



**The Influence of Feeding Dietary Sesame and Flaxseeds
on Immunity of Experimental Rats**

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Abstract

The present study aims to investigate the influence of feeding dietary sesame and flaxseeds on immunity in sixty male experimental rats of Sprague Dawley strain. Their weight ranged between (120 to 125 g). They were divided as follows: The first group as a negative control group which fed on basal diet. The other five groups were immune suppressed by the administration of Aflatoxin B1 and fed on basal diet. Group two was a positive immune control group and fed on basal diet. Groups three and four fed on basal diet with (5,10 %) sesame seeds powder. While groups five and six fed on basal diet with (5,10%) of flaxseed powder for four weeks. Measurement of body weight, performed serum samples were collected to determine lipid peroxide, nitric oxide and immunoglobulin G (antibodies level). The results showed that immune suppression group (2) resulted in a slight decrease in mean body weight as compared to the control negative group (1). Rats treated with 10% sesame seeds showed the greatest increase in weight. The lipid peroxide and nitric oxide levels were found to be increased in immune suppressed rats group (2) as compared to the control group (1). Treatment of rats fed on sesame and flaxseeds resulted in decreasing these levels especially in 10% sesame group. The immunoglobulin G was decreased in immune suppressed rats group (2) as compared to control group (1). Rats fed on sesame and flaxseeds showed an increase, especially in 10% sesame fed group of rats. Therefore, it is recommended to be used dietary sesame or dietary flaxseeds for improving immunity.

Key words Sesame seeds, Flaxseeds, Immunity , Nitric oxide, Lipid peroxide, Immunoglobulins, Rats.

Introduction

The human body is exposed to enter many of foreign bodies, being sources of variable risk, which call for a defense system against these objects. These may also treat the body with some cells that become abnormal, which can turn into cancerous tumors. The body defend itself by a complex structure called the immune system (*Melo et al., 2010*). It is a network of cells, tissues and organs that work together to defend the body against foreign objects such as microbes that cause infection such as bacteria, viruses and fungi. The basic feature of the immune system, the ability to destroy extraneous objects without affecting the rest of the healthy cells of the body. The immune system cannot protect the body from all diseases depending on himself only, but needs support by some of the food to be reinforced and strengthened(*National Institutes of Health, 2003 and Mishra,2015*).

The immunity is divided in the human body to a natural immunity and acquired immunity, which made up as a result of human exposure to antigen. The immune response divided into specialized and unspecialized immunity. The specialized immunity is composed of lymphocytes T , B and antibody immunity. There are five types of antibodies which are known immunoglobulin A,M,E,G and D (IgA, IgM, IgE, IgG and IgD) that devour microbes according to kind. Unspecialized immunity made up of physical and chemical barriers, such as skin, mucous membranes, tears, sweat, juices and macrophages dealing with all the exotic objects in the same way (*Medzhitovand Janeway, 2000 and Melo et al., 2010*).

Sesame are scientifically known as (*Sesamumindicum*), and belongs to the *Pedaliaceae* family, one of the oldest crops known to man, dating back to about 1600 before century (*Nagendraet al., 2012*). Sesame is an annual plant. The plant is usually from 60-120 cm, grown in tropical and subtropical warm areas all over the world ,particularly in the tropical areas of Asia and Africa, and believed to be native in Ethiopia. Sesame of Ethiopia, India, Burma, China and Sudan comprise 68% of the total production in the world (*Thomas,2000 and El Khier et al., 2008*). Its color varies from cream-white to charcoal-black but it is mainly white or black. Other colors of some sesame seeds varieties include, yellow, red or brown (*Naturland, 2002*).

The sesame-rich sources of oils with essential fatty acids as Omega 6, gives flavor to foods and garnish foods (*Gandhi,2009*), it is

characterized by a high nutritional value and resistance to damage. It is markedly different from other vegetable oils due to its high nutritional and therapeutic effect. The sesame seeds are rich in protein with good nutritional value similar to soybean (*Naerls, 2010*). Sesame is also rich in carbohydrates, fiber, vitamins (A, B, E) and some minerals such as calcium and phosphorus. Sesame uses in the preparation of many dishes, baked, desserts and tahini (*Nagendra et al., 2012*). The important antioxidants, phenolic compounds, such as sesaminol, sesamol, sesamol and sesamin; their antioxidant lignans have shown immunomodulatory effect (*Balan et al., 2009*).

Flaxseeds are scientifically known as *Linum usitatissimum*, an annual plant belongs to the *Linaceae* family (*Amin and Thakur, 2014*). The plant known blue-cyan and green leaves are oblique to the gray and flat seeds with color brown illustrious and golden yellow (*El Beltagi et al., 2007 and Rubilar et al., 2010*). This plant is growing in temperate areas with seasonal climate (*Thomas, 2000*). The most important producers of flaxseeds India, China, the United States, Ethiopia and Canada are of the world's biggest production countries of flaxseeds (*Oomah, 2001*).

Flaxseeds are sources of many vitamins and minerals as calcium, magnesium and phosphorus. Seeds are great importance, considering that a 30g portion of the seed constitutes 7% to 30% of the Recommended Dietary Allowances for these minerals (*Evelyn and Matias, 2013*). The most abundant vitamins constituting flaxseeds are tocopherols (α , β , γ) and niacin (*Winter, 2013*). Flaxseeds contain a good amount of phenolic compounds. These phenolic compounds are well known for anticancer and antioxidative properties. Basically, flaxseeds have three different types of phenolic compounds—phenolic acids, flavonoids and lignans (*Beejmohun et al., 2007 and Mazza, 2008*).

Flaxseeds are well-known for the content of chemical compounds with specific biological activity and functional properties, such as polyunsaturated fatty acids omega-3, omega-6, α -linolenic acid, lignans, high quality proteins, soluble dietary fibers and carbohydrates compounds which are biologically active in the prevention of some chronic diseases such as many types of cancer, diabetes, cardiovascular

diseases and cerebrovascular stroke (*Rubilaret et al., 2010 and Bernacchia et al., 2014*).

The present investigation aimed to determine the influence of feeding dietary sesame and flaxseeds on immunity of experimental rats.

Materials And Methods

Materials

About two Kg of both sesame and flaxseeds were obtained from Agricultural Research Center, Giza, Egypt. Nitric oxide, lipids peroxide and immunoglobulin G (IgG) kits were obtained from Bio-Diagnostic Company, Giza, Egypt. Aflatoxin B1 was administered in laboratory of Biochemistry in Faculty of Medicine, Assiut University.

Biological Evaluation

Experimental animals

The study was performed on sixty male rats of *Sprague Dawley* strain which were obtained from the animal house of the Faculty of Medicine, Assiut University. Their weight ranged (120±5 g). They were classified into 6 equal groups. Each group was housed individually in a wire cage, under the normal laboratory conditions. Rats included in the study were fed for a period of one week on basal diet as an adaptation period. The basal diet used consisted of corn oil 5%, salt mixture, 4% vitamin mixture 1% and corn starch 78.64% (*Pellet and Sossy, 1970*). The groups of rats were divided as follows: The first group as a negative control group fed on basal diet. Group two, three, four, five and six were immune suppressed by the administration of Aflatoxin B1 and classified on feeding as follows:

- Group (2): Fed on basal diet as a positive control group.
- Group (3): Fed on basal diet with (5%) sesame seeds powder.
- Group (4): Fed on basal diet with (10%) sesame seeds powder.
- Group (5): Fed on basal diet with (5%) flaxseeds powder.
- Group (6): Fed on basal diet with (10%) flaxseeds powder.

Methods

Biochemical Analysis

Determination of nitric oxide in serum

Colorimetric determination of serum nitric oxide was carried out **according to** the method of *Montgomery and Dymock,(1961)* using nitric oxide kits purchased from Bio Diagnostic Company, Giza, Egypt.

Principle

In acid medium and the presence of nitrite the formed nitrous acid diazotize sulphanilamide and the product is coupled with N-(1-naphthyl) ethylenediamine. The resulting azo dye has a bright reddish – purple color which can be measured at 540 nm.

Calculation

$$\text{mol/ L} = \frac{\text{A sampl}}{\text{A standard}} \times 50$$

Determination of lipid peroxide (malondialdehyde)

Colorimetric determination of serum MDA was carried out **according to** the method of *Satoh,(1978) and Ohkawa et al.,(1979)* using lipid peroxide kits purchased from Bio diagnostic Company, Giza, Egypt.

Principle

Thiobarbituric acid reacts with malondialdehyde in acidic medium at temperature of 95°C for 30 min. to form thiobarbituric acid reactive product, the absorbance of resultant pink product can be measured at 534 nm.

Calculation

$$\text{Serum} = \frac{\text{A sample}}{\text{A standard}} \times 10 \text{nmol/ml}$$

Determination of immunoglobulin G (IgG)

Total IgG levels were measured with ELISA technique according to the method of *Lavanchy et al., (1990)*. For measurement of total IgG, 50 ml of diluted serum sample was placed in each well of microtiter plates (Falcon Japan, Tokyo). Samples were kept overnight at 4°C. After washing twice with PBS-T (Phosphate Buffer Saline with 0.05% Tween-20), 200 ml of ELISA buffer per well were added, and the plate was incubated for 2 h at 37 °C. After washing four times with PBS-T 0.2 mg of a rat anti-mouse IgG monoclonal antibody (Vector Lab., Burlingame, CA) in 100 ml of PBS was added for 1h at 37 °C. Bound antibodies were detected with a three- stage indirect immunoperoxidase kit (Vectastain TM ABC kit, Vactor Lab., Burlingame, CA), using 2,2- azino- d(3-ethyl- benzthiazoline sulfate), (ABTS) as a substrate . Thirty minutes later, the plates were placed in a spectrophotometer (Immuno Mini- NJ2300; NalgeNunc International Japan, Tokyo), and absorbance was measured at 450 nm. The amount of IgG was calculated using mouse IgG, purified from mouse myeloma cells (Zymed, South San Francisco, CA), as a standard and expressed as g/L serum. The lowest detection limit in this assay was 50 mg/L.

Statistical Analysis

Data were analyzed using Statistical Package for Social Science (SPSS), according to the method of *Pallant, (2005)*.

Results and Discussion

Body weight gain of the experimental rats

The results given in table (1) revealed that body weight gain showed a significant differences among all the six studied groups in all feeding weeks, as well as interaction between studied groups and feeding weeks at ($P \leq 0.01$) in experimental period.

Table(1): Effect of different concentration of sesame and flaxseeds on the body weight gain (g)ofthe experimental rats

Weeks	Control(-) Group1	Immunesup- pression (control +) Group2	Immunesuppression			
			Sesame seeds		Flaxseeds	
			5%	10%	5%	10%
			Group3	Group4	Group5	Group6
1 st week	124.3 ^k ±4.11	126.3 ^k ±2.77	125.3 ^k ±6.26	122.4 ^l ±4.22	121.0 ^l ±2.78	120.8 ^l ±2.88
2 nd week	137.5 ^h ±4.10	128.0 ^j ±3.12	145.5 ^f ±2.44	140.7 ^g ±1.93	128.8 ⁱ ±1.98	134.3 ⁱ ±2.78
3 rd week	129.3 ^j ±3.31	138.9 ^g ±3.26	157.7 ^d ±2.55	160.4 ^c ±2.77	137.1 ^h ±2.99	144.3 ^f ±2.65
4 th week	134.5 ⁱ ±2.98	128.0 ^j ±4.16	154.7 ^e ±4.91	166.2 ^a ±2.78	141.4 ^g ±2.38	164.3 ^b ±5.13
Mean	131.4 ^d	130.3 ^e	145.8 ^b	147.4 ^a	132.1 ^d	140.9 ^c

- Means were compared by using Duncan method

- Values are presented as mean ± S.E

- For each group of rats n=10

S.E Standard Error

Values followed by the same letter within the same column were not significantly different .

F-test group(G)=(A)85.6** P≤ 0.01

F-test weeks(W)=(B)11.7** P≤ 0.01

F-test (GxW)=(AB)23.5** P≤ 0.01

However, the data showed that the immunesuppression group (2) resulted in a slight decrease in mean body weight (130.3g) as compared to control group (131.4g). The mean values of increment in the body weight gain for treated sesame and flaxseeds immune suppression groups 3,4,5 and 6 fed on (5%,10%) sesame seeds and(5%,10%)flaxseeds recorded 145,8(high), 147,4(high), 132.1(low) and 140.9 (low); respectively.

The most effective group in increasing body weight gain in the experimental rats was group (4) immune suppression group with (10%) sesame seeds recording 166.2±2.78g by the end of feeding time of experiments. However, the least decrement value was observed for group (2) immunesuppression group fed on basal diet recording 128.0± 4.16 g by the end of the feeding time of the experiments (the 4th week).

Such data agree with *Mohamed et al.,(2012)and Ibrahiem(2016)*who found that there were a significant difference in body weight gain of the rats fed on (5%) from pumpkin, sesame and flaxseeds.

While,*Oyinloye et al.,(2014)* reported that results of the study showed that diets fortified with *Nigella sativa*, sesame seeds and their combination increased body weight gain and improved feed efficiency ratio in diabetic rats.*Vijaimohan et al.,(2006),Saman et al.,(2011) andEL-Sayed, and Abor,(2014)*,showed thatthe groups fed on basal diet with different levels offlaxseed oil (20, 30, 40 g/ to kg) indicated that there weresignificant increases in body weight.

On the other hand,*Mahabadi et al.,(2013)*found that the body weight gain during the treatment period did not differ significantly among groups. Moreover,no clinical and behavioral changes were observed in the animals treated with the sesame seed. These changes in results could be attributed to several factors such as dose used, administration route and experimental period.

Effect of sesame and flaxseeds on lipid peroxide (malondialdehyde) of the experimental rats

The results given in table (2) revealed thatthe effect of two concentrations of sesame and flaxseeds on lipid peroxide (Malondialdehyde) levels of the experimental animals.

Table (2): Effect of sesame and flaxseeds on lipidperoxide (Malondialdehyde) of the experimental rats(n mol/ml)

Weeks	Control (-) Group1	Immunesup pression (control +) Group2	Immunesuppression			
			Sesame seeds		Flaxseeds	
			5%	10%	5%	10%
			Group3	Group4	Group5	Group6
The 4 th week	1.05 ^c ±0.04	2.23 ^a ±0.07	1.09 ^b ±0.03	0.95 ^d ±0.04	0.95 ^d ±0.06	1.21 ^b ±0.04

- Means were compared by using Duncan method

- Values are presented as mean ± S.E

- For each group of rats n=10

S.E Standard Error

Values followed by the same letter within the same column were not significantly different .

F-test group (G)=17.6** P≤ 0.01

The result showed that there were significant differences ($p \leq 0.01$) among all studied groups at the end of the experiment. However, the data confirmed that the most effected groups in decreasing serum lipid peroxide in the experimental rats were groups (4) and (5) (immunosuppression group fed on 10% sesame seeds and 5% flaxseeds) recording (0.95 ± 0.04 and 0.95 ± 0.06 nmol/ml) by the end of experimental period (the 4th week). High increment value was observed for group (2) (2.23 ± 0.07 nmol/ml) as compared to the healthy group fed on basal diet (1.05 ± 0.04 nmol/ml).

The results of this study showed that 10% of sesame seeds was effective in decreasing lipid peroxide levels, and (5%) flaxseeds, followed by (5%) sesame seeds and (10%) flaxseeds as compared with group (2).

Srivastava and Mittal, (2005) and Prakash and Naik, (2014) who reported that the generation of free radicals as reflected by increased malondialdehyde levels in patients could be one of the causes leading to the development of cancer. *Lei et al., (2012)* revealed that treatment with sesamin could reduce the content of the lipid peroxidation product malondialdehyde of cells, and increase the activity of the antioxidative defense system, and thus inhibit free radical generation. This suggested that sesamin has the anti-oxidative effect, which prevents and protects streptozotocin -induced oxidative stress and β -cell damage.

Fadlalla, et al., (2014) showed the effect of seeds mixture on serum malondialdehyde, in hypercholesterolemic rats. Lipid peroxidation was efficiently counteracted by the treatment with sesame and flaxseeds as compared with the untreated rats. Sesame and flaxseeds supplement might attenuate oxidative-stress-associated renal injury by reducing oxygen free radicals and lipid peroxidation. The sesame or flax powder prevents intoxication of the exposed animals to aflatoxin B1 during the experiment lasting for four weeks.

Effect of sesame and flaxseeds on the level of nitric oxide of the experimental rats

Results in table (3) showed, the effect of two concentrations of sesame and flaxseeds on nitric oxide in experimental rats.

Table (3): Effect of sesame and flaxseeds on the level of nitric oxide of the experimental rats(mol/L)

Weeks	Control (-) Group1	Immunesup pression (control +) Group2	Immunesuppression			
			Sesame seeds		Flaxseeds	
			5%	10%	5%	10%
			Group3	Group4	Group5	Group6
The 4 th week	4.92 ^e ±0.26	13.80 ^b ±0.71	8.52 ^d ±0.38	6.38 ^d ±0.33	10.35 ^d ±0.76	13.00 ^a ±1.8

- Means were compared by using Duncan method

- Values are presented as mean ± S.E

- For each group of rats n=10

S.E Standard Error

Values followed by the same letter within the same column were not significantly different .

F-test group(G)=17.86**

P1 =27.85** P≤ 0.01

P2 = 31.32** P≤ 0.01

The table (3) results showed a significant differences ($P \leq 0.01$) among all studied groups at the end of the experiment. Data confirmed that the most effected group in decreasing of serum nitric oxide in the experimental rats was groups (3) and (4) (immunesuppression group fed on 5% and 10% sesame seeds) recording 8.52 ± 0.38 and 6.38 ± 0.33 mol/L at the end of experimental period (the 4th week). On the other hand, the nitric oxide was found to be increased in immunesuppressed rats group (2) (13.80 ± 0.71 mol/L) compared to the control group fed on basal diet (4.92 ± 0.26 mol/L).

The low dose of sesame significantly reduced from the nitric oxide level which is related to the reducing anti inflammatory compounds in the sesame such as vitamin A and vitamin E. The reduction was progressed by increasing the load of sesame powder to the high dose. The low dose of flaxseeds reduced the level of nitric oxide which maybe due to present of carotenoids, while the high dose revealed higher level of nitric oxide due to the inflammatory effect of flaxseeds intoxicating linamarine that enhances the oxidative stress as well as level inflammation level of organs (Yamashita et al., 2003 and Monteiro et al., 2014).

The results (table 3) are in agreement with Hsu et al., (2013) findings, who reported that sesamol decreases the

inflammatory response, attenuates the associated organs damage and nitric oxide in serum of rats.

Effect of sesame and flaxseeds on the level of immunoglobulinG (IgG) of the experimental rats

Results in table (4) show the effect of two concentrations of sesame and flaxseeds on immunoglobulin G (IgG) in experimental rats.

Table(4): Effect of sesame and flaxseeds on the level of immunoglobulin G (IgG) of the experimental rats (mg/L)

Weeks	Control (-)	Immunesuppression (Control +)	Immunesuppression			
			Sesame seeds		Flaxseeds	
			5%	10%	5%	10%
	Group1	Group2	Group3	Group4	Group5	Group6
The 4 th week	224.0 ^e ±3.15	137.0 ^f ±4.1	230.0 ^b ±6.12	239.0 ^a ±3.12	220.0 ^d ±7.12	167.0 ^c ±3.12

- Means were compared by using Duncan method

- Values are presented as mean ± S.E

- For each group of rats n=10

S.E Standard Error

Values followed by the same letter within the same column were not significantly different .

F-test group (G)=26.3** P≤ 0.01

The table (4) results showed a significant differences (P≤ 0.01) among all studied groups. The results given in table (4) showed that immunoglobulin G level was found to be decreased in immunesuppressed rats group (2) (137.0±4.1 mg/L) as compared to control (-) group (224.0±3.15 mg/L). Rats fed on (10%) sesame seeds group (4) showed the greatest increase in immunoglobulin G level (239.0±3.12 mg/L), followed by (5%) sesame seeds group (3) (230.0±6.12 mg/L). Immunesuppressed group fed on (5%) flaxseeds was found to raise IgG (220.0±7.12 mg/L) as compared to group (6) fed on (10%) flaxseeds (167.0±3.12 mg/L).

Such data agree with findings *Nonaka et al., (2014)* who showed the effect of sesamin and sesaminolol on the level of immune globulins in rats that have been injected with ethanol. After examining the rats it was found a clear effect of these materials on raising the level of IgM, IgA, IgG in the blood plasma in spite of chronic effect of ethanol.

According to *Matusiewicz et al.,(2015)* dietary inclusion of flaxseed cake in experiment caused a decreased concentration of albumins in blood serum of rats, as compared to the control(-) group. In the longterm experiment, a higher ($p \leq 0.01$) concentration of globulins was shown in blood of the animals administered flaxseed cake. Differences in concentrations of albumins and globulins in blood serum of rats might result from the immunomodulating effect of diet. The response of the immune system is modified by flax components, fiber, fatty acids, and polyphenols including flavonoids. Also, *Barakat and Hamed,(2011)* showed that it is clear that the consuming seeds mixtures either flax/pumpkin or purslane / pumpkin had positive impacts to the immunity status of hypercholesterolemic rats by significant reduction in rat serum immunoglobulins to bring them near the normal levels.

Goyal et al., (2014) and Kajla et al.,(2015) reviewed on new flax facts. On the basis of several clinical studies, flax favorably effects on the immune system. Flax consumption may help prevent and treat disorders characterized in part by an overstimulated immune system. Such disorders include atherosclerosis, obesity, the metabolic syndrome, diabetes mellitus, rheumatoid, arthritis, multiple sclerosis and systemic lupus erythematosus.

Conclusion

Sesame and flaxseeds are important sources to improve immune system especially high dose of sesame seeds and low dose of flaxseeds. That are also protect the body from aflatoxicosis exposure by contaminate food. So, this research recommend the treatment to our foods by sesame and flaxseeds.

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تأثير التغذية ببذور السمسم والكتان على مناعة فئران التجارب

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يهدف البحث الحالي إلى دراسة تأثير التغذية ببذور السمسم والكتان على مناعة 60 من ذكور فئران التجارب سلالة سبراجو داولي، تتراوح أوزانهم ما بين (120 ± 5 جم). و تم تقسيمهم على النحو التالي المجموعة الأولى كمجموعة ضابطة سالبة تتغذى على الوجبة الغذائية الأساسية. بينما المجموعات الخمس الأخرى تم خفض المناعة لهم بالسهم أفلاتوكسين ب1. المجموعة الثانية كمجموعة ضابطة موجبه تتغذى على الوجبة الغذائية الأساسية. بينما المجموعات الثالثة و الرابعة تم تغذيتها على الوجبة الغذائية الأساسية مضاف إليها مسحوق بذور السمسم بنسب 5 و 10%. و المجموعات الخامسة و السادسة تم تغذيتها على الوجبة الغذائية الأساسية مضاف إليها مسحوق بذور الكتان بنسب 5 و 10%. وذلك لمدة أربعة أسابيع. تم قياس وزن الفئران، كما تم تجميع عينات الدم لقياس أكسيد النيتريك، بيروكسيد الدهون في سيرم الفئران . وقد أظهرت النتائج أن خفض (الاجسام المضادة G والجلوبولين المناعي مناعة المجموعة الثانية أدى الى انخفاض طفيف في متوسط وزن فئران التجارب مقارنة بالمجموعة الضابطة السالبة. وسجلت أكبر زيادة في وزن الفئران المجموعة الرابعة المغذاه على 10% سمسم . كما زادت مستويات أكسيد النيتريك و بيروكسيد الدهون فيالمجموعة الثانية مقارنة بالمجموعة الضابطة السالبة. وأدى إضافة كلا منبذور السمسم والكتان إلى الوجبة الغذائية الأساسية الى خفض هذه المستويات وخاصة في المجموعة الرابعة المغذاه على فيالمجموعة الثانية مقارنة G 10% سمسم. كما لوحظ انخفاض في مستوى الجلوبولين المناعي في المجموعات التي G بالمجموعة الضابطة السالبة وزيادة في مستوى الجلوبولين المناعي تغذت على بذور السمسم والكتان وخاصة المجموعة الرابعة المغذاه على 10% سمسم. وبناء على ذلك توصي هذه الدراسة باستخدام بذور السمسم والكتان في الغذاء لتحسين المناعة .

الكلمات المفتاحية: بذور السمسم - بذور الكتان - المناعة - أكسيد النيتريك - بيروكسيد - فئران التجارب G. الدهون - الجلوبولين المناعي