8±0.21[a]	1.2±0.05[a]	0.9±0.35[c]

Data are presented as means±SDM (*n*= 12 up to 30 days and n=6 up to the 60 days).
Data in a row with different superscript letters are statistically different ($P \leq 0.05$).

Blood lipids profile

Female rats were more susceptible to diet induced changes in serum cholesterol (c) level than male rats, there rate in female rats were chosen for the present investigation. The prevalence of obesity has increased dramatically over the past decade. It is reported that in the year 2000, more than 64% of the US population was either overweight or obese. In Egypt this ratio reaches about 75% in female over 30 years of age and 50 % in male of the same age group (MOHP, 2010). Epidemiologic support that dietary fiber intake prevents obesity is strong. Fiber intake is inversely associated with body weight and body fat. In addition, fiber intake is inversely associated with body mass index at all levels of fat intake after adjusting for confounding factors.

Data in Table (7) showed lipid profile of the different 6 groups of albino rats fed different diets. With regard to cholesterol level, control (-ve) group without added cholesterol showed significantly the lowest cholesterol (109 mg/dl). Addition of Psyllium husks powder to the hypercholesteremic diets was the most effective in reducing cholesterol formation in blood. Although groups 10 and 20% Psyllium husks powder contained 1% cholesterol, they showed only 18.3 and 5.5% increases in cholesterol level after 30 and 60 days, respectively, as compared to 241% increase in control +ve group. Doum powder addition to rat diets also was very effective in reducing the rate of cholesterol formation in the blood. Thus after 30 days the rate of increase in cholesterol level was about 85.3 and 84.4% in the diets containing 10 and 20 % Doum powder, respectively as compared with control -ve group. Again after 60 days the rate of increase in blood cholesterol dropped to about 50.5 and 32.4% in the same sample, respectively. Improvements in cholesterol levels as a result of fiber supplementation would be expected to result in low serum cholesterol levels based on the efficiency and structure of different fiber sources (Doum and Psyllium husks powder)

The group fed on the cholesterol-rich diet (control +ve) altered serum lipid concentration causing a marked hyperlipidemia. Serum lipids profile showed an increased level of total lipids, cholesterol and LDL as a result of induced hypercholesterolemia; their concentrations in control +ve group (without addition of dietary fiber) were 851, 372 and 203 mg/dl, respectively.

Supplementation of cholesterol rich diets (1% cholesterol +0.2% cholic acid) with dietary fiber sources (Doum and Psyllium husks powder) reduced cholesterol and total lipids formations compared to control +ve group. The rats fed on free cholesterol control –ve diet showed significantly the lowest cholesterol level in the serum when compared to those fed on fiber-rich diet with added cholesterol (Table 7). Generally lower serum cholesterol was obvious clear in the rats fed on a high fat cholesterol diet when given 10 or 20 % dietary fiber (Doum and Psyllium husks powder). High density lipoprotein (HDL) was high in rats serum fed on diets supplemented with dietary fiber

Table (7) showed atherogenic indices of the 6 rat groups. Very high atherogenic indices were obvious in control +ve group fed on the cholesterol–rich diet being 9.63 and 9.86 after 30 and 60 days, respectively. Far below levels were found in either the negative control group or the remaining 4 groups fed on diets supplemented with sources Doum and Psyllium husks powder rich in dietary fibers at levels of 10 and 20%.Most effective of dietary fiber were found in rat groups fed on diets supplemented with 20% Doum and Psyllium husks powder. Their athergenic indices were 1.28 and 0.24 after 30 days followed by 0.15and o.33 after 60 days. The results shown in Table (7) are optimistic that is to say, when dietary fibers especially from Doum and Psyllium husks powders were incorporated into cholesterol- rich diets, drastic improvement in atherogenic indices was obvious especially after 60 days of feeding.

Table (7): Effect of different levels of Doum and Psyllium husks powder in rats diet on lipid profile.

Lipid Profile (mg/dl)	Treatments					
	Control (-ve)	Control (+ve)	Treated by			
			Doum (%)		Psyllium husks (%)	
			10	20	10	20
After 30 days						
Total Lipid	391±10.3^{c}	851±13.8^{a}	506±18.0^{bc}	555±19.4^{b}	439±27.3^{cb}	598±17.5^{b}
Cholesterol	109±3.2^{b}	372±4.4^{a}	202±11.0^{b}	201±13.0^{b}	129±7.5^{c}	115±12.7^{c}
Triglyceride	56±5.0^{c}	72±1.3^{b}	70±10.1^{b}	63±12.0^{bc}	56±1.4^{c}	81±8.8^{a}
LDL	54±5.0^{dc}	203±6.0^{a}	90±4.4^{bcd}	68±9.7^{cde}	67±10.7^{cde}	39±4.3^{e}
HDL	57±4.0^{e}	35±1.4^{f}	59±2.3de	88±6.2^{ab}	78±6.6^{cd}	93±9.5^{a}
A.index	0.91	9.63	2.42	1.28	0.65	0.24
After 60 day						
Total Lipid	404±12.2^{d}	859±30.0^{a}	522±18.6^{bc}	526±20.2^{bc}	489±14.5^{cd}	598±22.2^{b}
Cholesterol	105±1.8^{f}	391±1.7^{a}	158±1.6cde	139±7.6^{def}	142±1.8^{def}	128±3.5^{ef}
Triglyceride	65±5.7^{b}	137±5.3^{a}	67±8.5^{b}	61±2.5^{b}	53±1.4^{bc}	77±2.6^{b}
LDL-C	55±5.6^{de}	199±4.9^{a}	88±2.0^{bc}	66±1.4^{cd}	63±8.4^{cde}	62±1.5^{e}
HDL-C	61±6.9^{dxc}	36±1.2^{e}	72±10.0^{cd}	121±4.9^{a}	81±6.5^{bcd}	96±1.3^{bc}
A.index	0.72	9.86	1.19	0.15	0.75	0.33

HDL,high-density lipoprotein; **LDL**, low-density lipoprotein
A.index (Atherogenic index) = (total cholesterol- HDL cholesterol) / (HDL cholesterol)$^{-1}$
Data are presented as means±SDM (n= 12 up to 30 days and n=6 up to the 60 days).
Data in a row with different superscript letters are statistically different ($P \leq 0.05$).

Histopathological examination:
Colon:

The colon is a dynamic organ involved in the absorption of salts, fluids, and nutrients, and it has a primary role in defecatory function. Also, the colon is an immunologically active tubular cavity playing an important part in host immune responses and defense from pathogens. The colon is a continuous structure originating at the ileocecal valve and extending to the anus (Maqbool, 2006) and Vogt *et al.,* (2014). Table (8) and Figure (1) show the histopathological alterations elucidated upon feeding rats on the different experimental diets for 30 and 60 day. The original rationale for adding dietary fiber to supplement rat's diets was to normalize bowel function. Dietary fiber is usually promoted as a preventive against constipation for normal healthy populations. Supplemented formulas containing fiber are also used in acute-care settings to prevent diarrhea associated with tube feeding (Fukata and Arditi, 2013). After 30 days feeding with diets containing 10 and 20% rich dietary fiber sources (Doum and Psyllium husks poewder) less histopathological alterations were induced in rat's colon when compared to rats fed hypercholesterolemic diets. After 60 days of feeding period, diet 3 still had the preventive effect on colon, whereas group 6 (with added 10% Psyllium husks) showed complete recovered of rats colon.

Table (8): Description of histopathological alterations induced in colon of rat groups fed different rich-sources of dietary fiber.

Group	No	Histopathological alteration
After 30 days		
Control -ve	1	Normal histological structure of the mucosal layer (mu) and muscularis (ml)
Control +ve	2	Focal lymphoid cell aggregation in the mucosal layer (m)
Doum 10%	3	
Doum 20%	4	Intact histopathological structure of mucosa (mu) and other layer
Psyllium husks 10%	5	Focal lymphoid cells aggregation in mucosal (m) with oedema (o)
Psyllium husks 20%	6	Inflammatory cells infiltration in mucosa and submucosal layers(m) with oedema
After 60 days		
Control -ve	7	Normal histological structure of mucosa (m), submucosa(sm) and senosa(s)
Control +ve	8	Lymphoid cells aggregation and proliferation were observed in submucosal layer
Doum 10%	9	Destruction of mucosa (m) with inflammatory cells infiltration in both mucosal and submucosal layerts(m)
Doum 20%	10	
Psyllium husks 10%	11	There was no histopathological alteration observed
Psyllium husks 20%	12	Showing fibrosis in mucosal layer

Aorta:

The aorta is the main blood vessel of the body. While congenital lesions and sinus of valsalva aneurysms are rare, they can present in adult life and lead to sudden death due to aortic rupture with haemorrhage. The most common disease of the aorta is atherosclerosis which may lead to obstruction with ischaemia, aneurysm formation with rupture and atherombolism. The normal aorta arises from the aortic sinus which contains the aortic valve and continues up and over to the left as the ascending aorta, the arch which arches over the left hilum of the lung and the descending thoracic and abdominal aorta.

In the present investigation, the control rats group fed on the control diets and subjected to examination after 30 and 60 days showed no histopathological alteration; a normal histological structure of myocardium was recorded in Figure (2). With regard to the rats of control + ve which suffers induced hypercholesterolemia their aorta showed diffuse vacuolization in the media after 30 and 60 days of feeding on diets with added cholesterol. The histopathological changes in aorta of rats fed on high-cholesterol diet supplemented with 10 or 20 % rich fiber sources (Doum and Psyllium husk powders) were shown in Figure (2).

In the rats of groups Doum 10 and 20%, there was vacuolization either mild or sever after 30 days. In contrast hypercholestremic diets supplemented with 10 or 20% Psyllium husk powders did not show histopathological alteration in rats aorta (Fig. 2)

Dietary interventions for lowering blood cholesterol (Table 7) are a major focus in prevention and treatment of cardiovascular diseases. Soluble fibers found in (Doum and Psyllium husk powders) have been considered and proved herein to be effective in the treatment of hypercholesterolemia. Along 60 days albino rats were fed on diets supplemented with 10 and 20% rich fiber sources (Doum and Psyllium husk powders) to elucidate different hypercholesterolemic effect of different dietary fiber on female rats. The aorta of rat group Psyllium husk 10 % showed complete recovery and repairing. Also Psyllium husk powder 20% was very effective in lowering plasma cholesterol and did not only reduce atherogenic

indices but also caused a complete recovery of diseased aorta due to hypercholesterolemia.

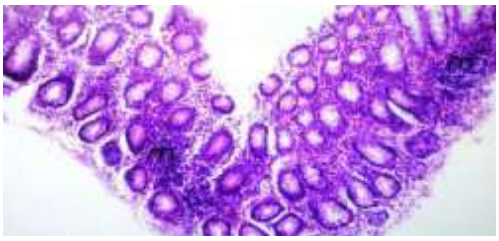
Heart

After 30 and 60 days, the hearts of rats of control –ve groups were fed on the standard diet did not show any histopathological alteration. A normal histological structure of myocardial bundle was recorded and illustrated in Figure (3)

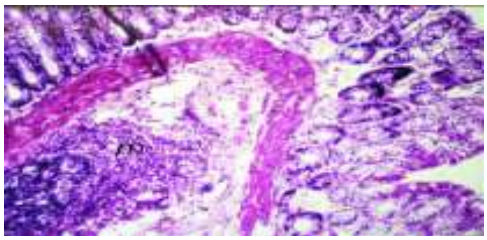
The experimental animals fed on diet containing 1% cholesterol +0.2% cholic acid without added dietary fibers (control +ve) suffered myocardium focal haemorrhage as well as focal inflammatory cells infiltration (after 30 days) whereas after 60 days degenerative changes were also observed in the myocardium in Figure (3-6). When the hearts of rats fed on diets supplemented with different level of (10 and 20%) dietary fiber (Dome and Psyllium husk powders) were subjected to examination under the light microscope, different symptoms and signs were observed either after 30 or 60 days

The animals of groups Doum 10%, Doum 20% and Psyllium husk 10% powders (described as hyper-cholesterolaemic) for 30 days, showed sclerosis in the wall of myocardial blood vessels (groups Doum 20% and Psyllium husk 10% powders) associated with focal inflammatory cells infiltration in the myocardium (group Doum 10%,) . It is of great importance to say that up to 20% of Psyllium husk powder in the diet could prevent and recover any histopathological alterations even in a short period (30 days) (Fig.3-4).

Doum and Psyllium husk powders added at both levels to hypercholesteremic diets could recover the indices of their diseased heart after 60 days of feeding. This means that both ingredients are effective therapeutic items and the other diets have their therapeutic effect in preventing and recovering atherosclerosis and cardiovascular disease. The benefits of increased dietary fibers intake were also confirmed in long term studies on rats. Additional benefits to experimental animal health in delaying the emergence of defects in either aorta or heart even with hypercholestremic diet intake

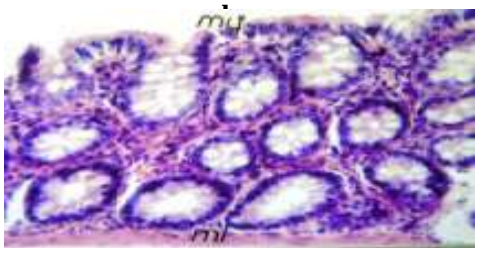


1) Control –ve after 30

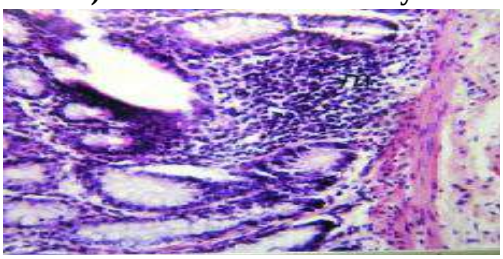


7) Control –ve after 60

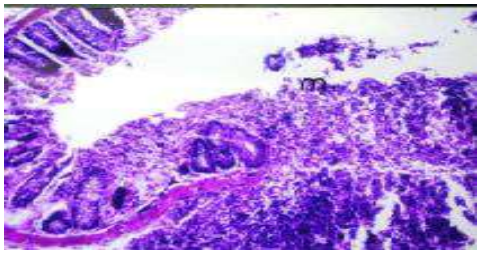
2) Control +ve after 30 days



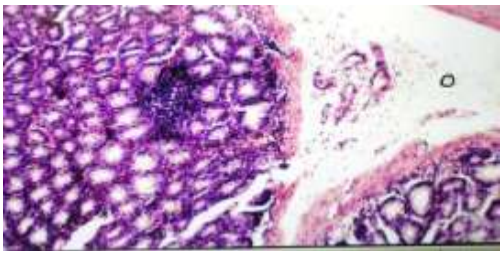
8) Control +ve after 60 days



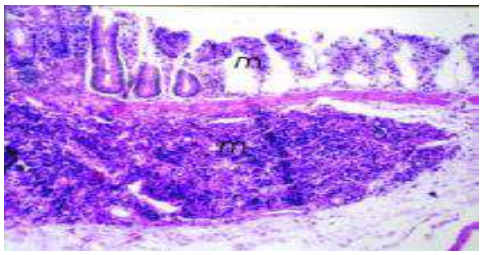
3) Doum 10% after 30 days



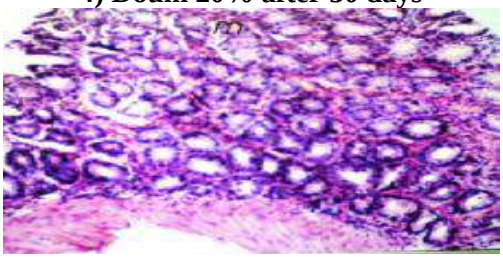
9) Doum 10% after 60 days



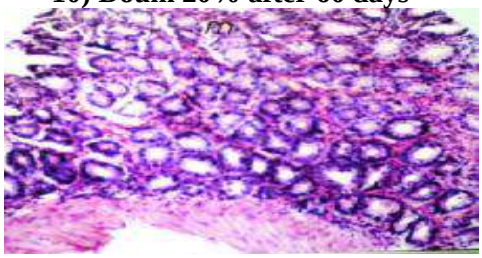
4) Doum 20% after 30 days



10) Doum 20% after 60 days



5) Psyllium husks 10% after 30



11) Psyllium husks 10% after 60

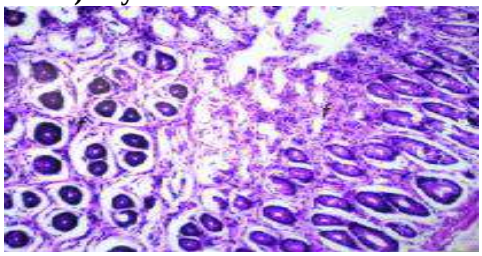
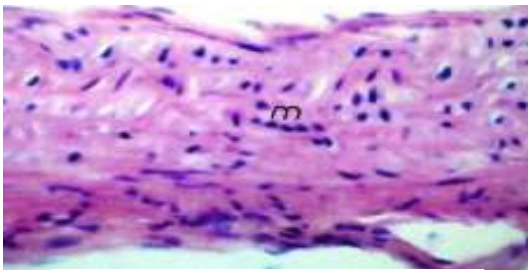
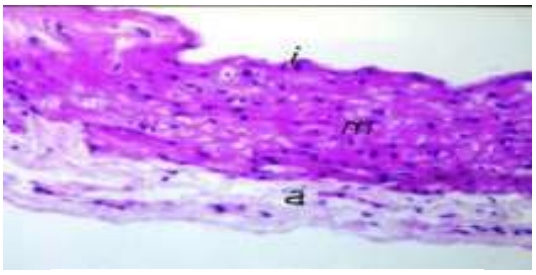


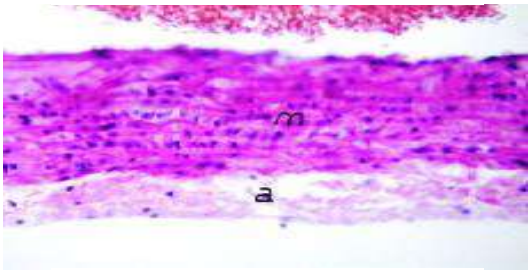
Fig. 1: Photomicrograph of colon of different rats groups, stained with H & E, X 400



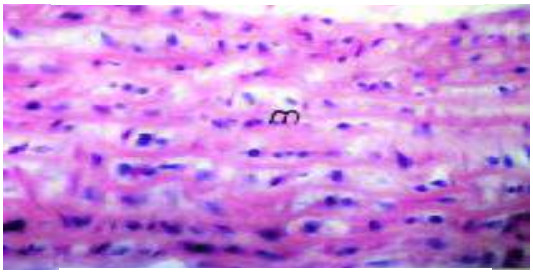
1) Control –ve after 30 days



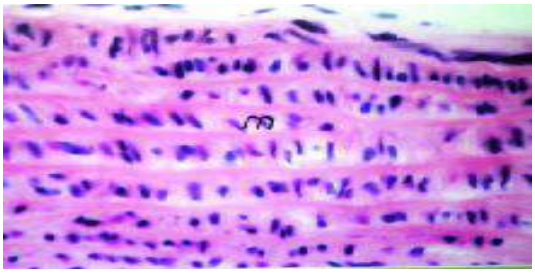
4) Control –ve after 60 days



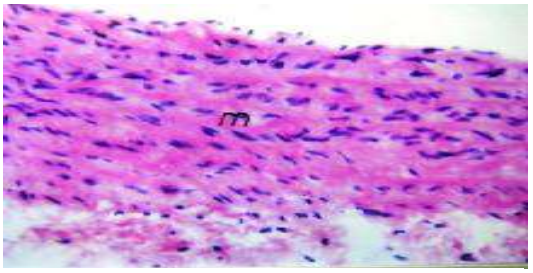
2) Control +ve after 30 days



5) Control +ve after 60 days



3) Doum and Psyllium husk 10, 20% after 30 days



6) Doum and Psyllium husk 10, 20% after 60 days

Fig. 2: Photomicrograph of aorta of different rats groups, stained with H & E, X 400

Fig. (2-1): Control negative group rat (fed on standard diet for 30 days) showing the normal histological structure of tunica media (tm).

Fig. (2-2): Control positive group (fed on standard diet + 1% cholesterol +0.2% cholic acid for 30 days) showing thickening in adventitia (a)

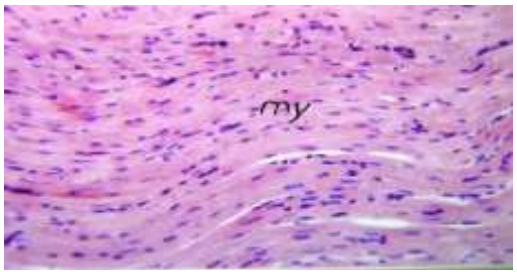
Fig. (2-3). hypercholestremic rats (fed on tandard diet + 1% cholesterol +0.2% cholic acid + Dome and Psyllium husk powders for 30 days) groups 10and 20% showing intact histolgical structure of the media (m).

Fig. (2-4): Control negative group rat (fed on standard diet for 60 days) showing normal histological structure of the Intima (i),media (m) and adventitia(a).

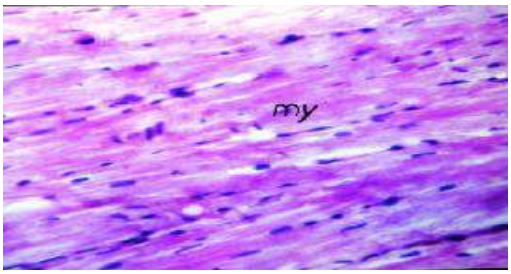
Fig. (2-5): Control positive group (fed on standard diet + 1% cholesterol +0.2% cholic acid for 60 days) showing vacuolization in the media (m)

Fig. (2-6): hypercholestremic rats (fed on standard diet + 1% cholesterol +0.2% cholic acid + Dome and Psyllium husk powders for 60 days) groups 10 and 20% showing intact histolgical structure of the media (m).

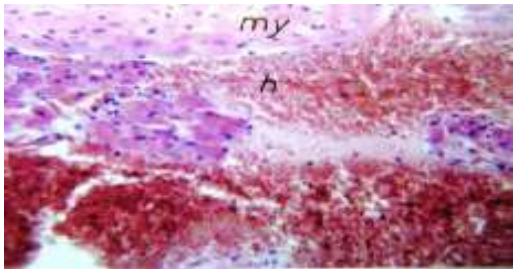
Biological and histopathological effects of psyllium husk seed and dome in hypercholesterolemic rats



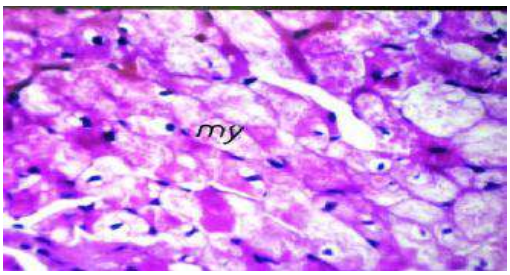
1) Control –ve after 30 days



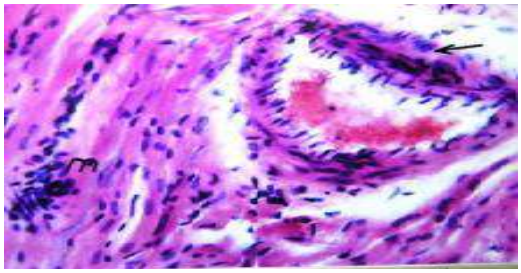
5) Control –ve after 60 days



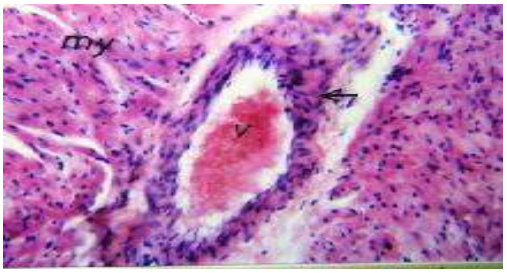
2) Control +ve after 30 days



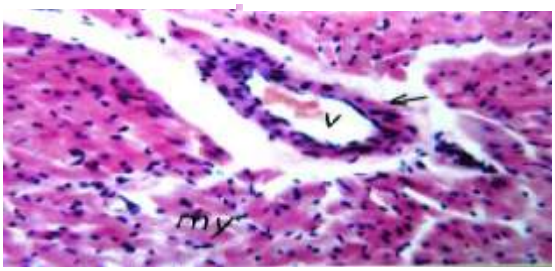
6) Control +ve after 60 days



3) Doum 10% after 30, 60 days



7) Doum 20% after 30, 60 days



4) Psyllium husk 10, 20% after 30, 60 days

Fig. 3: Photomicrograph of heart of different rats groups, stained with H & E, X 400

Fig. (3-1,5): Control negative group rat (fed on standard diet for 30 and 60 days) showing the normal histological structure of myocardium (my).

Fig. (3-2): Control positive group (fed on standard diet + 1% cholesterol +0.2% cholic acid for 30 days) showing focal haemorrhage (h) in myocardium (my)

Fig. (3-3). hypercholestremic rats (fed on standard diet + 1% cholesterol +0.2% cholic acid + Dome 10% for 30 and 60 days) showing sclerosis in blood vessels (arrow) and focal inflammatory and infiltration cells (m) in myocardium

Fig. (3-4): hypercholestremic rats (fed on standard diet + 1% cholesterol +0.2% cholic acid + Psyllium husk 10, 20% for 30 and 60 days) showing sclerosis in the wall of congested myocardial blood vessels

Fig. (3-6): Control positive group (fed on standard diet + 1% cholesterol +0.2% cholic acid for 60 days) showing myocardial degeneration (my)

Fig. (3-7): hypercholestremic rats (fed on standard diet + 1% cholesterol +0.2% cholic acid + Dome and Psyllium husk powders for 30 and 60 days) groups 10 and 20% showing sclerosis of myocardial blood vessels

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التأثيرات البيولوجيه والهيستوباثولوجية لقشور بذور لسان الحمل والدوم على الفئران المصابه بالكوليستيرول

سماح محمد إسماعيل
قسم الإقتصاد المنزلى - كلية التربية النوعية - جامعة عين شمس – القاهرة- مصر

المستخلص

تهدف هذه الدراسة إلى معرفة تأثير نسب مختلفة من قشور بذور لسان الحمل والدوم كمصدر نباتى للألياف الغذائية على خفض نسبه دهون الدم فى الفئران المصابه بأرتفاع نسبه الكوليستيرول. حيث أوضحت النتائج ارتفاع نسبه الكربوهيدرات فى قشور بذور لسان الحمل 94.94% بينما كانت 89.85% فى الدوم. فى حين كانت نسبه الألياف الخام 6.7% فى قشور بذور لسان الحمل و 13.62% فى الدوم. كما سجلت نتائج الألياف الغذائية أرتفاع نسبتها فى قشور بذور لسان لحمل 80.9% بينما سجلت 39% فى الدوم, وفيما يتعلق بمشتقات الألياف الغذائية فقد سجل الهيموسليلوز نفس الأتجاه بينما أظهرت نتائج السيليوز واللجنين أتجاه معاكس فقد كانت نسبتها أعلى فى الدوم عن قشور بذور لسان الحمل.

استخدمت في هذه الدراسة مجموعتان رئيسيتان. المجموعة الأولي (12 فأر) مجموعة ضابطة سالبة (غير مصابة) تم تغذيتها علي غذاء أساسي. المجموعة الثانية (60 فأر) تم تغذيتهم علي غذاء يحتوى علي 1% كوليستيرول ، 2% حمض الكوليك لإحداث ارتفاع فى نسبه الكلوليستيرول بالدم، و تم تقسيم هذه المجموعة الي خمسه م جموعات فرعية (12 فأر لكل منهم). المجموعة الفرعية الأولي تم تغذيتها علي غذاء يحتوى علي 1% كوليستيرول ، 2% حمض الكوليك مجموعة ضابطة موجبة (مصابة). المجموعات الفرعية الثانية والثالثة والرابعة والخامسه تم تغذيتهم علي وجبات مرتفعة فى الكوليستيرول وحمض الكلوليك ومحتوية علي 10، 20% من مسحوق قشور بذور لسان الحمل والدوم، علي التوالي.

أظهرت النتائج أن نسبه اخراج البراز فى الفئران التى تغذت على قشور بذور لسان الحمل أعلى من التى تغذت على الدوم وكذلك المجموعه الضابطه. كما أظهرت النتائج أنخفاض في النسبة المئوية للزيادة في الوزن، و كولسترول الليبوبروتينات في السيرم فى مجموعتى الفئران التى تناولت قشور بذور لسان الحمل مقارنة بالمجموعة الضابطة السالبة التى تم تغذيتها علي غذاء أساسي فقط ثم تلتها مجموعتى الفئران التى تغذت على الدوم. كما أظهرت نتائج التشريح الهستولوجى على كل من القولون والأورطى والقلب الأثر العلاجى فى الوقايه أو علاج الأصابه من جراء إرتفاع مستوى كوليستيرول الدم وذلك بأستخدام قشور بذور لسان الحمل والدوم كمصدر للألياف الغذائية.