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Potential Effects of Germinated Legumes in Dyslipidemic Rats

Fawzi A. El-Shobaki[1], Ibrahim H. Badawi[1], Zeinab, A. Saleh[1]*, Hagar F. Elbakry[1], Maha, M. Soltan[2] and Abdel-Razik, H. Farrag[3]

[1] *Nutrition and Food Science Dept., National Research Centre, Dokki, Cairo, 12622, Egypt*
[2] *Chemistry of Medicinal Plants Dept., National Research Centre, Dokki, Cairo, 12622, Egypt*
[3] *Pathology Department, National Research Centre, Dokki, Cairo, 12622, Egypt*

Abstract

Legumes have good nutritional value and are consumed by a lot of people all over the world and particularly the relatively low-income individuals. It is thus important to always try to further improve the nutritional value of these legumes using simple processing. In the present study, chickpea and soybean were soaked and germinated before involved in a diet given to animals suffering from dyslipidemia due to consumption of animal fat (Lamb fat). The generation of bioactive peptides after germination was followed by SDS PAGE. A feeding experiment was conducted where groups of albinorats were used, some were given the diet containing fat and the others were given a diet supplemented with either chickpea or soybean once non-germinated and the other germinated seeds. The produced dyslipidemia and the change in lipid parameters in all groups were biochemically and histopathologically assessed. The results proved that the deleterious effect of feeding lamb fat was corrected to a good extent when the diet contains germinated legumes. The conclusion is that germination of legumes generates bioactive peptides. This in addition to other compounds with bioactive action such as polyphenols possesses antioxidant power that helps to prevent the deleterious effect of dyslipidemia.

Keywords: Bioactive peptides, germination, chickpea, soybean, SDS –PAGE, dyslipidemia, histopathology

1. Introduction

Legumes are the main source of protein in the diet of people in developing countries. That is because animal protein sources are scarce and costly. In addition, most vegetarians depend on legumes in their diet, since it is the best food that contains most other nutrients needed [1]. Legumes are either consumed as it is or used in preparation of different processed food. They may be also used for preparation of food forms that suits animal feeding [2]. To satisfy the nutritional demand of the increasing population word wide, the situation presents a challenge including production and magnification of the nutritional and health value of legumes. Studies done in the field of food science and nutrition always concentrate on methods or means to improve the nutritional and health value of food to magnify the benefits obtained from the meals prepared from this modified food. There are several means by which food we eat become more nutritious and healthier, perhaps the most known method is by the action of proteases from microorganisms, addition of proteolytic enzymes during food processing, or the action of gastrointestinal enzymes once ingested [3, 4]. Improving the nutritional and health value of meals is essential particularly in poor countries where food is scarce and is not enough to satisfy the nutritional and health requirements of the individuals. Simple treatment as germination or addition of enzymes lead to the production of functional molecules that may help in correcting metabolic disorders such as obesity and the associated complication as hypertension, hyperlipidemia and hyperglycemia [5].

The amino acid composition and sequence in polypeptides in the protein source is the factor that determines their bioactivity [6,7]. Also, dipeptides

*Corresponding author e-mail: zsaleh_eg@yahoo.com; (**Zeinab, A. Saleh**).
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with the proline-proline sequence in their C-terminal are the least prone to degradation by proteolytic enzymes in humans [6]. Polypeptides have other beneficial functions such as acting as antioxidants, antihypertensive, antidiabetic and antitumor[8]. Bioactive peptides obtained by fermentation of legumes have been widely pursued for protection from and prevention of health metabolic disorders such as hypertension, hyperglycemia, hyperlipidemia, and obesity [5].

Soybean and chickpea are the most two legumes now produced in so many countries in the world particularly India, China and the United States. They are widely consumed in Asia and some African countries. Chickpea is also produced on a large scale in so many countries as India,11380 metric tons, Australia 998.23, Turkey 630, Russia 620.4, and the USA 577. 57. These two crops are widely used by the low-income people and are used in so many food products. Several studies were conducted on functional bioactive peptides derived from these two legumes [5]. The products are widely pursued for restoring health against some metabolic disorders. Among these metabolic disorders is atherosclerosis that affects considerable number of people in different countries and is one of the most important risk factors for coronary heart disease (CHD)[9]. Atherosclerosis occurs due to smoking, high blood pressure, obesity associated with high levels of glucose and cholesterol in the blood.

This study was done in a trial to improve the nutritional and health value of these two legumes that are widely used as protein sources in different countries of the world. Although some studies were previously done in this area, the location and other conditions of cultivation make a difference and add to the existing findings. Further studies to confirm and lead to the production of a variety of bioactive peptides that can serve different functions are needed. The effect of the produced bioactive molecules due to the treatment followed was tested upon hyperlipidemia occurred after excessive consumption of fat.

2. Materials and Methods:

The material used for this study wasseeds of Chickpea and soybean obtained from the Agriculture Research Center, Giza, Egypt.

2.1.Seed preparations

2.1.1.Preparation of germinated seeds

Chickpea and soybean samples were carefully washed and soaked in distilled water to rehydrate at room temperature (about 20-30 ºC) for 24 hours in case of Chickpea and 12 hours for soybean. After soaking, germination was carried out in special germinators. Sprouting of seeds was performed in the dark and the sprouting temperature was "kept at 20-30 C."During sprouting, seeds were watered every day to keep relatively high humidity to support their growth, and was frequently changed, twice a day, to remove the metabolites of germinated seeds and to inhibit the growth of microbes. After 72 hours germinated seeds were collected, washed dried at 40 ºC for 12 hours.

2.1.2. Preparation of protein isolates

Legume protein isolates were prepared according to the method of Papalamprou, et al. [10] with slight modifications. The germinated seeds were ground in a household mill (Braun, Pfungstadt, Germany). The flour samples were sieved with a 600 micrometer (μm) screen and defatted twice with petroleum ether for 40 min at 25°C. The resulting slurry was centrifuged at 5000 xg for 10 min. at room temperature, the pellet was air-dried overnight, ground, and stored in an airtight container at 5º C for analysis.

- Legume flour (100 g) was mixed with distilled water at a 1:10 ratio (w/v), adjusted to pH 8.00 using 1 M NaOH, and stirred at 500 rpm for 45 min at room temperature (20–22° C).
- The suspension was then centrifuged at 4500 g for 20 min at 4 °C to collect the supernatant.
- The resulting pellet was re-suspended in distilled water at a ratio of 1:5 (w/v), adjusted to pH 8.00, stirred for an additional 45 min, followed by centrifugation (4500×g, 20 min, 4 °C).
- Both supernatants were pooled and adjusted to pH=4.5 for chickpea and soybean using 0.1 M HCl to precipitate the protein. The protein was separated by centrifugation and collected.

- The precipitate obtained was washed twice with distilled water (4 °C) and re-dispersed in distilled water and pH adjusted to 7 with 1 M NaOH.

- Isolates were lyophilized and stored at 4 °C.

- The protein concentration of the isolates was determined with the Folin phenol reagent according to Waterborg[11]. It was found to be 53.83 %, 54.17 % for chickpea raw and germinated, and 42.50%, 53.6% for soybean raw and germinated, respectively

2.1.3. SDS-PAGE Electrophoresis

Separation and analysis of peptides of the germinated or the non-germinated seeds was done according to the method of peptide analysis by SDS PAGE electrophoresis adopted by Schagger [12] with slight modification.

3. Biochemical determinations

Cholesterol and total Lipids were measured using the commercial kits (Reactivos GPL, Barcelona, Spain) according to the manufacturer protocol.Triglycerides, HDL, and LDL were determined using the commercial kits (QuimicaClinicaAplicada S.A. Spain) according to the manufacturer protocol.

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated by colorimetric methods according to Reitman and Frankel [13].

4. Animal nutrition experiment.

The experiment was conducted using 36 Sprague Dawley albino rats obtained from the animal house of the National Research Center, body weight ranging from 120 – 166 g with a mean value of 142g. The animals were divided into six groups, each 6 rats. The experimental diets' contents are shown in (Table 1).

Group 1: (Control) Rats were fed on a standard diet [14].

Group 2: Rats were fed on a high fat and cholesterol diet.

Group 3: Rats were fed on a high fat and cholesterol diet containing percentage of non-germinated Chickpea.

Group 4: Rats fed on high fat and cholesterol diet with percentage of germinated Chickpea

Group 5: Rats fed on a high fat and cholesterol diet containing percentage of non-germinated soybean.

Group 6: Rats fed on a high fat and cholesterol diet with percentage of germinated soybean. The rats were maintained in individual cages, water and food were given ad-libitum for eight weeks. Food consumption and body weight were recorded weekly.At the end of the experimental period (after 8weeks) rats were fasted and blood samples were obtained from the orbital vein and were received into clean dry centrifuge tubes. Serum was separated by centrifugation at 3000 rpm for 15 minutes and kept in deep freezer at 20 °C until used for biochemical analysis. The experimental procedure was carried out according to the institutional Animals Ethics Community of the NRC, Egypt

5. Histopathological Method:

Heart and liver were excised from each animal. Samples of heart and livers were taken and fixed in 10% neutral formalin solution at least for 24 h. Tissues were then washed in successively changed water for 24 hours, dehydrated by rising in a series of alcohol (50% - 90%), then in 2 changes of ethanol. The samples were then cleaned in 2 changes of xylol and immersed in a mixture of xylol and paraffin in the oven at 60°C. The tissues were transported to 3 changes of pure paraffin wax (melting point of 58-60°C) in the oven, and then mounted in blocks left at 4°C until the time of use. The paraffin blocks were sectioned on the microtome at a thickness of 5 □m and mounted on clean glass slides then left in the oven at 40°C till dryness. The slides were deparaffinized in 2 changes of xylol then immersed in descending series of alcohol. The ordinary hematoxylin and eosin stain was used [15].

6. Statistical analysis

Data analysis was conducted by using the statistical program (SPSS, Version 20). Meanwhile, one-way analysis of variance (ANOVA) was used for comparison of different biochemical values in various experimental groups. It was followed by Duncan's multiple range tests [16] Differences between groups were considered significant at $p < 0.05$.

3. Results and discussion

Legumes are consumed in many countries all over the world particularly in low socioeconomic ones owing to being of high nutritional value and relatively low cost. It is thus important to always try to improve their nutritive value by different means and processing to help consumers making the best use of this widespread type of food. Among those methods is presoaking and germination that are known to improve not only the nutritive value but also the health value of these legumes. Among the most popular and widespread legumes consumed in the world are chickpea and soybean. Hence this article deals with and aims to improve the nutritional and health quality of these legumes applying simple technological processing. This is achieved through generation of nutritional or health compounds, getting rid of the anti-nutritional factors, and better chance of being digested. This study concentrates on the effect of soaking and germination on protein hydrolysis and generation of bioactive peptides. The soaking and germination processes were done following the traditional international methods with some modification concerning conditions and time of germination that allows the optimum yield of protein hydrolysis and generation of peptides. As shown from the results in table (2)and (Fig. 1) chickpea and soybean soaking, and germination generated new peptides at the molecular weight range between 5 -15 kDa. This was more marked in case of soybean. Previous studies proved the generation of polypeptides by germination of legumes [17] and concentrated on the health value of these compounds [18]. It was found that some of these polypeptides can lower apolipoprotein B secretion and decrease the level of low-density lipoprotein (LDL)-cholesterol oxidation. Recently, Wali*et al.*[19] reported that chickpea sprouts can be used as a source of natural antioxidant peptides for food and nutraceutical applications. Most studies done on legumes germination concentrate on the effect on characters such as the phytate content, the protein pattern, or the polyphenols present. Few studies concentrate on active polypeptides and their action. It is not clear whether germination of legumes always lead to hydrolysis of protein and generates active peptides to the same extent and whether this is similar in different types of legumes and conditions used for germination. The results obtained from the present study (table 2) show that not all legumes undergo protein hydrolysis to the same extent. As mentioned, hydrolysis was more marked in case of soybean. How far this rate of hydrolysis affects the health value of the germinated legume was studied through its ability to protect from hyperlipidemia as evidenced by serum lipid pattern, liver function, and histopathological examination.

An experiment was designed to see how far germinated seed can protect against hyperlipidemia and histopathological changes that occur in animals consuming Lamb fat as fat source in the diet. Studies on lamb fat are scarce. The trials to produce hyperlipidemia in animals is so old in date, may be more that 100 years ago. Several experimental diets were tried for several periods and on different animal models. High fat diet containing 30% vegetable oil + 3% pure cholesterol was used by Mani *et al.*[20].Munshi*et al.* [21] used male Wistar rats given a high fat (vanaspati ghee: coconut oil, 3:1) oral diet along with 25% fructose (high sugar) added in drinking water over a period of 6 weeks. Even gene manipulation was tried to produce hyperlipidemia [22]. In the present study two types of lipids were tested one is a vegetable oil (sunflower) added as recommended in the standard diet (4%) and the other is a saturated fat (Lamb fat). The purpose is to see whether the type of fat can affect the rate and extent of recovery when the protecting factor is given. The results of food intake, gain in body weight and food efficiency ratio (table 3) confirm the deleterious effect of saturated fat on the analyzed parameters pointing to the so harmful effect of Lamb fat compared to the vegetable fat. In addition, the results of the activities of each of ALT & AST enzymes (shown in table 4) of rats fed on the high fat, high cholesterol diet further confirm this finding. This rise was significant in case of ALT but not significant in case of AST. Adding chickpea or soybean either raw or germinated caused a return to near normal values of rats in the control group given the standard diet.

A study made by Muniz *et al.* [23] found that high-fat diet with lard (20%) and cholesterol (1%) triggered dyslipidemia. The high-lard diet without supplementation of cholesterol led to body weight gain, but not to dyslipidemia. The body weight gain in such study is most probably due to fat accumulation. In the present study there is a positive correlation between low food consumption, low gain in body weight and food efficiency ratio. In a network meta-analysis made by Schwingshackl, *et al.*[24] aim to compare the effects of different oils/solid fats on blood lipids, they reached the conclusion that it is safer as traditionally known to use vegetable oils better than saturated fats. Mrázová*et al.* [25] reported that regular consumption of meat lard caused general elevation of lipid parameters in a good percentage of consumers, however the general conclusion was that reduction of lipid parameters including HDL occurred when the whole experimental group was considered. The risk exerted due to elevation of lipid parameters is the incidence of obesity with all complications including

high blood pressure, type 2 diabetes, atherosclerosis, and heart diseases [26]. As previously motioned in the present results, all lipid parameters were increased in animals fed on the diet containing lamb fat. This agrees of findings reported in studies using lard in the diet; however, some studies reported the occurrence of overweight in animals fed on diet containing lard. In the present study no overweight was reported despite the high lipid parameters. However, tissue damage occurred in the heart and liver of the animals fed on the diet containing lamb fat (Fig. 2-4). This means that not only obesity that is associated with the reported health complications, but such deteriorating action can occur without incidence of obesity, just due to hyperlipidemia. As shown in the figures of histopathological examination, high amounts of the mononuclear cell infiltration associated with, dilated congested vascular changes related to damaged myocytes occurred in heart tissue, the liver section of the rats fed on the diet containing lamb fat showed distortion of tissue structure, associated with moderate hepatic steatosis, microvesicles and macrovesicles fatty changes (Fig. 3-C). This is an indication that the deteriorating action and health complication can occur in rats consuming diet containing lamb fat even if they do not suffer from clear obesity. Emelyanova*et al.* [27] reported that lard consumption is not the sole factor contributing to cardiac changes, but high calorie intake is mainly responsible for this. Those authors linked between high calorie intake, obesity, and cardiac changes. The results obtained from the present study show that lamb fat consumption without associated obesity lead to health complications that cause atherosclerosis which may develop heart diseases.

As shown in the results feeding rats on a diet containing either germinated chickpea or soybean protected the animals to a good extent against dyslipidemia and most of the histopathological changes that occurred in those fed on that diet containing lamb fat without the germinated legume. The effect was more marked than in animals fed on the non- germinated legume. The increased LDL was reported to have a direct relation to the development of atherosclerosis. This state causes an increase in the permeability of the intima to the plasma lipoproteins, and its retention makes it more susceptible to oxidation. The oxidized LDL cholesterol is pro-inflammatory and immunogenic. The reduced HDL cholesterol also substantially influences the prevalent atherogenic lipid profile. HDL cholesterol removes oxidized LDL cholesterol by inhibiting monocyte attachment to the endothelium. In addition reduction of HDL cholesterol stimulates the release of nitric oxide, thus prevents vascular bed atherogenesis. Thus the state of dyslipidemia manifested by increased LDL and decreased HDL is a main factor in development of atherosclerosis due to increased concentration of oxidized LDL [28]. Thus, any compound found in food that can participate in prevention of LDL oxidation may help in inhibition of atherosclerosis. As mentioned before the germinated chickpea and soybean are rich in bioactive compounds such as total flavonoid which was found to correlate with the reducing power of the legumes [29]. Those authors stated that the right selection of legume varieties and the suitable germination process produce good yield of bioactive compounds that can be used for nutritional or pharmaceutical application. There are other bioactive compounds that possess antioxidant power. Among those are the bioactive peptides that were followed in this study and proved to be increased in germinated seeds. Kamran and Reddy [30] stated that bioactive peptides obtained by enzymatic hydrolysis of legume proteins display biological activities that make them of health benefit against several diseases. So, this study confirms that germination of legumes results in major health benefits to the consumers particularly that the process of germination is relatively simple and not costly.

4. Conclusion

To conclude, in the present study a simple easy process was made on two legumes namely chickpea and soybean that signify the nutritive and health value of these legumes. This health value was manifested in improving dyslipidemia due to consumption of saturated fat (lamb fat) that is relatively not extensively studied. The improvement was evidenced from biochemical and histological examination. This effect is most probably due to enrichment of the legumes with compounds with antioxidant character and in particular bioactive peptides.

5. Acknowledgments

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6. Conflicts of interest

There are no conflicts to declare

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Figures

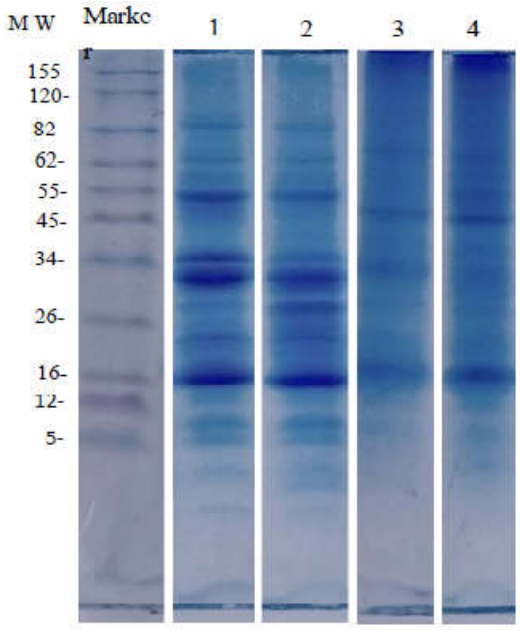

Fig. (1) Electrophoretic Tricine -SDS–PAGE protein profiles of chickpea and soybean protein extracts obtained by socking and germination.
Lane 1 = chickpea no germination,
Lane 2 = chickpea 72 h germination,
Lane 3 = Soybean no germination,
Lane 4= Soybean 72 h germination.

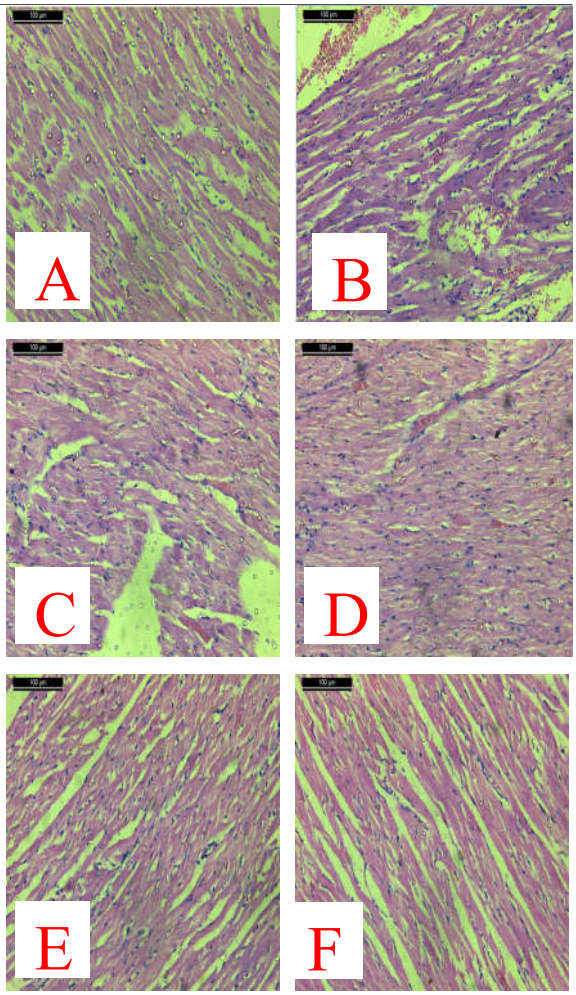

Figure 2: Sections of heart of **A)** control rat showing the cardiac muscle. It is bi-nucleated and the nuclei are located centrally. Intercalated discs are specialized junctions are found in between cardiac cells, **B)** positive control high cholesterol diet group showing higher amounts of the mononuclear cell infiltration associated inflammation, dilated congestion of vascular related to myocyte damage, **C)** high cholesterol diet group rat given ungerminated chickpea showed few amounts of the mononuclear cell infiltration associated inflammation, normal vascular related to myocyte, **D)** high cholesterol diet group and given germinated chickpea showing no mononuclear cell infiltration, dilated congestion of vascular related to myocyte damage, **E)** high cholesterol diet group and given ungerminated soybean showing no mononuclear cell infiltration, no congestion of vascular related to myocyte.Myocytes appeared more or less like normal control, **F)** high cholesterol diet group and given germinated soybean showing few mononuclear cell infiltration, Myocytes appeared more or less like normal control **(H & E, Scale Bar: 100 µm).**

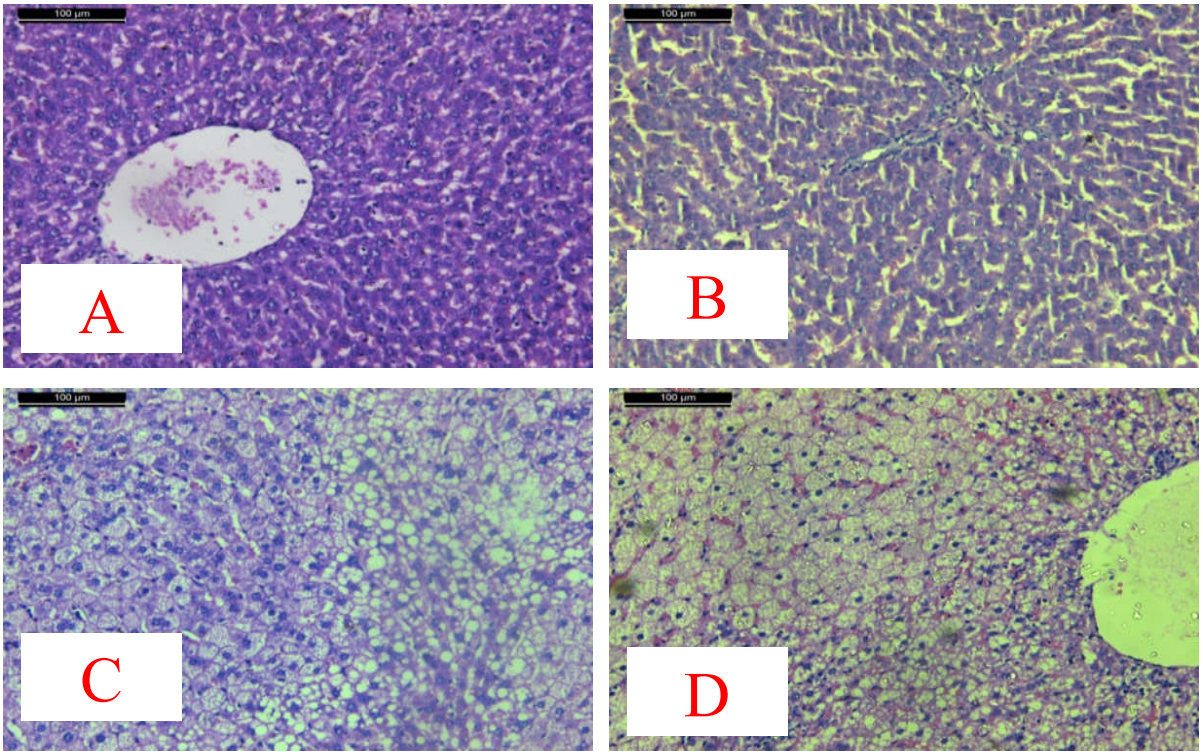

Figure 3: Sections of liver of **A)** rat of normal control showing normal structure of the hepatic lobule, **B)** rat of normal control showing normal structure of portal tract, **C)** positive control high cholesterol diet group showing distorted tissue structure with moderate hepatic steatosis, microvesicles and macrovesicles fatty changes were seen, **D)** high cholesterol diet group and given ungerminated chickpea showing few mononuclear cell infiltration, Hepatic tissue architecture with conserved vascular distribution**(H & E, Scale Bar: 100 µm).**

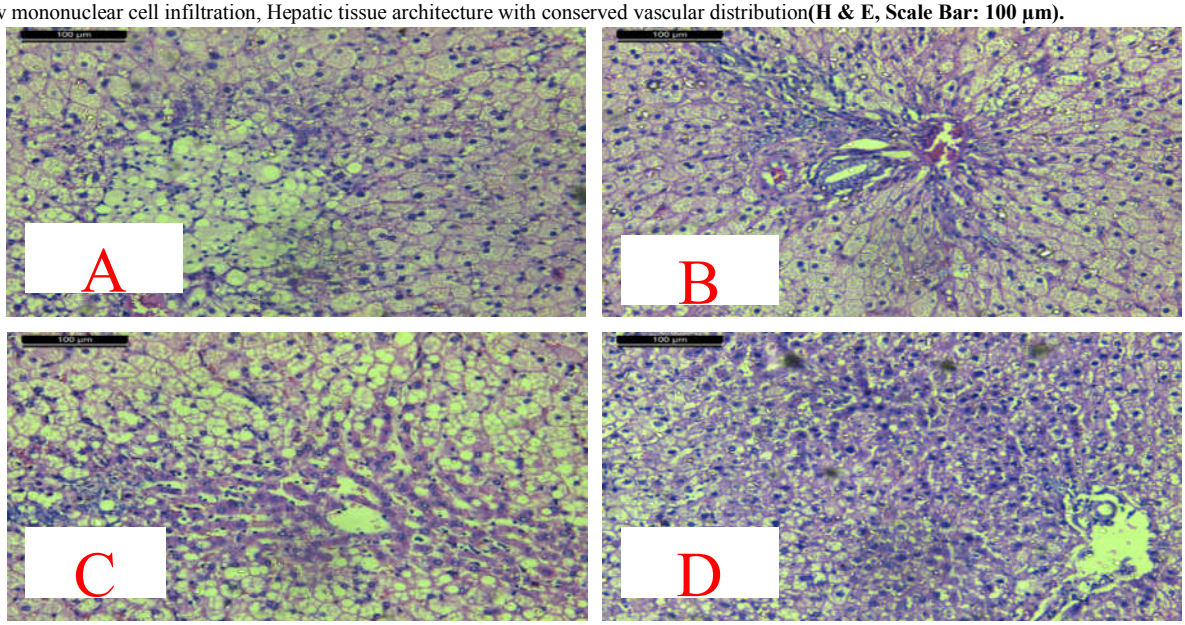

Figure 4: A section of liver of **A)** high cholesterol diet group that given germinated chickpea showing distorted tissue structure with moderate hepatic steatosis and vacuolar degeneration, **B)**high cholesterol diet group that given germinated chickpea showing distorted tissue structure with moderate vacuolar degeneration. Notice the congested portal tract that associated massive inflammatory infiltration, **C)** high cholesterol group that given ungerminated soybean showing moderate vacuolar degeneration. Notice many of hepatocytes appeared more or less like normal, D**)** high cholesterol diet group that given germinated soybean showing many of hepatocytes appeared more or less like normal. Mild vacuolar degeneration is noticed **(H & E, Scale Bar: 100 µm).**

Tables

Table 1: Composition of diets given to the control and experimental animals (g/100g)

Diet / Ingredients	1	2	3	4	5	6
Casein	14	14	7	7	7	7
Chickpea	0	0	25	25	0	0
Soybean	0	0	0	0	15	15
Sucrose	10	10	10	10	10	10
Sunflower oil	4	0	0	0	0	0
Lamb fat	0	20	20	20	20	20
Cellulose	5	5	4	4	4	4
Cholesterol powder	0	0.1	0.1	0.1	0.1	0.1
Bile salt	0	0.25	0.25	0.25	0.25	0.25
AIN-93 mineral mixture [a]	3.5	3.5	3.5	3.5	3.5	3.5
AIN-93 vitamin mixture [a]	1.0	1.0	1.0	1.0	1.0	1.0
D, L-methionine	0.18	0.18	0.18	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25	0.25	0.25	0.25
Corn starch to	100	100	100	100	100	100

[a] Vitamin and mineral mix. According to Reeves *et al.* (14)

Table 3: Mean values± SE of Food intake, Body weight gain and Food Efficiency Ratio (FER) of different groups

Group / parameter	1	2	3	4	5	6
Food intake (g)	940.28 ± 40.78	836.06 ± 53.51	862.14 ± 28.31	855.9 ± 59.04	881.77 ± 61.16	852.83 ± 63.68
P_1<		0.172	0.302	0.266	0.438	0.249
P_2<			0.728	0.792	0.544	0.823
Body weight gain (g)	146.02 ± 7.95	108.58 ± 12.81	114.40 ± 1.74	123.48 ± 10.47	113.08 ± 17.25	116.83 ± 16.23
P_1<		0.038	0.077	0.201	0.066	0.101
P_2<			0.738	0.394	0.796	0.636
FER	0.156 ± 0.008	0.128 ±0.009	0.133 ±0.004	0.144 ± 0.005	0.126 ± 0.012	0.134 ±0.011
P_1<		0.033	0.078	0.348	0.020	0.091
P_2<			0.690	0.212	0.821	0.632

P_1: p value in comparison with control group 1
P_2: p value in comparison with group 2

Table (2) Molecular weight (Mw) and band density

Band No.	Marker		Lane 1		Lane 2		Lane 3		Lane 4	
	Mw (kDa)	Density	Mw (kDa)	Density	Mw (kDa)	Density	Mw (kDa)	Density	Mw (kDa)	Density
Band 1	155.000	1866	94.819	2306	92.685	2427	77.964	3127	75.519	1882
Band 2	120.000	2102	72.488	2265	73.151	2020	66.483	2832	59.063	1891
Band 3	82.000	2708	55.924	3774	55.165	3620	47.472	2543	45.983	3650
Band 4	62.000	2634	32.984	1901	32.685	3383	31.516	2977	35.154	3258
Band 5	55.000	2491	29.037	4760	28.384	6544	23.127	2562	27.999	2988
Band 6	45.000	3091	22.607	7177	23.127	4269	17.842	2575	21.699	3003
Band 7	34.000	3546	18.253	2778	17.126	2464	13.213	7217	17.441	3197
Band 8	26.000	3220	12.915	3154	12.915	6558	8.772	2820	13.517	6312
Band 9	16.000	3143	10.523	7509	9.180	3583	-----	-----	10.333	2408
Band 10	12.000	4110	9.056	3523	7.617	1651	-----	-----	8.420	1710
Band 11	5.000	3490	7.793	1898	6.095	2328	-----	-----	6.496	1929
Band 12	-----	-----	6.095	2044	5.464	1353	-----	-----	-----	-----
Band 13	-----	----	4.596	1682	4.452	2155	-----	-----	-----	-----

Table 4: Mean values± SE of ALT and AST in different groups

Group / Parameter	1	2	3	4	5	6
ALT (U/L)	16.17 ± 0.40	31.5 ± 2.28	18.33 ± 2.30	18.17 ± 1.05	18.5 ± 0.73	18.00 ± 1.05
P_1<		0.000	0.332	0.370	0.296	0.410
P_2<			0.000	0.000	0.000	0.000
AST (U/L)	37.83 ± 2.39	42.83 ± 2.60	38.17 ± 1.66	34.33 ± 2.50	32.5 ± 2.39	30.5 ± 3.02
P_1<		0.161	0.924	0.322	0.136	0.043
P_2<			0.190	0.021	0.006	0.001

P_1: p value in comparison with control group 1
P_2: p value in comparison with group 2

Table 5: Mean values ± SE of total cholesterol, HDL, LDL, TG and TL of different groups

Group / Parameter	1	2	3	4	5	6
Total cholesterol (mg/dL)	77.02 ± 2.90	149.42 ± 3.42	85.11 ± 3.78	90.05 ± 2.56	93.48 ± 6.13	82.65 ± 3.33
P_1<		0.000	0.15	0.024	0.005	0.312
P_2<			0.000	0.000	0.000	0.000
HDL (mg/dL)	37.15 ± 2.93	29.74 ± 2.55	32.718 ± 2.53	34.47 ± 2.88	35.35 ± 2.27	35.92 ± 2.68
P_1<		0.57	0.246	0.479	0.633	0.744
P_2<			0.434	0.218	0.145	0.110
LDL (mg/dL)	44.93 ± 3.78	106.50 ± 7.94	56.02 ± 4.66	52.10 ± 6.32	55.61 ± 5.89	46.82 ± 3.87
P_1<		0.000	0.172	0.373	0.188	0.813
P_2<			0.000	0.000	0.000	0.000
TG (mg/dL)	105.04 ± 7.22	71.98 ± 2.40	64.53 ± 3.17	65.91 ± 3.58	66.12 ± 3.89	65.24 ± 2.97
P_1<		0.000	0.000	0.000	0.000	0.000
P_2<			0.217	0.312	0.329	0.263
TL (mg/dL)	260.98 14.45	462.81 27.91	276.62 26.17	271.90 19.7	295.30 24.15	253.34 23.11
P_1<		0.000	0.633	0.738	0.298	0.815
P_2<			0.000	0.000	0.000	0.000

HDL: High Density, Lipoprotein, LDL: Low Density Lipoprotein, TG: Triglyceride, TL: Total Lipids
P_1: p value in comparison with control group 1
P_2: p value in comparison with group 2