Biological Studies of high caloric foods supplemented with some probiotic bacteria

Sherief S.; Nehad R. EL-Tahan andDoaa E. A.
Nutrition and Food Science Dep., Faculty of Home Economics, Menofia University, Egypt

Abstract: Effect of different percent of high caloric food components (Lupine,chickpea and soyprotien) with probiotics bacteria on some biological and histological parameters of albino rats were studied. Thirty adult male rats were distributed into six groups, the first one was kept as control group, while the other five groups were fed on high caloric diet and supplemented with some probiotic bacteria for 6 weeks. Results showed that body weight gain markdely was higher especially in the mixture ofgroup(6) 1:1:1:1%) compared to control group and group which fed without probiotics. Also, this mixture with high probiotics lowered concentrations of total cholesterol, triglycerides, LDL-c, VLDL-c and decreased activity of AST and ALT enzymes to normal range. Furthermore, all mixture caused significant increase in HDL-c and total immunoglobulin production (IgG, IgM, IgE, IgA) were higher than the mixture (1%lupine, chickpea, soyprotien) without probiotics .

Histopathological examination showed amelioration of histopathological lesions seen in liver of rats received the mixture of seeds at different levels with probiotics. So, it could be recommended that intake of the mixture of lupine , chickpea , soyprotien and prpbiotics especially at 1:1:1:1 respectively may be useful for high caloric foods.

Key words: Malerats, lupine, chickpea, soyprotien, probiotics ,histopathological structure.

الدراسات البيولوجية للأغذية العالية الطاقة المدعمة ببعض انواع من بكتريا البروبيوتك

د/شريف صبري   د/ نهاد رشاد الطحان،
دعاء السيد عبدالرحمن المقدم
قسم التغذية وعلوم الأطعمة، كلية الاقتصاد المنزلي، جامعة المنوفية، مصر

الملخص العربى

تم فى هذا البحث دراسة الاغذية عالية الطاقة (الترمس والحمص وبروتين الصويا)المدعمة ببعض انواع من البربيوتك على بعض العوامل البيولوجية والنسيجية في ذكور الفئران الطبيعية الالبينو. وتم استخدام ثلاثين من الفئران البالغة تم تقسيمها إلى ستة مجموعات، المجموعة الاولى مجموعة ضابطة في حين أعطيت ٥ مجموعات الاخرى يوميا خليط من (الترمس و الحمص وبروتين الصويا بنسبة ١:١:١) ونسبة من البروبيوتك بنسبة ٠.٥ ، ١% لمدة ٦أسابيع، وأظهرت النتائج أن الزيادة في وزن الجسم كان اكثر بشكل ملحوظ خاصة في خليط من(الترمس والحمص وبروتين الصويا و البروبيوتك المضافة) بنسبة ١:١:١:١مقارنة مع المجموعة الضابطه السالبة و أيضا حدث خفض تركيزات الكوليسترول الكلي والدهون الثلاثية، LDL، VLDL وانخفض نشاط الانزيماتAST و ALT. وعلاوة على ذلك، حدثت زيادة كبيرة في تركيز –HDL و كذلك زيادة واضحة فى دلالات المناعة خاصة مع المجموعات التى تغذت على البروبيوتك.

وأظهر فحص الأنسجة تحسن الخلايا النسجية في كبد الفئران التى تغذت على خليط من الحبوب والبروبيوتك على مختلف المستويات. وتوصي هذه الدراسة أن تناول خليط الترمس والحمص وبروتين الصويا والبروبيوتك وخصوصا بنسبة ١:١:١:١ قد يكون من مفيدا هفى تحسين الهضم وزيادة الوزن بصورة صحية.

الكلمات الرئيسية: **الفئران الذكور من نوع الالبينو – الترمس– الحمص – بروتين الصويا – البروبيوتك(لاكتوباسليس والبيفيدوبكتريا) والتركيب التشريحي.**

INTRODUCTION

A probiotic is defined classically as a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract. This definition, however, was initially intended for use with animal feed products. For human nutrition, the following definition has been proposed: A live microbial food ingredient that is beneficial to health **(Salminen*et al.*, 1998)**.

A prebiotic is defined as a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. Modification by prebiotic of the composition of the colonic microflora leads to the predominance of a few of the potentially health-promoting bacteria, especially, but not exclusively, lactobacilli and bifidobacteria**(Gibson and Roberfroid, 1995).**

found that food can be said to be functional if it contains a component (which may or may not be a nutrient) that affects one or a limited number of functions in the body in a targeted way so as to have positive effects on health or if it has a physiologic or psychologic effect beyond the traditional nutritional effect **(Clydesdale, 1997).**

Among the most promising targets for functional foods are the gastrointestinal functions, including those that control transit time, bowel habits, and mucosal motility as well as those that modulate epithelial cell proliferation. Promising targets are also gastrointestinal functions that are associated with a balanced colonic microflora, that are associated with control of nutrient bioavailability (ions in particular), that modify gastrointestinal immune activity, or that are mediated by the endocrine activity of the gastrointestinal system. Finally, some systemic functions such as lipid homeostasis that are indirectly influenced by nutrient digestion or fermentation as lactobacilli and bifidobacteria represent promising targets

Legumes have played an important role in the traditional diets of many regions throughout the world. It is difficult to think of the cuisines of Asia, India, South America, the Middle East, and Mexico without picturing soybeans, lentils, black beans, chickpeas, and pinto beans, respectively. In contrast, in many Western countries beans play a less significant dietary role. In fact, bean intake has actually declined during the past century in many European countries(Roberfroid , 2000).

The lupin is devoid of starch, which is very unusual for a species of edible bean. Lupins have a thick seed coat (25%) which consists mainly of cellulose (insoluble fibre-bran) and its removal is the first step in lupin processing. The kernel (split) of lupin is rich in protein (40%), fibre (40%) and moderate in fat (8%) made up largely of unsaturated fatty acids. Intensive plant breeding programs have ensured that modern lupin varieties have relatively low levels of the alkaloids found in their ancestral genotypes. Lupins also contain moderate amounts of carotenoids; beta carotene, lutein, zeaxanthin and tocopherols (Vitamin E). Australian sweet lupin features a higher calcium and phosphate content than cereals with trace element content varying in line with the mineral content of the soil in which the lupin is grown. Lupin oils have high antioxidant capacities due in part to the presence of tocopherol (Vitamin E – the total vitamin E content is about 2.3-4.6 mg/kg of oil) **(Williamson *et al*., 1994).**

Chickpea (Cicerarietinum L.) is one of the world's most important grain legumes because it is a valuable source of protein, minerals and vitamins, and plays a very important role in human diets in many areas of the world. Chickpea is an excellent source of protein, carbohydrates, and fiber, and provide many essential vitamins and minerals. Their highly nutritional properties have been associated with many beneficial health-promoting properties, such as managing high cholesterol and type-2 diabetes and in the prevention of various forms of cancer. The main antioxidant compounds in legumes are vitamins C and E, phenolic compounds and reduced glutathione (GSH). Different studies have shown that they have a protective antioxidant effect on cancer and cardiovascular diseases**(Mallillin*et al.*, 2008 and Murty*et al.*, 2010)**

Soybean protein is a "complete protein" since it provides all of the essential amino acids for human nutrition. Soybean protein is essentially identical to that of other legumepulses and is one of the least expensive sources of dietary protein. For this reason, soy is important to many vegetarians and vegans. Soy flour contains 50% protein. The digestibility of some soy food are as follows: Steamed soybeans 65.3%, tofu 92.7%, soy milk 92.6%, and soy protein isolate 93–97%. Consumption of soy protein in place of animal protein has been found to reduce serum concentrations of total cholesterol, low-density lipoproteins (LDLs) and triglycerides. One theory proposes that cholesterol absorption is impaired or altered. Another theory postulates that phytoestrogens (plant compounds that have hormone-like effects; isoflavones are the phytoestrogens found in soy products) bind to estrogen receptors and produce similar effects including lowering LDLs and increasing high-density lipoproteins, vasomotor tone changes, and arterial wall function **(**Hasler, 2002**)**.

So, this research aimed to study the effect of these legumes mixture with probiotic on some biological and histopathological parameters.

MATERIALS AND METHODS

lupine, ckickpea and soyprotien were obtained from the local market of seeds andprobiotics((lactobacilliscasei, pifidobacterium acidophilus)were obtained from(Lacteol fort) from Tenth Raadan for phamaceutical Industries and Diagnostic Reagents(rameda)6th of October City A.R.E and scientifically identified at Horticultural Research Institute, Agriculture Research Center, Egypt. All chemicals and diagnostic kits were purchased from El-Gomhoria Co., Cairo, Egypt.

Experimental animals: This study was carried out on thirty adult male Sprague Dawley albino rats weighing 160±5 g body weight. The rats were obtained from Laboratory Animal Colony, Helwan, Egypt. Before their use in the experiment, the rats were kept for one week for acclimatization to the laboratory conditions. They were fed on basal diet and provided with water and food ad libitum.

The basal diet consisted of casein (10%), cellulose (5%) salt mixture (4%), vitamin mixture (1%), corn oil (10%) and corn starch (70%) according to **Reeves *et al*. (1993)**

Experimental procedure: Rats were divided into six groups consisting of five rats each. The first group was fed on the basal diet and kept as a control group, while the other groups were fed on lupine ckickpea and soy protein with probiotics for 6 weeks as the following : Group (2) mixture of lupine, ckickpea with as(1:1 0.5%) probiotics. Group (3) mixture lupine, soyprotien and probioticsas(1:1:0.5%). Group (4) lupine ,ckickpea and soyprotien without probiotics as(1:1:1 %). Group (5)mixture lupine , ckickpea, soyprotien and probiotic as(1:1:1:0.5%). Group (6) 1% mixture lupine ,ckickpea , soyprotien and probiotics as(1:1:1:1 %).

During the experiment period, the feed intake and body weight were recorded weekly. Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) were calculated at the end of the experimental period according to the following equations:

BWG (g) = final weight (g) - initial weight (g) FER = weight gain (g)/feed intake (g).

Collection of blood samples and organs: At the end of the experimental period, rats were sacrificed following a 12 h fast. The rats were lightly anaesthetized by ether and about 7 ml of blood was withdrawn from the hepatic portal vein into dry centrifuge plastic tubes. Blood portal vein into dry centrifuge plastic tubes. Blood separate the serum samples which were kept in tubes at -20 ^{o}C till biochemical analysis **(Jeyakumar*et al*., 2006).**

Biochemical analysis: Serum total cholesterol was calorimetrically determined according to **Allain*et al*. (1974)** and triglyceride was determined calorimetrically according to High Density Lipoprotein cholesterol (HDL-c) was determined calorimetrically according to **Richmond (1973)**. Low Density Lipoprotein cholesterol (LDL-c) and Very Low Density Lipoprotein cholesterol (VLDL-c) were calculated mathematically according to **Friedewald*et al*. (1972).**

LDL-c = TC-[HDL-c + (TG/5)] VLDL-c = Triglycerides/5.

The activity of Aspartate Aminotransferases (AST) and Alanine Aminotransferases (ALT) enzymes were assigned by the method of total immunoglobulin (IgG, IgM, IgE and IgA) determined by Radioimmunoassay as described by the method of **Patrono and Peskar (1987).**

Histopathological study: Livers of the scarified rats were dissected, removed, washed with normal saline and put in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. The tissue specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin (H and E) and then studied under an electronic microscope according to (**Carleton ,1979)**.

Statistical analysis: Results are expressed as mean values with their standard deviation of the mean. Statistical differences between groups were evaluated using one-way ANOVA followed by Duncan post hoc test using SPSS version 11.0 for Windows (SPSS, Chicago, IL, USA). Differences were considered significant at ($p<0.05$) according to **Snedecor and Cochran ; 1986)**.

RESULTS AND DISCUSSION

1-Effect of mixture of lupine, chickpea, soyprotien and probiotic on feed intake (FI), body weight gain (BWG) and feedefficiency ratio (FER) in normal rats.

Data in table (1) showed that feeding on mixture in group (6) which fed on 1% mixture lupine ,ckickpea , soyprotien and %1 probiotics led to increase the feed intake which was near to the normal group. The feed intake of the other tested groups were lower than the group (6) and normal group. There is no significant changes between group 2, 3 and 5. The group (4) which fed on %1lupine , 1%ckickpea and1% soyprotien without probiotics was the lowest feed intake. For body weight gain , there is no significant differences between group (2) and group(5) and also between groups (1) and (6) . There were significant differences between control group and the tested groups except group 6. In case of feed efficiency ratio, the sixth group was the best group and there is no significant change between this group and the control group. The lowest group for FER was the fourth group. This results were matched with **Salminen*et al.*(1998)** that probiotic improve the feed intake and body weight of rats. Probiotic as functional foods enhanced the gastrointestinal functions, including those that control transit time, bowel habits, and mucosal motility as well as those that modulate epithelial cell proliferation**(Clydesdale, 1997).**

Table (1): Effect of mixture of lupine, chickpea, soyprotien and probiotic on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) in normal rats.

Groups / parameters	G1	G2	G3	G4	G5	G6
Feed intake g/day	$13.9^{a}\pm$ 0.18	12.58^{b} ±0.38	12.4^{b} ±1.35	$11.2^{c}\pm$ 0.03	12.73^{b} ±1.01	14.08^{a} ±0.75
BWG g/42days	70.27^{a} ±3.01	55.41^{c} ±0.56	50.12^{d} ±1.46	40.28^{e} ±2.19	57.17^{c} ±4.42	70.58^{a} ±2.32
FER	0.120^{a} ± 0.01	0.104^{b} ± 0.01	0.096^{c} ±0.06	0.086^{d} ± 0.12	0.107^{b} ± 0.06	0.119^{a} ±0.01

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at ($p<0.05$)

2-Effect of mixture of lupine, ckickpea ,soyprotien and probiotic on serum lipids

Administration of the mixture of lupine, chickpea ,soyprotien, and probiotics at different levels caused significant decreases in serum of total cholesterol, triglycerides, LDL-c and VLDL-c compared to control group (Table

2). Serum HDL-c levels increased but not significantly by the administration of the mixture of lupine, chickpea ,soy protein and probiotics. Rats that were given mixture of lupine, chickpea, soyprotien and probiotic bacteria at the levels 1:1:1:1 (group 6) showed significantly higher levels of HDL-c compared to control group. The value of group four which fed on the mixture of seeds without probiotics were lower for HDL-c than control group while it was higher for the other lipid parameters than control group and the group (6). The obtained results in the same line of(Hasler; 2002**)**.

who found that soy protein can decrease LDL-c, total cholesterol and increase the level of HDL-c. Also,**Clydesdale (1997)** reported that probiotics reduce the absorption of lipids from diet .

Table (2): Effect of mixture of lupine,chickpea ,soyprotien and probiotic on serum lipids

Serum lipids	G1	G2	G3	G4	G5	G6
Total cholesterol	75.43[b] ±2.19	76.33 [b] ±3.15	75.47[b] ±1.13	105.57[a] ±3.21	72.36[b] ±1.12	75.43[b] ±0.31
Triglycerides	76.48[c] ±0.13	76.8[c] 1.03±	81.4[b] ±3.01	119.36[a] ±1.02	76.68[c] ±2.63	71.96[d] ±0.56
HDL-cholesterol	53.94[a] ±0.12	47.87[b] ±1.15	48.89[b] ±0.04	33.90[c] ±0.97	47.92[b] ±0.03	54.94[a] ±0.05
LDL-cholesterol	20.2[d] ±1.17	24.9[b] ±4.34	22.5[c] ±0.74	47.8[a] ±0.24	23.1[b] ±0.91	18.1[d] ±0.91
VLDL-cholesterol	1.29[c] ±1.17	1.56[b] ±4.34	1.28[c] ±0.74	3.87[a] ±0.24	1.24[c] ±0.91	1.29[c] ±0.91

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at ($p<0.05$)

3- **Effect of mixture of lupine, chickpea ,soyprotien and probiotic on liver function enzymes in normal rats.**

From data presented in table (3) the administration of mixture of lupine, chickpea, soyprotienand probiotics group as 1:1:1:1 significantly reduced AST and didn't effected on ALT level when compared with the other treatment groups. There is no significant differences between group 1 and 5 also between group 2 with 3. On the other hand, there were no significant between group 1, 5 and 6. While, there significant changes between group 4 and the other groups. From the above results, It could be noticed that crude fiber in legumes is a group of indigestible carbohydrates. can improve the function of the alimentary tract and also lower blood glucose , cholesterol levels and liver functions (**Roberfroid , 2000)**.

Table (3): Effect of mixture of lupine, chickpea, soyprotien and probiotic on liver function enzymes in normal rats.

Parameters	G1	G2	G3	G4	G5	G6
AST(U/L)	27.8^{c} ±0.07	30.2^{b} ±1.11	32.5^{b} ±0.21	55.1^{a} ±2.50	27.7^{c} ±0.15	25.1^{d} ±0.56
ALT(U/L)	19.8^{c} ±1.91	28.9^{b} ±1.41	27.4^{b} ±0.5	44.4^{a} ±2.01	20.7^{c} ±3.25	19.7^{c} ±0.52

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at ($p<0.05$)

4- Effect of mixture of lupine, chickpea, soyprotien and probiotic on immunological productions .

From table (4), it could be observed that administration of the tested legumes and probiotics at (1:1:1:1)it is Effect to rats activity (group 6). The mixture of seeds (lupine ,ckickpea ,soyprotien and probiotics bacteria induced significant increased in serum levels of immunological profile compared to control group. All other tested mixture of legumes caused non significant changes in serum level of immunological productions.The main antioxidant compounds in legumes are vitamins C and E, phenolic compounds. So, different studies have shown from where that they have a protective antioxidant effect on immunity status, cancer and cardiovascular diseases**(Mallilline*t al.*, 2008 and Murty*et al.*, 2010)**

Table(4): Effect of mixture of lupine, chickpea, soyprotien and probiotic on immunological productions .

Immuno logical Profile mg/dl	G1	G2	G3	G4	G5	G6
IgE	64.17^{a} ±0.05	60.5^{b} ±0.2	59.87^{b} ±1.34	45.54^{c} ±1.05	60.76^{b} ±0.05	62.99^{a} ±1.11
IgM	108.2^{a} ±0.005	106.65^{a} ± 0.65	106.33^{b} ±3.5	82.33^{c} ±10.96	105.66^{b} ±9.6	107.66^{b} ±2.5
IgA	111.1^{a} ±0.1	109.5^{a} ±0.5	109.5^{a} ±1.5	78.33^{b} ±2.08	108.5^{a} ±1.5	111.06^{a} ±6.02
IgG	1100.05^{a} ±9.05	1089^{c} ±10.87	1089.66^{c} ±25.16	778.36^{d} ±20.85	1085^{c} ±15.4	1096.66^{b} ±6.27

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at ($p<0.05$)

Histopathological examination of liver of the negative control rats fed on basal diet revealed normal histological picture of hepatic lobule which consists of central vein surrounded by normal hepatocytes as shown in photo(1). Examination of liver of group (2) showed of hepatocytes and infiltration of leucocytes in hepatic sinusoid photo(2). Liver of rats given the mixture and probiotic showed little vacuolar degeneration of hepatocytes and some improvement in degeneration (photo. 3). In addition, portal edema and few leucocytes infiltration in hepatic lobule were observed the second mixture of seeds photo(4). Liver and the third mixture showed marked improvements with no observed pathological lesions photo(5) and photo (6). These results were according to who found that probiotic at level 1% and the legumes at the levels 1:1:1 can keep(**Mallillin ; and Murty *et al.(2008;* 2010**).

tissue in normal status without any changes and improve the cells structure more than control group.

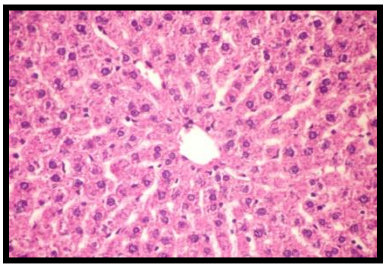
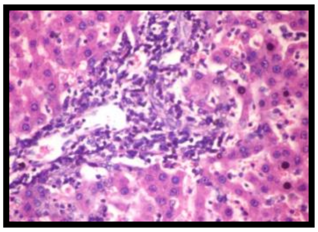


Photo (1): Liver of rat from group (1)
It could be observed that liver's rat in photo (1) which fed on basal diet it showed Kupffer cells activation of rat's liver.
(H and E X 400)



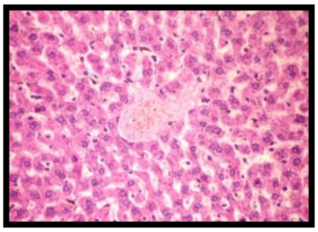
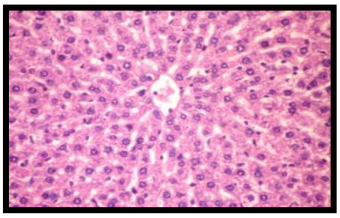


Photo (2): Liver of rat from group (2) It could be observed that liver's rat in photo which fed on lupine, chickpea and probiotic it showed Kupffer cells activation of rat's liver (H and E X 400)



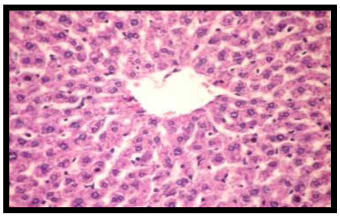


Photo (٣): Liver of rat from group (4) the obtained results from photos it could be observed that liver's rat in photo which fed on lupin, chickpea and soy protein without probiotic. It showed Kupffer cells activation or rat's liver (H and E X 400)

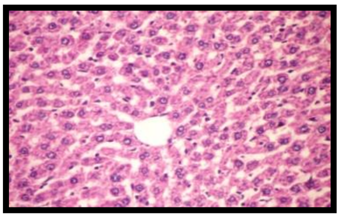
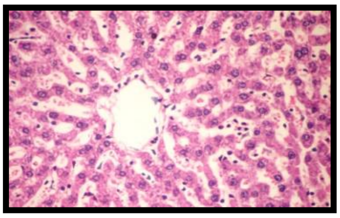



Photo (4): Liver of rat from group (6) It could be observed that liver's rat in photo which fed on lupine, chickpea , soy protein and probiotic it showed Kupffer cells activation of rat's liver

(H and E X 400)

the kidney tissue in normal status without any changes and improve the cells structure more than control group.

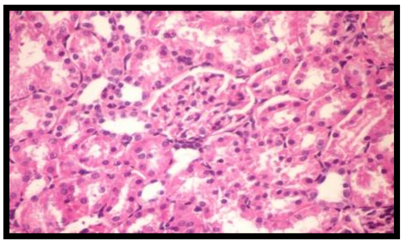


Photo (1):

Kidney of rat from group (1) Iit could be observed that Kidney 's rat in photo (1) which fed on basal diet it showed Kupffer cells activation of rat's Kidney

(H and E X 400)

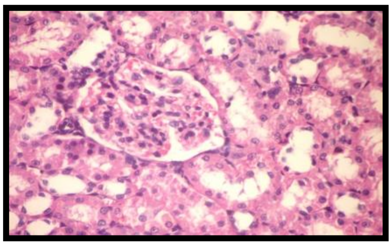
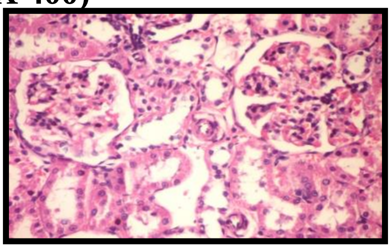



photo (2): Kidney of rat from group (2) It could be observed that Kidney 's rat in photo which fed on lupin, chickpea and probiotic it showed Kupffer cells activation of rat's Kidney

(H and E X 400)

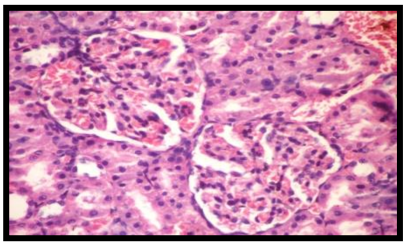
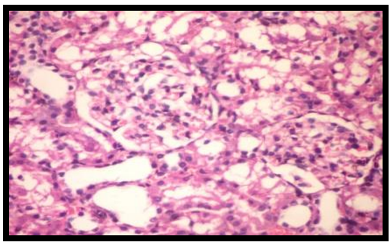
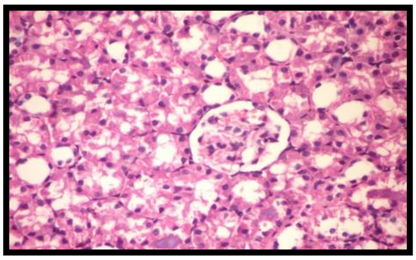



Photo (3): Kidney of rat from group (4) It could be observed that Kidney 's rat in photo which fed on lupine, chickpea and soy protein without probiotic. It showedKupffer cells activation of rat's Kidney

(H and E X 400)



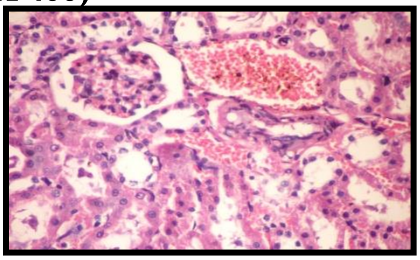


Photo (4): Kidney of rat from group (6) It could be observed that Kidney's rat in photo which fed on lupine, chickpea , soy protein and probiotic it showed Kupffer cells activation of rat's Kidney
(H and E X 400)

DISCUSSION

Several studies have showed that each of soy protien have long been recognized as an excellent source of high-quality protein. The soyprotien also contains a wide variety of chemical compounds that have potent bioactivity. Among these compounds are the isoflavones and the saponins. The goal of this research was to quantify isoflavone and saponin concentrations in elite soybean cultivars grown in different environments and to identify a naturally occurring high and low variety that could be used in animal studies of colon cancer they reportedsignificant environment genotype interactions for the cultivars and selected 2 that provided the range of concentration for isoflavones and saponins. These were grown in an adequate quantity for animal studies, which are ongoing. They explored the influence of isoflavones and saponins on human colon tumor cells in culture, Caco-2, to determine potential mechanisms through which these compounds influence the carcinogenic process. it could be observed that the inhibition of Caco-2 cell proliferation by isoflavones and saponins, suggesting a protective effect of these compounds in colon cancer. Using purified soy saponins, we found no negative effects on mouse growth, organ weights, or intestinal morphology when the diet contained up to 3% saponins by weight. Hence, soy isoflavones and saponins are likely to be protective of colon cancer and to be well tolerated. Continuing studies will explore the cancer-protective effects of these compounds in animal models **(Ruth *et al* ., 2012).**

Ingesting oligosaccharides such as raffinose and stachyose, namely, encouraging igenous bifidobacteria in the colon against putrefactive bacteria.

The insoluble carbohydrates in soyprotien consist of the complex polysaccharides cellulose, hemicellulose, and pectin. The majority of soybean carbohydrates can be classed as belonging to dietary fiber.

Within soybean oil or the lipid portion of the seed is contained thephytosterols: Stigmasterol (17–21%), sitosterol(53–56%)
and campesterol(20–23%) accounting for 2.5% of the lipid fraction.

Saponins, a class of natural surfactants (soaps), are sterols that are present naturally in a wide variety of food-plants: vegetables, legumes, and cereals–ranging from beans and spinach to tomatoes, potatoes and oats. Whole soybeans contain from 0.17 to 6.16% saponins, 0.35 to 2.3% in defatted soy flour and 0.06 to 1.9% in tofu. Legumes such as soybean and chickpeas are the major source of saponins in the human diet. Sources of non-dietary saponins include alfalfa, sunflower, herbs and barbasco. Recent studies have shown that saponins are potential functional food ingredients because of their physiological properties.

Soy contains isoflavones like genistein and daidzein. It also containsglycitein, an O-methylated isoflavone which accounts for 5–10% of the total isoflavones in soy food products. Glycitein is a phytoestrogen with weak estrogenic activity, comparable to that of the other soy isoflavones(**Song et al. ,1999**).

Chickpea (Cicerarietinum L.) is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries. It is a good source of carbohydrates and protein, and the protein quality is considered to be better than other pulses. Chickpea has significant amounts of all the essential amino acids except sulfur containing types, which can be complemented by adding cereals to daily diet. Starch is the major storage carbohydrate followed by dietary fiber, oligosaccharides and simple sugars like glucose and sucrose. Lipids are present in low amounts but chickpea is rich in nutritionally important unsaturated fatty acids like linoleic and oleic acid. β-sitosterol, campesterol and stigmasterol are important sterols present in chickpea oil. Calcium, magnesium, phosphorus and especially potassium are also present in chickpea seeds. Chickpea is a good source of important vitamins such as riboflavin, niacin, thiamin, folate and the vitamin A precursor, β-carotene. Like other pulses, chickpea seeds also contain anti-nutritional factors which can be reduced or eliminated by different cooking techniques. Chickpea has several potential health benefits and, in combination with other pulses and cereals, it could have beneficial effects on some of the important human diseases like cardiovascular disease, type 2 diabetes, digestive diseases and some cancers. Overall, chickpea is an important pulse crop with a diverse array of potential nutritional and health benefits(**Jukanti1, 2012**).

Australian Sweet Lupins (Lupinusangustifolius) are high in protein, dietary fiber and antioxidants, very low in starch, and are gluten-free. Lupins can be used to make a variety of foods both sweet and savoury including everyday meals, traditional fermented foods, baked foods and sauces.

The lupin is devoid of starch, which is very unusual for a species of edible bean. Lupins have a thick seed coat (25%) which consists mainly of cellulose (insoluble fiber-bran) and its removal is the first step in lupin**.**

processing. The kernel (split) of lupin is rich in protein (40%), fiber (40%) and moderate in fat (8%) made up largely of unsaturated fatty acids. Intensive plant breeding programs have ensured that modern lupin varieties have relatively low levels of the alkaloids found in their ancestral genotypes. Lupins also contain moderate amounts of carotenoids; beta carotene, lutein, zeaxanthin and tocopherols (Vitamin E).

Australian sweet lupin features a higher calcium and phosphate content than cereals with trace element content varying in line with the mineral content of the soil in which the lupin is grown.

Lupin oils have high antioxidant capacities due in part to the presence of tocopherol (Vitamin E the total vitamin E content is about 2.3 - 4.6 mg/kg of oil(**Gladstone *et al*. ,1998).**

Effect of seeds and probiotic on immunological its effect on increasing antioxydantenzyems could be indirect result of their effect on lipids metabolism. The histopathological results showed that rats supplemented with mixture of lupine chickpea , soy protien and probiotic bacteria at ,5 and 1% can prevent/reduce diet induce fatty liver. This fat reduction in the liver was confirmed by serum lipid analysis and by measurement of liver specific marker enzymes as mentioned before (**Zafar and Mujahid ;1998 and Ahmed ;et al ; 2003).**

On the basis of the present results, it could be conclude that seeds mixture of lupine ,chickpea ,soyprotien and probiotics especially at 1:1:1 and 0,5% may have synergistic effect and its intake of be useful for product high caloric food supplemented with probiotic bacteria (lactobacillis ,bifidobacterium) accompanied by hyperlipidemia as it reduces feed intake and body weight, improves serum lipid profile, liver function and immunological activity in rats. Moreover, this mixture has a promising effect on the liver tissues as it ameliorates the histopathological lesions seen in this organ of rats.

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Jukanti1, A .K; Gaur, P. M. and Gowda1 C. L. L.(2012): Chibbar2 International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, AP 502 324, India Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5A8.

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