

Study the effect of supplemented snacks with some plants and seeds on the healthy status of rats

Adel, A. Elmotty; Nehad ,R. EL-Tahan and Radwa ,A .A.

Nutrition and Food Science Dep, . Faculty of Home Economics
Menoufia University, Egypt.

Abstract:

The effect of supplemented snacks with some plants and seeds on some biological of albino rats were studied. Twenty five (25) adult albino rats, weighing ($90 \text{ g} \pm 5$) were used in this study and were distributed into 5 groups each of 5 rats the first one fed on basal diet as control group with cheese crackers, while the other four groups were fed on basal diet with crackers contained olive leaf, licorice, garden cress and flax seed for 28 days. Results showed that body weight gain was markedly higher especially in the flax seed followed by licorice and olive leaf compared to control group and group which fed crackers with garden cress . Also, the same supplemented crackers lowered concentrations of total cholesterol, triglycerides, LDL-c, VLDL-c and decreased activity of AST and ALT enzymes in normal range. Furthermore, all supplemented crackers with natural plants and seeds caused significant increase in HDL-c and total immunoglobulin production (IgG, IgM, IgE, IgA) were higher than the control group which fed on crackerswith artificial cheese.

Key words: Male rats, crackers, flax seed, licorice, olive leaf.

INTRODUCTION

The immune system is a network of cells, tissues^f, and organs that work together to defend the body against attacks by “foreign” invaders. These are primarily microbes (germs)—tiny, infection-causing organisms such as bacteria, viruses, parasites, and fungi. Because the human body provides an ideal environment for many microbes, they try to break in. It is the immune system’s job to keep them out or, failing that, to seek out and destroy them. When the immune system hits the wrong target or is crippled, however, it can unleash a torrent of diseases, including allergy, arthritis, or AIDS. The immune system is amazingly complex. It can recognize and remember millions of different enemies, and it can produce secretions and cells to match up with and wipe out each one of them. The secret to its success is an elaborate and dynamic communications network. Millions and millions of cells, organized into sets and subsets, gather like clouds of bees swarming around a hive and pass information back and forth. Once immunecells receive the alarm, they undergo tactical changes and begin to produce powerful chemicals. These substances allow the cells to regulate their own growth and behavior, enlist their fellows, and direct new recruits to trouble spots (**Beck and Habitat , 1996**).

Licorice is one of the most widely prescribed herbs in Chinese medicine. It is used to treat gastric ulcers when administered 20 to 30 minutes before meals through lining the stomach wall. The processed form of licorice (DGL) is not associated with adverse effects and can be used to treat peptic ulcer disease in combination with antacids (this combination has been marketed as Caved-S). However, licorice is rarely used nowadays because of its side effects and the emergence of other more powerful classes of medications for treatment of peptic ulcers. In Japan, glycyrrhizin has been given intravenously for treatment of patients with chronic hepatitis B with improvement in liver functions and occasionally complete recovery. It was suggested that glycyrrhizin is able to suppress the secretion of both hepatitis B surface antigen and its intracellular transport (**Takahara *et al.*, 1994** and **Sato *et al.*, 1996**). In women, licorice has been used in conjunction with spironolactone in the treatment of polycystic ovary syndrome (PCOS)

(**Oerter et al., 2003**). This estrogenic activity of licorice has been well documented (**Armanini et al., 2004**).

Flaxseed, or Linseed (*Linum Usitatissimum*), popularly known as Alsi, Jawas, Aksebija in Indian languages, is a blue flowering rabi crop and a member of family Linaceae (**Anonymous, 2000**). As the source of linen fiber flax has been cultivated since at least 5000 BC, today it is mainly grown for its oil Flaxseed is one of the richest vegetarian source of α -linolenic acid (omega 3 fatty acid) and soluble mucilage. In present era, consumer's trend towards functional food has increased significantly as health awareness rose. Flaxseed can be one stop for novel high quality source of nutrition (**Berugland, 2002 and Oomah, 2001**).

Lepidium sativum (Garden cress) is an annual herb, belonging to Brassicaceae family. It is a fast-growing, edible plant botanically related to watercress and mustard and sharing their peppery, tangy flavor and aroma. Seeds, leaves and roots are economically important, however, the crop is mainly cultivated for seeds. In some regions garden cress is known as garden pepper cress, pepper grass or pepperwort. It is also known as Asalio or chandrasur in India and it is an important medicinal crop in India. Garden cress is a perennial plant, and an important green vegetable consumed by human beings, most typically as a garnish or as a leaf vegetable (**Tiwari and Kulmi, 2004**).

Olive tree (*Olea europaea L.*) is one of the most important fruit trees in Mediterranean countries, where they cover 8 million ha, accounting for almost 98% of the world crop. This demonstrates the great economic and social importance of this crop and the possible benefits to be derived from utilisation of any of its by products (**Guinda et al., 2004 and Tabera et al., 2004**). Olive leaves are one of the byproducts of farming of the olive grove; they can be found in high amounts in the olive oil industries (10% of the total weight of the olives) and they accumulate during pruning of the olive trees. Popular medicine and phyto-therapy use olive leaves to treat and prevent hypertension and for their hypo-glycaemic, antiseptic and diuretic properties. They were formerly used as a folk remedy for combatting fevers and other diseases, such as malaria, but this use was dropped. Several reports have shown that olive leaf extract has the capacity to lower blood pressure in animals and increase blood flow in the

coronary arteries, relieve arrhythmia and prevent intestinal muscle spasms (**Garcia et al., 2000**). There is an increasing interest in the phenolic compounds in olive by-products, due to their biological properties. Olive oil polyphenols possess good antioxidant activities. Also olive leaves are a source of several antioxidants (**Ranalli et al ., 2006**).

MATERIALS AND METHODS

This study was carried out using Licorice (glycyrrhizic acid), Flaxseed, or Linseed (*Linum Usitatissimum*) , Garden cress (*Lepidium sativum*) ,and Olive Leaves powder were obtained from Harraz , Tala , Menoufia Governor . Cheese Crackers was obtained from local market, Tala , Menoufia Governor. Wheat flour (72%) extraction , corn flour , oil , salt for processed crackers were obtained from local market. All chemicals and diagnostic kits were purchased from El-Gomhoria Co., Cairo, Egypt.

Experimental animals: This study was carried out on twenty five adult male Sprague Dawley albino rats weighting ($90 \text{ g} \pm 5$). 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 five groups consisting of five rats each. The first group was fed on the basal diet with chesse crackers and kept as a control group, the other four groups were fed on basal diet with crackers contained olive leaf, licorice, garden cress and flax seed for 28 days.

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 °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 **Wahlefeld (1974)**. 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)**.

$$\text{LDL-c} = \text{TC} - [\text{HDL-c} + (\text{TG}/5)] \quad \text{VLDL-c} = \text{Triglycerides}/5.$$

The activity of Aspartate Aminotransferases (AST) and Alanine Aminotransferases (ALT) enzymes were assigned by the method of **Bergmeyer et al. (1978)**, total immunoglobulin (IgG, IgM, IgE and IgA) determined by Radioimmunoassay as described by the method of **Patrono and Peskar (1987)**.

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

1-The effect of supplemented snacks with some plants and seeds on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) in normal rats.

Data in Table (1) showed that feeding on flax seed followed by licorice and olive leaf led to increase the feed intake which was higher than the control group. The feed intake of the other tested group was lower than control group. There is no significant changes between group 2, 3 and 4. The group (5) which fed on crackers with garden cress was the lowest feed intake. For body weight gain , there is no significant differences between group (2), group(3) and group (4) . There were significant differences between control group and the tested groups. In case of feed efficiency ratio, the second group was the best group and there is no significant change among this group, licorice and olive leaf groups. The lowest group for FER was the control group.

Table (1): The effect of supplemented snacks with some plants and seeds on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) in normal rats.

| Groups parameters | G1 | G2 | G3 | G4 | G5 |
|----------------------|------------------------------|-----------------------------|-----------------------------|------------------------------|------------------------------|
| Feed intake g/day | 13.9 ^a ± 0.18 | 14.08 ^a ±0.75 | 12.4 ^b ±1.35 | 12.73 ^b ±1.01 | 11.2 ^c ± 0.03 |
| BWG g/42days | 70.27 ^a ±3.01 | 70.58 ^a ±2.32 | 50.12 ^d ±1.46 | 57.17 ^c ±4.42 | 40.28 ^e ±2.19 |
| FER | 0.120 ^a ± 0.01 | 0.119 ^a ±0.01 | 0.096 ^c ±0.06 | 0.107 ^b ± 0.06 | 0.086 ^d ± 0.12 |

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

2- The effect of supplemented snacks with some plants and seeds on serum lipids.

Administration of supplemented snacks with some plants and seeds at 15% level 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 supplemented crackers with flax seed followed by licorice , olive leaf group and garden cress group .

Rats that were given crackers with flax seed (group 1) showed significantly higher levels of HDL-c compared to control group and the others. The value of group five which fed on garden cress cracker group was lower for HDL-c than control group while it was higher for the other lipid parameters than the other groups .

Table (2): The effect of **supplemented snacks with some plants and seeds on serum lipids.**

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

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

3- The effect of supplemented snacks with some plants and seeds on liver function enzymes in normal rats.

From data presented in Table (3) the administration of crackers with flax seed followed by licorice and olive leaf significantly reduced AST and didn't effected on ALT level when compared with the other treatment groups. There is no significant differences between group (2) with(3). On the other hand, there is no significant between group (1) and (4) . While, there were significant changes among groups(2),(3) and the other groups. From the above results, it could be noticed that crude fiber in flax seeds is a group of indigestible carbohydrates. It can improve the function of the alimentary tract and also lower blood glucose , cholesterol levels and liver functions (Roberfroid , 2000).

Table (3): The effect of supplemented snacks with some plants and seeds on liver function enzymes in normal rats.

| Parameters | G1 | G2 | G3 | G4 | G5 |
|------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| AST(U/L) | 27.8 ^c ±0.07 | 30.2 ^b ±1.11 | 32.5 ^b ±0.21 | 27.7 ^c ±0.15 | 55.1 ^a ±2.50 |
| ALT(U/L) | 19.8 ^c ±1.91 | 28.9 ^b ±1.41 | 27.4 ^b ±0.5 | 20.7 ^c ±3.25 | 44.4 ^a ±2.01 |

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

4- The effect of supplemented snacks with some plants and seeds on immunological productions .

From Table (4), it could be observed that administration of flax seed followed by licorice and olive leaf cracker induced significant increases in serum levels of immunological profile compared to control group. The other tested cracker which was with garden cress caused non significant changes in serum level of immunological productions. The main antioxidant compounds in flax seed are vitamins C and E, phenolic compounds. So, different studies have shown that they have a protective

antioxidant effect on immunity status, cancer and cardiovascular diseases (Oomah, 2001).

Table(4): The effect of supplemented snacks with some plants and seeds on immunological productions .

| Immunological Profile mg/dl | G1 | G2 | G3 | G4 | G5 |
|-----------------------------|-----------------------------|----------------------------|---------------------------|--------------------------|-----------------------------|
| IgE | 45.54 ^c ±1.05 | 64.17 ^a ±0.05 | 60.5 ^b ±0.2 | 60.76 ^b ±0.05 | 59.87 ^b ±1.34 |
| IgM | 82.33 ^c ±10.96 | 108.2 ^a ±0.005 | 106.65 ^a ±0.65 | 105.66 ^b ±9.6 | 106.33 ^b ±3.5 |
| IgA | 78.33 ^b ±2.08 | 111.1 ^a ±0.1 | 109.5 ^a ±0.5 | 108.5 ^a ±1.5 | 109.5 ^a ±1.5 |
| IgG | 778.36 ^d ±2 0.85 | 1100.05 ^a ±9.05 | 1089 ^c ±10.87 | 1085 ^c ±15.4 | 1089.66 ^c ±25.16 |

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

DISCUSSION

Several studies have showed that each of flax seeds have long been recognized as an excellent source of protein. The flax seeds also contains a wide variety of chemical compounds that have potent bioactivity. Among these compounds are the isoflavones and the saponins. The goal of our research was to quantify isoflavone and saponin concentrations in elite flax seeds 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. We observed significant environment \times 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. We observed the inhibition of Caco-2 cell proliferation by isoflavones and saponins, suggesting a protective effect of these compounds in colon cancer. Using purified flax seeds

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, flax seeds 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 indigenous bifidobacteria in the colon against putrefactive bacteria.

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

Within flax seeds oil or the lipid portion of the seed is contained the phytosterols: 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 flax seedss contain from 0.17 to 6.16% saponins, 0.35 to 2.3% in defatted flax seeds flour and 0.06 to 1.9% in tofu. Legumes such as flax seeds and licorices 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.

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

licorice 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. Licorice 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 licorice is rich in nutritionally important unsaturated fatty acids like linoleic and oleic acid. β -sitosterol, campesterol and stigmasterol are important sterols present in licorice oil. Calcium, magnesium, phosphorus and especially potassium are also present in licorice seeds. Licorice is a good source of important vitamins such as riboflavin, niacin, thiamin, folate and the vitamin A precursor, β -carotene. Like other pulses, licorice seeds also contain anti-nutritional factors which can be reduced or eliminated by different cooking techniques. Licorice 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, licorice is an important pulse crop with a diverse array of potential nutritional and health benefits(**Jukanti1 *et al.*,2012**).

Olive leaf are high in protein, dietary fiber and antioxidants, very low in starch olive leaf can be used to make a variety of foods both sweet and savoury including everyday meals, traditional fermented foods, baked foods and sauces.

The olive leaf have a thick seed coat (25%) which consists mainly of cellulose (insoluble fiber-bran) and its removal is the first step in olive leaf (**Putnam *et al.*, 1997**).

Olive leaf 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 plants on immunological its effect on increasing antioxydant enzyems could be indirect result of their effect on lipids metabolism.

On the basis of the present results, it could be conclude that seeds of flax and flax seed followed by licorice and olive leaf may have synergistic effect reduced feed intake and body weight, improved serum lipid profile, liver functions and immunological activity in rats.

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دراسة تأثير المفروشات المدعمة ببعض النباتات والبذور على الحالة الصحية للفئران

أ.د/ عادل عبد المعطي أحمد
 أستاذ التغذية وعلوم الأطعمة
 كلية الاقتصاد المنزلي - جامعة
 المنوفية

أ.د/ نهاد رشاد الطحان
 أستاذ التغذية وعلوم الأطعمة
 جامعة -كلية الاقتصاد المنزلي
 المنوفية

أ/ رضوة عادل عبد الحميد السرسناوي
 ماجستير كلية الاقتصاد المنزلي
 جامعة المنوفية

الملخص العربي

تهدف هذه الدراسة إلى دراسة تأثير المقر مشات المدعمة ببعض النباتات والبذور على الحالة الصحية للفئران ولتحقيق ذلك تم استخدام خمسة وعشرين (25) فأر من ذكور الفئران البيضاء وزنهم (90 جم \pm 5) وتم توزيعهم إلى (5) مجموعات كل مجموعته بها (5) فئران وتم تغذيته أول مجموعته منهم على الوجبه الاساسيه مضاف إليها الدوريتوس بالجبنه أما الأربع مجموعات الأخرى تم تغذيتهم على الدوريتوس المدعم بورق الزيتون، العرق سوس، حب الرشاد، بذور الكتان لمدة 28 يوم . وأظهرت النتائج أن الزيادة في وزن الجسم كانت بشكل ملحوظ في بذور الكتان تليها عرق سوس ثم ورق الزيتون مقارنة بالمجموعة الضابطة والمجموعة التي تغذت على حب الرشاد وأيضاً لاحظ انخفاض في مستوى تركيزات الكوليسترول الكلى بأنواعه (ALT , AST) والدهون الثلاثية وكذلك انخفاض في إنزيمات الكبد مثل (LDL-c ,VLDL-c) عن المعدل الطبيعي و على ذلك وجد أن هذه المقرمشات المدعمة بالنباتات والبذور تسببت IgG, واجمالي إنتاج الجلوبيولين المناعي (HDL-c) في زيادة كبيرة في نسبه من الكوليسترول وكانت أعلى من المجموعة الضابطة التي تغذت على الدوريتوس بالجبنه . (IgM, IgE, IgA).