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ORIGINAL ARTICLE

Experimental study of the chemical and immunological properties of functional cookies fortified with pumpkin seed powder and its effects on hypoimmune experimental Rats

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Abstract

This study was conducted to estimate the influence of pumpkin seed powder supplementation on the chemical composition of cookies also immune system function in immunocompromised laboratory rats. The chemical composition of cookies made by replacing wheat flour with 15 and 30% of pumpkin seed powder was high in protein, fat, ash (minerals) and fiber, but low in carbohydrates. Twenty white male rats were divided into four groups, the first group, the negative control group (-V) were fed cookies made of 100% wheat flour. Laboratory animals were dosed in the following groups (the infected positive control group was fed cookies made from 100% wheat flour, groups (3 and 4) were fed cookies containing 15 and 30% of pumpkin seed powder, respectively) orally 0.3 µl Sandimmune Immunosuppressive for 7 days until symptoms of reduced immunity appear. The results indicated the immunocompromised rats that fed cookies containing (15 and 30%) of pumpkin seed powder led to a weight gain of the rats in groups 3 and 4, which was at 68 and 77 gm, respectively, compared to the infected control group, which was at 36 g. There was also a significant decrease ($P < 0.05$) in cholesterol, triglycerides, low-density lipoproteins and very low-density lipoproteins and a significant increase ($P < 0.05$) in high-density lipoproteins compared to the positive control group (infected). Moreover, immunosuppressed laboratory rats treated with cookies made with pumpkin seed powder had a significant effect on improving blood parameters (RBCs, Hb, HCT, WBC and Net) and the values average of immunoglobulins (IgM, IgA and IgG) compared to The positive (infected) control group. The current study recommended eating pumpkin seeds for patients with low immunity.

1. Introduction

Currently, there is a growing request for health-encouraging foods, as some food sources dried in powdered form make available a means for producers to develop the health advantages of grain products [1, 2]. Bakery products, for example, cookies, biscuits, bread and cakes, are a great group of general food produces, which are used up by an extensive range of people around the world because of the variation of tastes, relatively long shelf life and little cost [3]. These products can be fortified plus minerals, vitamins, proteins, polyphenols and fiber by incorporating rich sources [4]. Cookies are bakery products and have become fast food for each age group because they are simple to carry, delicious to

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eat, cholesterol-free, contain vital digestive and nutritional values and are not expensive [5]. In recent years, seeds and nuts have received increasing attention due to their bioactive components' high nutritional and therapeutic value [6-8] stated that pumpkin seed powder can be used to strengthen soups, biscuits, cookies, pies and bread, in addition, to enhancing wheat flour to produce bakery products, for example, unique-tasting pastries. Pumpkin seeds may be small in size, but they are packed with beneficial nutrients, for example, phytosterols, amino acids, phenolic compounds, unsaturated fatty acids, essential minerals and tocopherols, all of which are bioactive compounds necessary for a life of healthy [9]. In vivo experiments have demonstrated that natural bioactive compounds in pumpkin seeds, such as carotenoids, tocopherols and sterols, have a range of biological activity in disease prevention and immune enhancement [10]. Nutritionally, pumpkin seeds are expensive in proteins, fats, minerals and fiber. The chemical composition of pumpkin seeds reveals the following proportions: 33.48% protein, 30.66% fat, 3.07% fiber, 3.98% ash and 524.58 kcal of energy [11]. Linoleic acid is the most abundant fatty acid in pumpkin seeds [12]. Studies of the response to immune enhancement evaluate the response to immune enhancement using cookies mixed with pumpkin seed powder, and its activity as an antioxidant and immune booster. have not been conducted. Therefore, this study aimed to evaluate the response to immune enhancement using cookies mixed with pumpkin seed powder and its activity as an antioxidant and immune booster.

2. Materials and methods

2.1 Materials

Pumpkin seeds, wheat flour and other ingredients of the cookie mix such as cornstarch, margarine, sugar flour and baking powder were obtained from the local market in Tikrit.

2.2 Preparation of pumpkin seed powder

Pumpkin seeds were washed well with running water to remove dirt, dust and impurities, then dried by oven at a temperature of 65 °C until reaching a moisture content of 5-6%, then the pumpkin seeds were ground by an electric grinder into powder and kept in sealed polyethylene bags until use.

2.3 Cookies preparation

The cookies were made according to the method used [13] where the wheat flour was replaced by 15 and 30% of the pumpkin seed powder, and the components of the cookie mix were clarified in Table (1). The margarine was mixed with Crushed sugar using a mixer for 5 minutes until a good cream was formed, then the egg yolk was added to the cream. Wheat flour is also added with the rest of the components to the cream and mix in the mixer until the dough is formed well and ready to be rolled and cut.

Table 1: The components of cookies

The components	Treatments ratios and quantities		
	The control	Pumpkin 15%	Pumpkin 30%
Wheat flour	450gm	382.5gm	315gm
pumpkin seed powder	0	67.5gm	135gm
Corn Starch	50gm	50gm	50gm
Margarine	350gm	350gm	350gm
Crushed sugar	180gm	180gm	180gm
Egg yolk	32gm	32gm	32gm
Baking powder	5gm	5gm	5gm

Put the pieces of dough in the oven at 150 ° C for 18 minutes. After that, the cookies were taken out of the oven and left to cool down to room temperature, and they were packed in sealed polyethylene bags until use.

2.4 Chemical composition of raw materials and cookies

The moisture, fiber, lipid and ash content were estimated using AOAC (2000) methods [14]. The protein content was estimated by Kjeldahl method (6.25 N) and total carbohydrates were calculated by difference.

2.5 The Vital Experience

2.5.1 Preparation of laboratory animals

Healthy and disease-free laboratory animals were brought and prepared from the University of Tikrit /College of Veterinary Medicine with numbers of 20 adult male Albino rats, whose weights ranged at 175-178 gm, unsystematically dispersed into four groups of near weights, all group contained five animals. These animals were placed inner cages prepared of plastic. Afterward preparing these cages, their floors were covered with sawdust, which was substituted three times a week. The animals were regularly fed on a basic diet formulated according to (NRC, 1995) for one week for adaptation. Animals were kept under a clean, environmentally controlled air room at 24 ± 5 °C, illumination (12 h light/12 h dark), 60 ± 4% relative humidity, and water availability. the main food during the trial period was cookies, which were manufactured. The animals were raised below the staff of supervision of a specialized veterinary, taking into account the aspect of cleanliness.

2.5.2 Induction of infection

Laboratory animals in the following groups (infected positive control group, third group fed cookies containing 15% pumpkin seed powder, fourth group fed cookies containing 30% pumpkin seed powder) orally 0.3 µl of the immunosuppressive Sandimmune for 7 days. After that, their health condition was monitored and the changes that occurred were recorded daily, as symptoms began to appear from the fourth day of dosing, which included lethargy, recluse, lack of movement and lack of appetite. Blood was drawn from them to verify their immunity, then the feeding period described above began to study the effect of pumpkin seed powder added to functional cookies on blood parameters, blood lipid levels and immunity in rats with low immunity.

2.5.3 Experiment design

- G1: Group of healthy control animals (which are fed cookies made of 100% wheat flour).
- G2: Group of infected control animals given the immunosuppressant Sandimmune + cookies made from 100% wheat flour.
- G3: Group of infected animals given the immunosuppressant Sandimmune + cookies made from pumpkin seed powder 15%.
- G4: Group of infected animals given the immunosuppressant Sandimmune + cookies made from pumpkin seed powder 30%.

After the end of the experiment, which lasted 28 days, the animals were starved for 20 hours and next anesthetized with chloroform, then the rats were dissected from the chest part and blood was drawn directly from the heart to make the essential tests. In two tubes the blood was drawn, the first including EDTA anticoagulant to determine CBC, the another tubes free of it, including nearly 3 ml of blood, which were centrifuged by use a centrifuge at 3000 rpm for 15 minutes to obtain the serum that was preserved. At a temperature of -20 ºC until the analysis is carried out as in (Tietz, 2005). The blood tests included the following parameters:

The erythrocyte count (RBCs), hemoglobin concentration (Hb) and the whole number of white blood cells (WBCs) were estimated according to (Bregman, 1987) and (Schottelius et al., 1988) and (John and Leuis, 1984) respectively and the proportion of lymphocytes (LYM) and total numbers of neutrophil white cells (Neut) and (HCT) as stated in (Tietz, 2005). Serum levels of TC, TG, and HDL-c were measured according to Cohn et al. (1988) and Foster and Dumns (1973) and Young (1995, respectively. (LDL-c) and (VLDLc) were calculated according to (Fried et al., 1972).

IgM, IgA and IgG antibodies were determined using a special kit produced by Genius and using the Shenzhen Genius Electronics co. Following the manufacturer's instructions, according to (Burtis and Ashwood, 1999).

2.5.4 Statistical Analysis

The results of the experiments were examined by use the Linear Model General contained by the ready-made statistical program [15].

To study the influence of factors according to the complete unsystematic design (CRD), Duncan's test [16] was similarly conducted to determine the significance of changes amid the means of factors affecting the tested traits at the level ($p<0.05$).

3. Results and discussion

3.1 Chemical composition of cookies

The chemical composition of the manufactured cookies was estimated by replacing wheat flour with pumpkin seed powder at percentages of 15 and 30% as in Table (2), where a significant increase was found at $P< 0.05$ for protein, fat, ash and fiber. Protein increased significantly with an increase in the replacement rates, which were at 12.4 and 16.8% for cookies made from pumpkin seed powder and at rates of 15 and 30%, respectively, compared to the control treatment (wheat flour), which had a protein percentage of 10.92%. As for fat, it increased significantly for the two treatments T2 and T3 and its percentage was at 19.8 and 20.6%, respectively, compared to the treatment of T1 in which the percentage of lipid was at 17.31%. With regard to ash, its percentage increased significantly with an increase in the percentage of replacement with pumpkin seed powder 15 and 30%, and it was at 2.2 and 2.8%, respectively, compared to the control treatment, in which the percentage of ash was at 0.53%. At the same time, we find that the percentage of fiber also witnessed a significant increase in its percentage by increasing the percentage of replacement, and it was at 2.18 and 2.5% for cookies made from pumpkin seed powder at percentages of 15 and 30%, respectively, compared to the control sample in which the percentage of fiber was 0.89%.

As for carbohydrates, there was a significant decrease in the percentage of replacement with pumpkin seeds powder, which was at 60.02 and 53.28% compared to the control treatment, which was at 66.4% of carbohydrates. This decrease in the proportion of carbohydrates was due to the increase in the proportions of protein, fat, ash and fiber.

These results agreed with what was found [17], as it was found that the highest carbohydrate content was in the control treatment and then decreased more significantly in the rest of the treatments. The decrease in carbohydrates could be due to reduced levels of refined wheat flour [18]. The results are also in agreement with what researchers found [19] that the chemical composition of cookies made from replacing wheat flour with sprouted pumpkin seed powder is 20% Ash 2.31%, lipid 25.29%, protein 17.42%, fiber 2.22% and carbohydrates 52.7%.

The results also agreed [20, 21], who found that pumpkin is a rich source of nutrients and various elements. The percentage of pumpkin seed powder supplementation increased from 0 to 15% (w/w), the protein content, ash content and crude fiber were also increased [22].

Table 2: The chemical composition of cookies

Treatments	Humidity	protein	fat	Ash	Fiber	Carbohydrates
	%					
T1	3.95+0.028b	10.92+0.005c	17.31+0.057c	0.53+0.057c	0.89+0.005c	66.4+0.057a
T2	3.4+0.057c	12.4+0.057b	19.8+0.057b	2.2+0.057b	2.18+0.057b	60.02+0.057b
T3	4.02+0.057a	16.8+0.28a	20.6+0.05a	2.8+0.057a	2.50+0.057a	53.28+0.057c

*Similar letters in one column mean that there are no significant differences between them at the level of probability (0.05).

T1 = cookies made from 100% wheat flour. T2 = Cookies made by replacing 15% wheat flour with pumpkin seed powder. T3 = Cookies made by replacing wheat flour with pumpkin seed powder at 30%.

3.2 Effect of feeding with functional cookies made by replacing wheat flour with 15 and 30% of pumpkin seed powder on the weight of immunocompromised rats.

The effect of feeding with cookies prepared by substituting wheat flour with pumpkin seed powder at percentages of 15 and 30% on the weights of experimental animals was clarified in Table (3), which shows that there are significant differences in the final weight. Where the final weights of the group animals fed with cookies made of pumpkin seed powder by 15 and 30% were at 246 and 253 gm, respectively, compared with the weight of the animals of the group with reduced immunity, which was at 212 gm, while the weight of the animals of the negative control group was at 247 gm. While the weight gain in which there was a significant increase for the studied groups that were fed functional cookies prepared by substituting wheat flour with pumpkin seed powder at percentages of 15 and 30%, it was the highest weight gain for the group of animals fed with cookies made by replacing 30% of pumpkin seed powder and this increase was at 77 gm. Negative control, the weight gain was at 72 gm, followed by the group fed functional cookies made with the replacement of 15% of pumpkin seed powder and it was at 68 gm compared to the group of animals with reduced immunity, which was at 36 g. The pumpkin seeds contain a high percentage of crude protein, about 35%, and this translates into a great and various amount of amino acids [23]. These amino acids take essential roles as construction blocks of proteins and as an intermediary in the metabolism process. Dietary provide of an appropriate quantity and quality of important amino acids is so important for physiological functions in the body of human [24].

Table 3: The effect of feeding functional cookies on the weights of rats with reduced immunity

Treatments	*Initial weight*	*final weight*	*weight gain*
	gr		
G1	175a±3.26	247 b ± 1.77	72
G2	176a ± 4.15	212 d ± 2.38	36
G3	178a ± 2.30	246 c ± 0.21	68
G4	176 a ± 3.47	253 a ± 1.58	77

*Similar letters in one column mean that there are no significant differences between them at the level of probability (0.05).

G1 = the negative control group that was fed cookies made of 100% wheat flour. G2 = the positive control group (with reduced immunity) that was fed cookies made of 100% wheat flour. G3 = group of immunocompromised animals that were fed cookies made with 15% substitute of wheat flour with pumpkin seed powder. G4 = group of immunocompromised animals that were fed cookies made with 30% substitute of wheat flour with pumpkin seed powder.

The polyunsaturated fatty acids in pumpkin seeds have been studied because of their protective effect versus diseases and their role in enhancing immunity [25]. They are essential for the healthy growing and development of the brain and nervous system, individually; Research also reports that it has health benefits in improving coronary heart disease, high blood pressure, and arthritis [26]. Undoubtedly, this high content of protein and polyunsaturated fatty acids caused the rats to regain their weight in the nutrient groups with the pumpkin seed powder cookies.

3.3 Effect of feeding with functional cookies made by replacing wheat flour with 15 and 30% of pumpkin seed powder on the level of lipids in the blood of immunocompromised rats

Table (4) shows the effect of feeding immunocompromised laboratory rats with functional cookies partially replaced with pumpkin seed powder at rates of 15 and 30% on the level of lipids in the blood. The results showed that there were significant differences at ($P<0.05$), as cholesterol decreased significantly in the groups fed with cookies manufactured by replacing wheat flour with pumpkin seed powder at percentages of 15 and 30% and it was at 126 and 122 (mg/100ml), respectively, compared to the animal group. The immunocompromised group was 136 (mg/100ml), while the negative control group fed cookies made of 100% wheat flour recorded the lowest concentration at 118 (mg/100ml). The same was true for the triglycerides (TG), which also decreased significantly for the same groups and were at 105 and 102 (mg/ 100 ml), respectively when compared with the triglycerides of the negative control group, which was at 98 (mg / 100 ml), which is the lowest among all groups. As for the high-density lipoproteins (HDL-C), and as we can note from the table, it had a significant rise at ($P<0.05$) in the groups fed with cookies, in which wheat flour was substituted with pumpkin seed powder at percentages of 15 and 30%, and their values were at 41 and 48 (mg/100ml), individually, compared to the group of immunocompromised animals, which had a concentration of 30 (mg/100ml). On the contrary, there was a significant decrease in the concentration of low-density lipoproteins (LDL-C) in the groups given functional cookies replaced by wheat flour with pumpkin seed powder at percentages of 15

and 30% and their concentration was at 64 and 53.6 (mg/100ml) in comparison. With the group of infected control animals whose concentration was at 82 (mg/100ml). Likewise, for very low density lipoproteins, a significant decrease occurred when feeding groups of animals with functional cookies with replacement ratios of 15 and 30%, and their concentration was at 21 and 20.4 (mg/100ml) when compared with the group of animals affected with reduced immunity, whose concentration was at 23.2 (mg/100 ml). The results agreed [27] that eating pumpkin seeds led to a decrease in the concentrations of TC, TG, and LDL-c, and that this effect is due to the high content of unsaturated fatty acids and fiber in pumpkin seeds, which can contribute in lowering the level of cholesterol and increasing the level of HDL in the blood.
The results obtained showed that supplementation and various levels of pumpkin seed powder reduced the level of lipids in laboratory rats, while it increased the concentration of high-density lipoprotein (HDL-c) in the serum. This effect may be due to the active chemical compounds in the pumpkin seeds and the unsaturated oils and fatty acids they contain. These results were steady [28] who reported that administration of pumpkin can significantly reduce serum TG, TC, and LDL-c levels. The fiber reduces the level of LDL in the plasma by suppressing the absorption of cholesterol and bile acids and rising the experience of LDL receptors, and thus it can be completed that pumpkin decreases the influence of triglycerides during its fiber content. Dietary fiber can too decrease triglyceride levels by inhibiting lipogenesis in the liver [29].

Table 4: Effect of functional cookie feeding on blood lipids of immunocompromised rats

Treatments	*TC*	*TG*	*HDL-C*	*LDL-C*	*VLDL-C*
	(mg/100ml)				
G1	118d ± 3.26	98 d ± 1.77	46 b ± 3.26	52.4 d ±3.26	19.6 c ± 3.26
G2	136 a ± 4.15	116 a ± 2.38	30 d ± 4.15	82.8 a ±4.15	23.2 a ± 4.15
G3	126 b ± 2.30	105 b ± 0.21	41 c ± 2.30	64 b ± 2.30	21 b ± 2.30
G4	122 c ± 3.47	102 c ± 1.58	48 a ± 3.47	53.6 c ± 3.47	20.4bc ± 3.47

*Similar letters in one column mean that there are no significant differences between them at the level of probability (0.05).

G1 = the negative control group that was fed cookies made of 100% wheat flour. G2 = the positive control group (with reduced immunity) that was fed cookies made of 100% wheat flour. G3 = group of immunocompromised animals that were fed cookies made with 15% replacement of wheat flour with pumpkin seed powder. G4 = group of immunocompromised animals that were fed cookies made with 30% replacement of wheat flour with pumpkin seed powder.

3.4 Effect of feeding with functional cookies made by replacing wheat flour with different percentages of pumpkin seed powder on blood parameters of immunocompromised rats.

The effect of feeding cookies made with replacement of wheat flour with 15 and 30% of pumpkin seed powder on CBC parameters of immunocompromised rats is shown in Table (5). The results showed that feeding with functional cookies caused a significant increase in RBC count at ($p<0.05$). Its numbers were at 5.23, 6.17 and 6.84 ($10^6/mm^3$), respectively, compared to their numbers in the animals of the immunocompromised group, which was 4.91 ($10^6/mm^3$). The negative control group, which was fed biscuits made of 100% wheat flour and without Any replacement ratio was at 6.38 ($10^6/mm^3$).
This feeding also had an effect on the hemoglobin concentration of the rats, which resulted in a significant increase, as feeding functional cookies made by replacing percentages of 15 and 30% of pumpkin seed powder increased the concentration of hemoglobin at 11.92 and 12.45 (mg/dl), respectively compared to the group of animals affected with low immunity, as the hemoglobin concentration was at 10.46 (mg/dl). As for the uninfected control group, the hemoglobin concentration was 11.92 (mg/dl). The results showed when estimating the percentage of red blood cells (HCT) that the effect of feeding led to a significant increase in the percentage of red blood cells, as it was at 37.71 and 38.62%, respectively, compared to its percentage in the animals of the group with low immunity, which was 34.92%. The value of the red blood cells percentage of the negative control group was 38.26%. The results revealed that feeding with functional cookies began a significant rise ($p<0.05$) in the total numbers of white blood cells (WBC), Their numbers were at 7.75 and 8.38 ($\times10^6/mm^3$), respectively, compared with their numbers in the blood of the group of low-immune animals, which were at 6.12($\times10^6/mm^3$), while the total numbers of white blood cells in the negative control group were at 7.92($\times10^6/mm^3$).
As for the percentage of lymphocytes, feeding with cookies prepared by substituting wheat flour with pumpkin seed powder at percentages of 15 and 30% led to a significant decrease in their percentage, reaching 51.5 and 48%, respectively, compared to their numbers in the blood of the group of low-immune animals that were at 60.2%, while the percentage of lymphocytes (Lym) in the negative control group that was fed 100% wheat flour cookies at 47.6%, which was the lowest percentage among the animal groups in the table. The results also showed that feeding with functional cookies led to a significant increase in the percentage of the total numbers of white cells, neutrophils, reaching 53.5 and 56.3%, respectively, compared to their numbers in the blood of a group of low-immune animals that were at 50%, while it was The percentage of total numbers of neutrophil white cells in the negative control group that were fed 100% wheat flour cookies at 54.8%. Feeding pumpkin began a significant ($P < 0.05$) increase in blood parameters such as RBC, HCT and

hemoglobin in treated rats. This can be attributed to the free radical scavenging activities of flavonoids and other plant phenols or to the metal-binding activity and polyphenol interesting properties of the immune system [30]. Elevation in these variables indicates increased production of the vast majority of immune-enhancing cells that are produced in bone marrow stem cells, and this is believed to have a stimulating effect on immune reactions because increased production of immune system cells may mean greater function of the immune system [31]. Since pumpkin seeds are rich in flavonoids, which have health-promoting properties due to their high antioxidant capacity in vivo, eating pumpkin seeds enhances immunity and thus enhances overall health [32].

Table 5: Effect of feeding cookies made from pumpkin seed powder at different rates on blood parameters of immune compromised rats

Treatments	RBCs	Hb	HCT	WBC	Lym	Net
	10^6/mm^3	mg/dl	%	106/mm3	%	
G1	6.38 b ±0.21	12.21 a +0.24	38.26 b +0.22	7.92 b +0.13	47.6 d +1.08	54.8 c +0.19
G2	4.91 c ±0.17	9.85 c +0.43	34.92 d +1.45	6.12 c +0.66	60.2 a +0.31	50 e +0.24
G3	6.17 b ±0.32	11.92 b +0.21	37.71 c +0.14	7.75 b +0.15	51.5 b +0.2	53.5 d +1.41
G4	6.84 a ±0.18	12.45 a +0.2	38.62 a +0.31	8.38 a +1.25	48 c +1.25	56.3 b+0.02

* Similar letters in the same column mean that there are no significant differences between them at the 0.05 probability level.

G1 = the negative control group fed cookies made from 100% wheat flour. G2 = the positive control group (with reduced immunity) that was fed cookies made of 100% wheat flour. G3 = group of immunocompromised animals fed a cookie made with a 15% replacement of wheat flour with pumpkin seed powder. G4 = group of immunocompromised animals fed cookies made with 30% replacement of wheat flour with pumpkin seed powder.

3.5 Effect of feeding with functional cookies made by replacing wheat flour with different percentages of pumpkin seed powder on immunoglobulins (IgM, IgA and IgG) of immunocompromised rats.

The effect of feeding with functional cookies made by replacing wheat flour with 15 and 30% of pumpkin seed powder on some immunological parameters in immunocompromised rats was shown by the results in Table (6). The results revealed that feeding with functional cookies made by replacing wheat flour with 15 and 30% of pumpkin seed powder began a significant ($p<0.05$) increase in IgM concentration, as its concentrations were at 154 and 159 (mg/dL), individually, compared with Its concentration in the blood of low-immune positive control group animals was 130 (mg/dL).These results also showed the effect of feeding with functional cookies made by replacing wheat flour with percentages of 15 and 30% of pumpkin seed powder, which caused a significant increase in the concentration of IgA, as its concentration was at 1267 and 1278 (mg/dL), respectively, compared with its concentration in The blood of the experimentally immunocompromised group animals was 1108 (mg/dL), while the negative control group fed cookies made of wheat flour only had the IgA concentration at the highest value of 1291 (mg/dL).Whereas, feeding laboratory animals with functional cookies made from pumpkin seed powder in different proportions led to a significant increase in the concentration of IgG at 2722 and 2774 (mg/dL) for each of 15 and 30% concentrations, respectively, compared with the low group of animals. Immunity was tested experimentally, as the concentration of IgG immunoglobulin was at 8124 (mg/dL).

Pumpkin seeds and their oil are an essential source of phytosterols [33]. While there are more than 100 various kinds of specific phytosterols in plant species, the predominant phytosterols mentioned in pumpkin seed oil is 7-sterols, unlike most vegetable oils this dominance is left behind by sitosterol 7,22,25-stigmatatrienol, 7-stigmastenol, spinasterol [34]. Pumpkin seeds are too a better source of vitamin E (tocopherol). These seeds as well contain a small amount of α-tocotrienol and β-tocotrienols. Tocopherols and tocotrienols, which are powerful antioxidants that have the capability to inactivate greatly active radicals by freeing the H+ ion from their ring, thus activating and enhancing the immune factor [35]. The pumpkin seeds are some excellent food source rich in minerals essentially magnesium, phosphorous, zinc, selenium and potassium that are responsible for fighting diseases and can act as a weapon to fight these diseases and boost immunity [36].

Table 6: Effect of feeding cookies made from Pumpkin seed powder with percentages of 15 and 30% on the concentration of immunoglobulins in immunocompromised rats

Treatments	IgM	IgA	IgG
	mg/dL		
G1	157 b + 1.04	1291 a +1.08	2806 a + 1.02
G2	130 d + 2.06	1108 d + 0.14	2481 d + 0.25
G3	154 c + 0.51	1267 c +1.24	2722 c + 1.13
G4	159 a + 1.08	1278 b + 2.21	2774 b + 1.33

*Similar letters in one column mean that there are no significant differences between them at the level of probability (0.05).

G1 = the negative control group that was fed cookies made of 100% wheat flour. G2 = the positive control group (with reduced immunity) that was fed cookies made of 100% wheat flour. G3 = group of immunocompromised animals who were fed cookies made by replacing wheat flour with pumpkin seed powder at a rate of 15%. G4 = group of immunocompromised animals who were fed a cookie made of 30% replacement of wheat flour with pumpkin seed powder.

Improved immune function might be connected to the chemical structure of pumpkin seeds, which includes in height levels of antioxidant composites for example flavonoids, amino acids, phenols,vitamins (ascorbic acid and carotene), minerals and alpha-tocopherol. These results established with [37, 38], who shown that pumpkin contains β-carotene, which is an antioxidant that really helps increase immunity. These results were also consistent with [39] which indicated that the addition of pumpkin powder contributed to the activation of the immune system in the blood. These findings were in agreed [40] who described that pumpkin seeds are a rich source of polyphenols, carotene and antioxidants. Pumpkin contains a significant amount of antioxidants, tocopherols, and carotenoids [41]. Soluble antioxidants in lipids (tocopherols and carotenoids), vitamin C is a powerful water-soluble antioxidant that protects cells and cellular components from free radicals by donating electrons, and regenerates other antioxidants, such as vitamin E (tocopherol) [42]. Therefore, the Eating pumpkin has various benefits for improving overall health. The addition of pumpkin at various levels developed IgM and IgG concentrations in laboratory rats [43]. Polyphenols are classified by chemical buildings into phenolic acids and bioflavonoids. Polyphenols from few sources have indicated a modulatory influence on epigenetic mechanisms for example gene methylation, histone modifications, and post-transcriptional regulation by microRNA. In change, these mechanisms can modulate the immune system affecting both activation and differentiation of multiple cell types related to the immune system response [44].

4. Conclusion

Functional cookies, which substituted part of wheat flour with various percentages of pumpkin seed flour, indicated an immune stimulating influence in rats with decreased immunity because of the presence of active plant substances for example flavonoid antioxidants, total phenols, unsaturated fatty acids, a number of vitamins and important minerals for example zinc. Also, clinical trials in humans can be conducted to test patients with decreased immunity and see the influence of pumpkin seed supplementation on them.

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