

Effect of feeding spinach and lentils on some immunological traits in male rats

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ABSTRACT

This study aims to some natural food sources as effective factors to enhance the immune in male rats. Spinach and lentils were carried of chemical, biological and Sensory evaluations of prepared a vegetarian burger using a mixture of these sources.

Moisture, protein, fat, ash, fiber, carbohydrates and energy content of spinach powder as dry weight 6.22, 12.84, 4.79, 23.43, 11.72, 40.99 g/100 g and 258.44 kcal/100 g, respectively. So, higher in phenolic compounds compared to lentils. In germinated lentils increased protein and fiber but fat, ash, carbohydrates and energy decreased compared to untreated lentils. Soaking and cooking of lentils increased iron, zinc, copper, selenium and phenolic compounds compared to germinated and cooked lentils.

Results indicated the rats were fed on spinach and lentil led to increase of white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB). while, were decrease serum lipid profile and glucose contents. Therefore, significant decrease in the values of Triglycerides (T.G) Very Low-density Lipoprotein (VLDL), Low-density Lipoprotein (LDL). As, they were improved the Immunoglobulin G (IgG) and Immunoglobulin A (IgA).

Sensory evaluation indicated that there was a high degree of acceptance in the burger samples, as it achieved a degree greater than 80%, compared to the control burger.

Conclusion: feeding rats of spinach and lentils led to improvement in immunity due to they contain the many bioactive ingredients. Also, all samples of preparation vegan burger were acceptable in all sensory characteristics from all members.

Key words: bioactive compounds, soaking, blood picture and Veggie burger.

INTRODUCTION

Immunity is affected by various factors including age, gender, sleep cycle, stress, foods, exercise, genetic factors, etc. Certain environmental factors also affect immunity. Women have a stronger immune response than men. Therefore, they are high susceptible to autoimmune disorders such as rheumatoid arthritis and inflammatory bowel disease. A person with a weakened immune system is high susceptible to various infections that lead to various disorders (Patil *et al.*, 2021).

An optimal immune response depends on an adequate diet and nutrition in order to keep the infection at bay. For example, adequate protein intake is crucial for optimal antibody production. A low micronutrients intake, such as vitamin A or zinc, is associated with an increased risk of infection. Oftentimes, bad nutrient status is linked to inflammation and oxidative stress, which in turn can affect the immune system. Food components with particularly high anti-inflammatory and antioxidant potential include vitamin C, vitamin E, and phytochemicals such as carotenoids and polyphenols (**Iddir et al., 2020**).

Vegetables are important for human nutrition, whereas; provide the body by fibers, vitamins, minerals, and non-nutritive phytochemical compounds (phenolic compounds, flavonoids, bioactive peptides, etc.), which have proven health-promoting effects (**Zhang et al., 2019**).

Among plants, legumes are considered a vital source of protein, rich in bioactive co-nutrients, and shown to provide high levels of other nutrients, such as soluble and insoluble fibers, potassium, magnesium, folate, selenium, and phosphorous. Contrarily, legumes may include anti-nutritional compounds (**Cheng et al., 2019**). Consuming legumes had positive health effects and reduces the risk of heart disease, cancer, diabetes, weakness, high blood pressure, bowel disorders, and reduced LDL cholesterol (**Anderson and Major, 2002**).

Spinach (*Spinacia oleracea*) is a good source of protein and minerals, thus constituting a functional ingredient in a new product with high nutritional and biological values. Also, spinach is a rich source of major micronutrients such as iron, manganese, zinc, and magnesium and also contains small quantities of vitamin E, A, C, K, folate, thiamine (B1), pyridoxine (B6) and riboflavin (B2). Moreover, it is a rich source of fiber and has an added benefit of low-calorie content. It is present in food in many forms such as raw, canned boiled, pureed, frozen, dehydrated, cooked and baked (**Slavin and Love 2012**).

The saponins in spinach help lower blood pressure and cholesterol, and interact with surface molecules of the intestinal cell membrane, forming insoluble complexes with the cholesterol that are excreted through the bile duct with the feces. This mechanism interferes in the absorption of cholesterol by the intestine (**Hidaya, 2021**).

Lentils are one of the most popular grown and marketed dried legumes worldwide. Also, good sources of protein, carbohydrates, fiber and low fat (**Yu-Wei and Wang, 2015 and Khazaei et al., 2019**). Lentil proteins contain all the essential amino acids (39.3 g of essential amino acids per 100 g of proteins) and are rich in lysine, leucine, arginine, aspartic and glutamic acid. However, they are limited in sulfur-containing

amino acids (methionine and cysteine) and tryptophan, and thus the consumption of lentils mixed with other plant protein sources, such as cereal grains, represents an efficient way to obtain an adequately well-balanced amino acid profile (**Romano et al., 2021**).

Cooking lentils by boiling improves taste and flavor, and also increases the bioavailability of nutrients (**Pal et al., 2017**). Global health authorities recommend that people maximize their protein intake from plant sources (such as legumes), reduced protein intake from animal sources and increased vegetable protein intake is positively associated with reduced both cardiovascular disease-related deaths and all-cause mortality (**Chelladurai and Erkinbaev 2020**). Also, epidemiological studies have shown that pulse consumption is inversely proportional to the incidence of various chronic diseases such as coronary heart disease, type II diabetes mellitus, cardiovascular diseases, cancer, and aging (**Villegas et al., 2008**).

According to **Ganesan and Baojun (2017)** lentil intake caused to significant decrease of risk of developing degenerative illnesses like diabetes, cardiovascular disease (CVD) and cancer. Also, lentils are rich source of bioactive components which preventing degenerative disorders in people as well as having a substantial positive impact on health. Moreover, lentil is a low glycemic index (GI) as reported by (**Kendall et al., 2010**). Additionally, treated diabetic rats by diet contained lentil had increase of HDL-C level. These findings may aid in the prevention and treatment of diabetes and cardiovascular disease CHD (**Al- Tibi et al., 2010**).

Germination process plays an important role in reducing nonnutritive compounds in legumes and increasing the levels of available carbohydrates, dietary fiber, and other components. Germination is one of the most common and effective processes to improve the nutritional quality of legumes (**Vidal-Valverde et al., 2002**).

Alkaltham et al., (2022) reported the lipid contents of lentil between 1.02% (germinated) and 1.23% (boiled), total phenolic and flavonoid between 45.32 mg GAE/100 g (germinated) and 68.02 mg GAE/100 g (control) to 70.95 mg QE /100 g (germinated) and 199.52 mg QE/100 g (control), respectively. Also, antioxidant activity values were detected between 0.70 mg TE/kg (germinated) and 3.35 mg TE/kg (boiled). The major phenolic compounds of raw, germinated, and boiled lentil seeds were gallic acid, 3,4-dihydroxybenzoic acid, and catechin. While oleic acid amounts of lentil oils between 33.22% (control) and 47.72% (germinated), linoleic acid 26.40% (germinated) and 40.91% (boiled). In addition, linolenic acid amounts between 4.12% (germinated) and 6.97% (boiled). The key minerals of raw, germinated, and boiled lentil seeds were P, K, Mg, and S. However, according to the results, it was determined that lentils are a good source of potassium.

In general, the oil, total phenol, total flavonoid contents, and antioxidant activities of germinated lentils were recorded to be low when compared to the results of control and boiled lentils. In addition, the highest antioxidant activity value was established in boiled lentil seeds. The moisture contents of raw, germinated, and cooked pulses were determined between 6.58%–9.19%, 50.39%–54.26%, and 70.40%–71.24%, respectively (Poblete et al., 2020).

On the other hand, Veggie burger is a convenient processed food product prepared completely from non-meat ingredients usually based on plant protein (De Silva et al., 2011). Legume (e.g., pea, lentil and chickpea) are used to partially substitute meat to obtain low-calorie burgers with high protein and fiber contents (Argel et al., 2020).

Therefore, this research aims to study the effectiveness for some biologically active compounds some natural food sources to improve immunity in rats. Also sensory evaluation of burger samples prepared using the different mixtures of these vegetable sources used in the study.

MATERIALS and METHODS

Spinach and Lentil were obtained from local market in Benha, EL- Qalyubia Governorate, Egypt. Soybean flour, salt, onion, starch, burger spices, and oil were purchased from local market at Giza, Egypt.

(30) normal albino male rats of strain Sprague Daley, average weight (150±10) were purchased from the Laboratory Animal Department, Food Technology Research Institute, Agriculture Research Center (ARC), Giza, Egypt.

Folin -Ciocalten phenol reagent (2N), Sodium Carbonate (99.8%) (NaCO₃), sodium nitrite (NaNO₂), Alanonium chloride (AlCl₃), sodium hydroxide (NaOH) ,2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and Azinobis-(3-ethylbenzothiazoline- 6- sulfonic acid (ABTS) were purchased from Sigma-Aldrich (St. Louis, Mo, USA). The kits were punched from Gamma- Tread Company, Cairo, Egypt.

Preparation of dried spinach powder

Whole spinach were cleaned and washed with water to remove dirt and other impurities. Then, it was dried in solar energy unit in the Center Laboratory - Central Research, Dokki, Egypt.

The dried spinach was finely ground and placed in polyethylene bags and kept in the refrigerator at 4°C until use.

Preparation of lentil

Soaking lentil was soaked in tap water for 12 hr. in ratio 15 w/v. At the end the soaking period, the soaked water was discarded and rinsed twice in tap water. The soaked seeds lentil was divided into two parts, the first part was subjected to germination process and the second part was subjected to cooking process.

Germination The soaked seeds were allowed to germinate under a wet muslin cloth for 48 hours. After that, the germinated seeds were cooked.

Cooking The soaked and germinated samples were cooked by boiling 40 and 30 min in a covered stainless-steel pot respectively, on a moderate flame with water retention. All cooking treatments, the minimum cooking time to reach a similar tenderness for an adequate palatability and taste according to the Egyptian eating habits.

Chemical Composition of Spinach, Lentil and product samples

Chemical analysis (moisture, fat, crude protein, ash and crude fiber) for dried powder of spinach and lentil powder was determined according to the method of **AOAC, (2000)**. Carbohydrates were calculated by difference as the following equation

$$\text{Carbohydrates} = 100 - [\text{moisture} + \text{Protein} + \text{fat} + \text{Ash} + \text{Fiber}]$$

The results of chemical analysis calculated as (g/100g dry weight basis). Caloric values of the materials were calculated using the appropriate factor as described by **(FAO/ WHO/ UNU 1985)** as following equation

$$\text{Energy value (Kcal)} = 4 (\text{protein} + \text{total carbohydrates } \%) + 9 (\text{fat } \%)$$

Determination of Minerals

Some element contents (Zinc, Iron, Selenium and copper) were determined using a Pye Unicam SP 1900 Atomic Absorption Spectroscopy instrument (Perkin Elmer model 4100 ZL) at Soils, Water and Environment Research Institute (SWERI), Giza, Egypt. as described by the **A.O.A.C. (2000)**.

Antioxidant Activity

Total phenols and flavonoids were determined using the method described by **Batista et al., (2011)**. Antioxidant activity was determined by two tests as follows The

electron donation ability of the obtained ethanol extracts was measured by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) according to the method of **Hanato *et al.*, (1988)**. And, Azinobis-(3-ethylbenzothiazoline- 6- sulfonic acid (ABTS) assay was estimated according to the method described by (**Olszowy and Dawidowicz 2018**).

Biological assay

The selected of 30 adult albino rats average weighted (150 ± 10 g) were used in the present experiment. The animals were housed in plastic cages and the room temperature was maintained at $21 \pm 2^\circ\text{C}$ with time lighting 12h and relative air humidity of 40% to 60%. They rats were fed on basal diet as reported by **Reeves *et al.*, (1993)**.

Experimental Design

After the adaptation period, the rats were randomly divided into (6) groups, then each group (5 rats) as shown in Table (1).

Table (1) Diet composition with powder spinach and lentil treatments

Items	G1	G2	G3	G4	G5	G6
Protein	12	10.67	8.937	9.2	9.80	9.94
Fat	10	9.53	9.61	9.56	9.57	9.55
Fiber	5	7.326	3.91	4.04	4.114	4.18
Sugar	10	10	10	10	10	10
Mixture Minerals	3.5	3.5	3.5	3.5	3.5	3.5
Mixture Vitamins	1	1	1	1	1	1
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2
DI methionine	0.3	0.3	0.3	0.3	0.3	0.3
Starch	58	47.48	52.55	52.2	51.52	51.34
Spinach	-	10	-	-	5	5
Lentil	-	-	10	-	5	-
Lentil Germinated	-	-	-	10	-	5

G₁ Control group, G₂10 % Spinach powder, G₃10 % soaked and cooked lentil, G₄10 % germinated and cooked lentil, G₅5 % Spinach powder and 5% soaked and cooked lentil powder, G₆ 5 % Spinach powder and5% germinated and cooked lentil.

The duration of the study was 8 weeks. Body weight of rats was measured once a week. The total body weight gain was calculated at the end of experiment. Liver, kidney, and heart were removed from each rat, washed by saline solution and weighed then stored in formalin solution 10% it was calculating the absolute and relative organ weight. Relative organ weight (Liver, kidney, and heart) was calculated according to **Drury and Wallington (1980)**.

Blood samples

At the end of the experiment period, fasted rats overnight then anaesthetized by diethyl ether and sacrificed. Blood samples were collected from eye plexuses and divided into two parts. The first patch was collected in heparinized tube to obtain the whole blood to measure the hematological profile. The second patch was collected in dry clean centrifuge glass tube without any coagulation to prepare serum by leaving the samples for 15 minutes at refrigerator. Then, the tubes were centrifugation for 15 min at 3000 rpm. After that, the clean supernatant serum was collected and kept frozen at -18°C until analysis.

Hematological analysis

White blood cell (WBC), Lymphocytes (LYM), Monocytes (MON), Granulocytes (GRA), red blood cell (RBC), hemoglobin (HGB), Hematocrit (HCT), Platelet Count Test (PLT) and Procalcitonin (PCT) was measured as according to **Jane (1986); Moser et al., (2001) and Ochei and Kolharktar (2008)**.

Biochemical analysis

Serum glucose was determined as according to the procedure of **Trinder (1969)**. Total cholesterol was calorimetrically determined as according to the enzymatic method of (**Rifai et al., 1999**). Triglycerides were determined in serum obtained from by described method of **Fassati and Prencipe, (1982)**. High density lipoprotein cholesterol [HDL-C] was determined using the method of **Lopez-Virella et al., (1977)**. Very low-density lipoprotein cholesterol [VLDL-C] and low density of lipoprotein cholesterol [LDL-C] were calculated as according to **Lee and Nieman (1996)**. Coronary risk index [CRI] was calculated according to (**Adeneye et al., 2010**).

Quantitative Determination of Immunoglobulin (IgA & IgG)

Total serum **IgA** and **IgG** were quantified with Bethyl Mouse IgA and IgG (ELISA) quantization set (E 90-103; Bethyl Laboratories, Inc.). Assays were performed as according to **Yongping et al., (2017)**.

Preparation of Veggie burger formulae (as application)

Burger samples (five formulae) were prepared as according to (**El Hadidy et al., 2014**) and shown in Table (3). The ingredients were mixed and homogenized by Braun Cutter Machine (Combi Max 700, USA). Then, burger samples were shaped manually using patty maker (hand metal machine) to obtain round discs of 80g weight, 8 cm

diameter and 1cm thickness). All samples were aerobically packed in a foam plate, wrapped with polyethylene film and stored at - 18°C for further analysis.

Table (2) Veggie Burger formulas of spinach and lentil (g/100g)

Items	F1	F2	F3	F4	F5
Soybean flour	85	-	-	-	-
Soaked and cooked lentil	-	85	-	83	-
Germinated and cooked lentil	-	-	85	-	83
Spinach	-	-	-	2	2
Salt	1.5	1.5	1.5	1.5	1.5
Onion	5	5	5	5	5
Spices mixture	1	1	1	1	1
Starch	6	6	6	6	6
Garlic	1.5	1.5	1.5	1.5	1.5

*Water as need requirement.

Texture profile analysis

Texture profile analysis (TPA) of different burger treatments was determined by Bourne (2003).

Sensory evaluation

Burger samples were subjected to evaluate sensory attributes (appearance, taste, color, odor, and tenderness) by ten trained panelists of Food Technology Research Institute (FTRI). Giza, Egypt, as according to Jeffery and Lewis (1983).

Statistical Analysis

Statistical analyses were carried out by SPSS program (Version 19). Data were expressed as means \pm SEM and the statistical analysis was per formed using one way analysis of variance followed by Duncan's tests (Snedecor and Cochran, 1989).

RESULTS and DISCUSSIONS

Chemical composition of spinach and lentil powder

Results in Table (3) showed that, chemical composition of spinach and effect of soaked, germinated and cooked of lentils seeds on chemical contents. Spinach was a high content of ash and fiber. And, it was a low content of fat and energy. Soaking process and cooked lentil seeds caused to decrease in protein, fat and energy. Also, it was increased in fiber and carbohydrates compared to raw lentil seeds. The same trend was showed for germinated and cooked lentil seeds. Meanwhile, the germinated and cooked lentil seeds were higher protein and fiber than soaked and cooked lentil seeds.

These results are in the line with (**Abdelfattah 2023 and Helal *et al.*, 2023**) found that lentils were high in crude proteins, and dietary fiber as well as rich source of energy and other nutritional components, but it was low in fat. Also, **Khalil *et al.*, (2007)** found the effect of germination lentil showed a rise in protein and a decrease in NFE contents. This apparent increase in the total protein was observed with the time of germination may be attributed to oxidation and consumption of the other water-soluble classes (like sugars and minerals) in the germination process. The increase in crude fiber during germination was reported to be mostly due to changes in the polysaccharides found in the cell wall such as cellulose, glucose and mannose, suggesting that the changes were due to an increase in the cellular structure of the plant during germination (**Rumiyati *et al.*, 2012**). Also, fat used for energy production (**Kaushik *et al.*, 2010**). Accordingly, **D'souza (2013)** who found ash content was decreased with germinated and cooked might be due to the leaching out of both macro and micro elements into the soaking water. While crud fiber was significantly increased by cooking treatment, this increase could have been due to protein fiber complex formed after possible chemical modification induced by the soaking and cooking of dry seeds (**Bressani 1993**).

Table (3) Chemical composition of spinach and lentil powder (g/100g DM)

Materials Items	Spinach	Lentil		
		Raw	Soaked and cooked	Germinated and cooked
Moisture (g)	6.22±0.03	3.12±0.00 ^c	8.87±0.03 ^a	7.79±0.05 ^b
Protein (g)	12.84±0.28	37.21±0.16 ^a	28.42±0.24 ^c	29.79±0.48 ^b
Fat (g)	4.79±0.54	11.62±0.03 ^a	4.43±0.33 ^b	3.92±0.11 ^b
Ash (g)	23.43±0.29	2.25±0.15 ^b	3.73±0.32 ^a	2.75±0.25 ^b
Fiber (g)	11.73±0.55	4.99±0.53 ^c	7.95±0.29 ^b	9.61±0.01 ^a
Carbohydrates (g)	40.99±0.12	40.81±0.85 ^b	46.60 ±0.39 ^a	46.14 ±0.40 ^a
Energy (Kcal.)	258 ±4.05	417 ±2.57 ^a	340 ±1.27 ^b	339 ±3.01 ^b

*Each value in a row followed by the same letter is not significantly different at (p≤ 0.05).

Immune elements content in spinach and lentil powder

Data in Table (4) showed the Fe, Zn, Se and Cu content in spinach and lentil. The results indicated that, spinach was the highest level of Fe (44.7mg/100g DM). Moreover, both soaked and germinated and cooked lentil seeds caused to decrease in all immune elements under study (Fe, Zn, Se and Cu). Germinated and cooked lentil had more decrement than soaked and cooked lentil for immune elements. The great decrement was showed in Fe thus; germinated and cooked lentil had decrease (50.20%) and soaked and cooked lentil (43.56%) compared to raw lentil seeds. These results were consistent with **Sharma (2006)** reported that lentils there was increases in Zn and Fe contents upon ordinary and pressure cooking. **Soltan (2013)** who reported that in lentil seeds were excellent vegetable sources of Fe. Studies have shown that the consumption of cooked lentil in the diet prevents iron deficiency anemia iron being a very important mineral,

which is required daily, especially for adolescents and pregnant women. Several minerals (zinc, copper, manganese, molybdenum, selenium and boron) have been well documented in lentils (**Rodriguez *et al.*, 2008 and USDA 2016**). However, spinach has remarkable abilities to restore energy, increase vitality and improve the quality of the blood due to its high iron content (**Szalay 2015**).

There are natural chelating agents in pulses due to adversely affected on bioavailability of Fe. So, could be minimized by cooking, germinating and fermentation of lentil seeds prior to ingestion (**Umata *et al.*, 2005**).

Table (4) Immune Elements content (Iron, Zinc, Selenium and copper) in spinach and lentil powder

Materials Items	Spinach	Lentil		
		Raw	Soaked and cooked	Germinated and cooked
Fe (mg/100g)	44.70	20.5	11.53	10.21
Zn (mg/100g)	3.54	5.40	4.90	4.82
Se (µg/100g)	1.5	13.20	10.26	9.58
Cu (mg/100g)	2.53	3.00	2.38	1.92

Total phenol, Flavonoids and Anti-Oxidants

Table (5) showed total phenol, total flavonoids and antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and Azinobis-(3-ethylbenzothiazoline- 6-sulfonic acid (ABTS) for spinach and lentil seeds powder.

Results indicated that spinach powder had a high content of total phenols and total flavonoids (34.14 ± 1.20 g/100g) and (8.28 ± 0.66 g/100g), respectively. Antioxidant activity by DPPH was higher than antioxidant activity by ABTS (77.58% and 36.51%), respectively. These results correspond with the results mentioned by **Kaur *et al.*, (2016)** who said the spinach is an excellent source of micronutrients not only vitamins but also flavonoids, phenols, and carotenoids. The dark green color of spinach leaves indicates they contain high levels of chlorophyll which have anti-inflammatory and anti-cancerous properties (**Roberts and Moreau, 2016**).

Generally, results indicated the raw lentil had the highest levels of T. phenols (27.70 ± 0.70 g/100g) and total flavonoids (8.13 ± 0.07 g/100g). As well as antioxidant activity by both DPPH and ABTS compared to treated lentil seeds. Soaked and cooked lentil recorded a higher value in flavonoids and ABTS than germinated and cooked lentil seeds. While, it was recorded lower value of T. Phenols and DPPH (26.88 ± 0.57 g/100g and $80.42 \pm 0.33\%$) respectively compared to germinated and cooked lentil seeds. These results were agreement with **Helal *et al.*, (2023)** said that, a lentil was recorded lower total phenols. On the other hand, flavonoids, such as glycosides of flavone's and flavones, are mainly present in the seed coat of lentils (**Amarowicz *et al.*, 2009**).

Saharan *et al.*, (2002) explained that soaked and cooked lentil led to decrease in phenolic compounds may be a result of leaching into the soak water or binding of phenols with other organic substances such as carbohydrate or protein. And, decrease in phenolic compounds as a result of cooking could be due to thermal degradation, as well as breakdown of phenols as reported by **Duhan *et al.*, (2000)**.

Table (5) Total Phenols, Flavonoids, and Anti - Oxidants activity (DPPH and ABST) in Spinach and Lentil

Items		Spinach	Lentil		
			Raw	Soaked and cooked	Germinated and cooked
T. Phenols (g/100g)		34.14 ±1.20	27.70±0.70 ^a	26.88±0.57 ^c	27.48±0.59 ^b
Flavonoids (g/100g)		8.28±0.66	8.13±0.18 ^a	7.29 ±1.10 ^b	7.16±0.58 ^c
Anti-Oxidants activity	DPPH %	77.58±0.34	83.45±0.89 ^a	80.42±0.33 ^b	82.26±0.01 ^{ab}
	ABTS %	36.51±0.01	42.96±0.01 ^a	41.23±0.01 ^b	32.67±0.01 ^c

* Total phenol as Gallic acid and total flavonoids as quercetin.

**Each value in a row followed by the same letter is not significantly different at ($p \leq 0.05$).

Effect of feeding male rats on spinach and lentil on body weight gain (BWG) and some organs weight

The effect feeding male rats on spinach and lentil on body weight gain (BWG) and some organs weight of illustrated in Table (6). Generally, rats fed on germinated and cooked lentil (10%) were the lowest BWG. Rats fed on soaked and cooked were the highest BWG. While, feeding rats on spinach combine with soaked and cooked lentil caused to decrease in BWG compared to rats feeding each alone.

Results in the same Table (6) showed that, rats fed on diet contained (10%) of germinated and cooked lentil had the lowest weight of liver compared other groups. Also, rats fed on germinated and cooked lentil alone or combine with spinach had lower liver weight than rats fed on soaked and cooked lentil alone or combine with spinach. The similar results for kidney and heart weight except rats fed on spinach combine with soaked or germinated and cooked lentil had the same results (0.67g) for heart weight.

These data were in line with those obtained by **Radwan *et al.*, (2008)**, they stated that there were no significant differences between liver, kidney and heart weight in groups of rats fed soaked and cooked lentil and control group. **Mokhtari *et al.*, (2021)** said that Spinach has high antioxidants and polyphenols and showed protective effects against liver diseases.

Table (6) Effect of feeding male rats on spinach and lentil on body weight gain (BWG) and some organs weight

Groups	Initial	Final	BWG (g)	%	Liver (g)	%	Kidney (g)	%	Heart (g)	%
G1	151.50±0.86 ^a	263.33±27.42 ^{ab}	111.83±26.55 ^a	73.81	8.22±1.06 ^{ab}	3.12	1.86±0.12 ^{ab}	0.71	0.82±0.05 ^a	0.32
G2	150.66±11.46 ^a	262.33±22.80 ^{ab}	111.66±11.78 ^a	74.13	8.42±0.30 ^a	3.21	1.78±0.08 ^{ab}	0.68	0.79±0.02 ^a	0.31
G3	151.66±5.54 ^a	279.00±12.70 ^a	127.33±7.26 ^a	83.96	7.97±0.79 ^{ab}	2.86	2.11±0.22 ^a	0.76	0.78±0.00 ^{ab}	0.28
G4	151.33±5.20 ^a	198.00±6.92 ^c	46.66±12.12 ^{bc}	30.84	5.14±0.16 ^c	2.60	1.54±0.09 ^{bcd}	0.78	0.68±0.05 ^{ab}	0.35
G5	151.33±9.49 ^a	214.33±19.34 ^{bc}	63.01±10.81 ^{bc}	41.64	7.79±0.66 ^{ab}	3.64	1.42±0.10 ^{bcd}	0.67	0.67±0.03 ^{ab}	0.32
G6	151.33±1.76 ^a	211.33±6.06 ^{bc}	60.01±5.68 ^{bc}	39.65	6.70±0.39 ^{abc}	3.18	1.17±0.04 ^d	0.56	0.67±0.06 ^{ab}	0.32

*Each value in a column followed by the same letter is not significantly different at ($p \leq 0.05$).

**G1 Control group, G2 10 % Spinach powder, G3 10 % Soaked and cooked lentil powder, G4 10 % Germinated and cooked lentil powder, G5 5 % Spinach powder and 5% Soaked and cooked lentil powder, G6 5 % Spinach powder and 5% Germinated and cooked lentil powder.

Effect of feeding male rats on spinach and lentil on blood picture

The results in Table (7) illustrated feeding effect by spinach and lentil on blood picture in male rats. Generally, rats fed on basal diet (control group) had the lowest WBC, WRC and HGB compared to rats feeding on diet contained spinach or lentil seeds. Thus; feeding on spinach powder alone or treated lentil seeds caused to improve levels of WBC, WRC and HGB. Exception, feeding on spinach combine with germinated and cooked lentil caused to decrease of HGB (12.50g/dl). While, rats fed on germinated and cooked lentil seeds had the highest level of HGB (14.90g/dl). The results follow the same trend for HCT, PCT and PLT.

These results are agreement with those reported by **Soliman *et al.*, (2010)** and **Soltan, (2013)**, who found that the three blood parameters (RBCs, WBCs and Hb) were increased in the diabetic rats treated with lentils. Blood is an important body fluid, which contains the red blood cells, white blood cells and platelets suspended in the serum in homeostatic concentrations. Blood examination is a good way of assessing the health status of a plays a vital role in physiological, nutritional and pathological status of organisms submitted that assessment of hematological parameters can be used to determine the extent of deleterious effect on blood constituents of an animal as reported by **Ashafa *et al.*, (2009)**. Moreover, **Jenkins *et al.*, (2008)** and **Helal *et al.*, (2023)** reported that, could be reduce incident diabetes to improve glycemic control by consuming increased number of pulses as replacement food for more rapidly digested carbohydrate. Low (GI) diets resulted in moderately reduced level of hemoglobin (Hb).

Table (7) Effect of feeding male rats on spinach and lentil on blood picture

Groups		G 1	G 2	G 3	G 4	G 5	G 6
WBC ($10^3 / \mu\text{L}$)		11.05±0.72 ^d	14.53±1.22 ^{bc}	16.78±0.96 ^b	13.33±0.82 ^{cd}	16.21±0.99 ^b	21.46±0.37 ^a
Differential white cell count%	LYM	81.93±0.20 ^{ab}	82.60±1.90 ^{ab}	86.93±0.72 ^a	74.16±7.13 ^b	79.76±1.70 ^{ab}	85.93±0.49 ^a
	MON	8.40±0.23 ^b	9.86±0.49 ^{ab}	7.30±0.63 ^b	13.26±2.91 ^a	8.76±1.41 ^b	7.66±0.37 ^b
	GRA	9.66±0.43 ^{ab}	7.56±1.41 ^{ab}	5.76±0.08 ^b	12.60±4.21 ^a	11.50±0.28 ^{ab}	6.40±0.11 ^b
RBC ($10^6 / \mu\text{L}$)		7.34±0.18 ^d	7.90±0.03 ^{bc}	7.95±0.02 ^{bc}	8.35±0.26 ^b	8.90±0.04 ^a	7.53±0.13 ^{cd}
HGB (g/dl)		12.90±0.11 ^{cd}	14.33±0.03 ^{ab}	13.60±0.05 ^{bc}	14.90±0.17 ^a	14.05±0.08 ^b	12.50±0.51 ^d
HCT (%)		40.65±0.43 ^b	44.10±0.34 ^a	42.73±0.08 ^a	44.33±0.60 ^a	44.43±0.20 ^a	37.23±1.24 ^c
PCT (%)		0.42±0.06 ^a	0.44±0.01 ^a	0.44±0.03 ^a	0.42±0.03 ^a	0.20±0.04 ^b	0.27 ±0.03 ^b
PLT ($10^3 / \mu\text{L}$)		788.00±36.95 ^a	762.50±2.59 ^a	663.00±33.48 ^b	630.00±23.67 ^{bc}	507.50±0.28 ^d	581.50±10.10 ^c

*Each value in a column followed by the same letter is not significantly different at ($p \leq 0.05$).

**G1 Control group, G210 % Spinach powder, G310 % Soaked and cooked lentil powder, G410 % Germinated and cooked lentil powder, G55 % Spinach powder and 5% Soaked and cooked lentil powder, G6 5 % Spinach powder and 5% Germinated and cooked lentil powder.

Effect of feeding male rats on spinach and lentil on glucose and immune system

The effect of spinach and lentil on immune system including (IgG and IgA) of male rats illustrated in (Table 8). Generally, rats in control group had the lowest IgG and IgA levels as indicators for immune system. Feeding rats on spinach or lentils seeds resulted in improvement of IgG and IgA. But, combating between spinach with lentil seeds (both soaked and germinated) caused to decrease of IgG and IgA compared to each alone.

These results were agreement with **Mallillin *et al.*, (2008)** and **Murty *et al.*, (2010)** who reported that, the main antioxidant compounds in legumes are vitamin E and phenolic compounds. So, different studies had suggested that, a protective antioxidant effect on immunity status, cancer and cardiovascular diseases.

Serum glucose for male (mg/dl)

The results in the same Table (8) showed that, rats in control group had the highest serum glucose content (163.16mg/100dl) compared to other groups. Feeding rats on spinach or lentils seeds resulted in decrement serum glucose content. Also, germinated and cooked lentil seeds caused to improve serum glucose better than soaked and cooked lentil seeds. On the other hands, diet contained spinach and lentil seeds (both soaked and germinated) cooked improved serum glucose more than each alone.

These results were confirmed by **Maruyama *et al.*, (2013)** they found that consumption of a regular-sized dish of spinach significantly ($p \leq 0.05$) decreased blood glucose. **Da Silva and Imai (2017)** found that spinach is being used for conditions associated with insulin resistance such as diabetes mellitus because of the beneficial properties of inositol or vitamin B₇. Inositol helps liver dissolve fat and together with choline, produces lecithin, preventing the fat from being deposited. These compounds have similar physical properties to sugars but a low calorie content, low glycemic index, and low response to insulin. Spinach is contained sugar such as glycosyl glycerol, glucaric acid, myo inositol and mannitol.

Also, **Al-Tibi *et al.*, (2010)** found the ability of lentils to alleviate the glycemic load (GL) has been demonstrated in experimentally induced diabetic rats, healthy volunteers, and insulin-dependent and nondependent diabetic patients. **Shams *et al.*, (2008)** and **EL-Akhras *et al.*, (2016)** who found that addition of 50 g cooked lentils to diet led to a significant decrease in fasting blood glucose. The glucose lowering effect of lentils was ascribed by previous studied to probable influences of low GI diets on glucose metabolism. Comparing lentil consumption to starchy control foods consistently reduces acute blood glucose and insulin response, according to **Clarke *et al.*, (2022)**.

Swieca *et al.*, (2013) observed that the regular consumption of the germinated lentils is beneficial for the prevention and management of diabetes. Lentils have the

ability to improve blood glucose, lipid and lipoprotein metabolism in diabetic and healthy human beings (Aslani *et al.*, 2015).

Table (8) Effect of feeding rats on spinach and lentil on glucose and immune system (mg/dl)

Groups \ Items	IgG	IgA	Glucose
G 1	815.50±0.86 ^c	85.33±1.20 ^d	134.16±1.01 ^a
G 2	836.00±1.15 ^a	94.50±0.28 ^a	129.16±1.48 ^b
G 3	828.00±1.73 ^b	94.00±1.15 ^a	127.67±1.20 ^b
G 4	826.00±1.73 ^b	92.00±0.57 ^{ab}	126.33±1.20 ^c
G 5	825.50±0.86 ^b	88.50±0.86 ^c	127.50±0.28 ^d
G 6	825.50±2.02 ^b	90.50±0.86 ^{bc}	123.50±1.25 ^c

*Each value in a column followed by the same letter is not significantly different at ($p \leq 0.05$).

**G1 Control group, G210 % Spinach powder, G310 % Soaked and cooked lentil powder, G410 % Germinated and cooked lentil powder, G55 % Spinach powder and 5% Soaked and cooked lentil powder, G6 5 % Spinach powder and 5% Germinated and cooked lentil powder.

Effect of feeding male rats on spinach and lentil on serum lipid profiles

The results in Table (9) showed effect of feeding male rats on spinach and lentil on serum lipid profile including (cholesterol; TC, triglycerides; TG, high density lipoprotein –cholesterol; HDL-c, low density lipoprotein –cholesterol; LDL-c, very low-density lipoprotein –cholesterol; VLDL-c and coronary risk index; CRI). Rats in control group which fed on basal diet were the highest contents of TC, TG, LDL-c, VLDL-c and RF. Rats fed on germinated and cooked lentil seeds with spinach had the great decreased of TG, LDL-c and VLDL-c (12.38, 12.37 and 26.90%), respectively. Also, germinated and cooked lentil seeds had decrease better than soaked and cooked lentil seeds.

These results were agreement with **Elvira-Torales *et al.*, (2019)** said that, the consumption of spinach led to a significant reduction in cholesterol and triglycerides. However, spinach is considered one of the richest plant sources of phytochemicals such as carotenoids, contributing to the intake of lutein and zeaxanthin. The lipid lowering properties of spinach phytochemicals were examined by rats treated with spinach extract. The results showed that, 62.3% reduction in serum triglycerides when compared to control rats. Spinach extracts normalized plasma triglycerides in rats when observed in non-diabetic rats (**Kumar and Loganathan 2010**).

Consumption of lentils actively reduced the TC, TG, LDL-c and pathological manifestations of cardio morphometric analysis (**Bazzano *et al.*, 2011**). According to

Ahmed (2014) who observed that lentils given to type 2 diabetes patients, resulted in an increasing HDL-c and reduce TC without changing other lipid fractions. **Ludvik et al., (2004)**, which contend that HDL-function c in reverse cholesterol transfer makes it a preventative, measure against atherosclerosis. The lipoprotein lipase enzyme, which is involved in the metabolism of triglyceride-rich lipoprotein, is stimulated by HDL-c. Additionally, **Ahmed (2017)** observed that feeding on lentil seeds had significantly lowers the atherogenic index (AI) and boosts TC/TG in rats.

Table (9) Effect of feeding male rats on spinach and lentil on serum lipid profiles (mg/dl)

Items Groups	Cholesterol	T.G	HDL	VLDL	LDL	Risk Factor
G1	119.00±0.57 ^a	126.50±0.28 ^a	43.50±0.86 ^b	25.30±0.05 ^a	49.70±0.05 ^a	2.87±0.04 ^a
G2	115.50±0.28 ^b	123.00±0.57 ^b	46.00±0.57 ^{ab}	24.60±0.12 ^b	44.90±0.98 ^b	2.67±0.02 ^b
G3	112.00±0.57 ^c	116.50±1.25 ^c	47.00±0.57 ^a	23.30±0.25 ^c	41.70±0.75 ^c	2.48±0.05 ^c
G4	103.00±1.73 ^d	112.50±0.86 ^{de}	43.67±0.88 ^b	22.50±0.17 ^{de}	36.83±1.05 ^d	2.57±0.07 ^{bc}
G5	104.50±0.28 ^d	114.00±0.57 ^d	44.83±1.01 ^{ab}	22.80±0.11 ^d	36.86±0.85 ^d	2.54±0.06 ^{bc}
G6	102.00±0.57 ^d	110.84±0.60 ^e	44.00±0.57 ^b	22.17±0.12 ^e	36.33±1.32 ^d	2.55±0.06 ^{bc}

*Each value in a column followed by the same letter is not significantly different at ($p \leq 0.05$).

**G1 Control group, G2 10 % Spinach powder, G3 10 % Soaked and cooked lentil powder, G4 10 % Germinated and cooked lentil powder, G5 5 % Spinach powder and 5% Soaked and cooked lentil powder and G6 5 % Spinach powder, 5% Germinated and cooked lentil powder.

Chemical composition of prepared burgher samples (per 100g DM)

This investigation studied to prepare of veggie burger from soybeans as control, spinach and lentil seeds (both soaked and germinated) and cooked too. Then, the samples were analyzed for chemical composition and immune elements. The results are presented in Table (10).

The results indicated that, veggie burger of soybeans (as control) had the highest protein, ash and energy contents (37.97, 3.63 and 393), respectively. It had the lowest levels of fat, fiber and carbohydrates contents (3.33, 2.40 and 52.67) respectively. Veggie burger made by germinated and cooked lentil had higher protein, fiber and ash contents than burger made by soaked and cooked lentil. While, it had decreased in fat, carbohydrates and energy contents than burger made by soaked and cooked lentil.

The same Table (10) showed immune elements content (Iron, Zinc, Selenium and cooper) in burger samples. Generally, burger made by soybeans had the lowest levels of Fe, Zn and Se (7.08mg/100g, 1.02mg/100g and 6.37µg/100g) respectively. Germination process caused to decrease in the immune elements. So, the results in veggie burger made

by germinated and cooked lentil had decrease in immune elements more than veggie burger made by soaked and cooked burger. On the other hands, burger made by spinach combine with soaked and cooked lentil had a higher level of Fe, Zn, Se and Cu compared burger made by spinach combine with germinated and cooked lentil.

Table (10) Chemical composition and immune elements for raw veggie burger samples (DM)

Items	Control	Lentil burger		Lentil with spinach burger	
		Soaked and cooked	Germinated and cooked	Soaked and cooked	Germinated and cooked
Protein (g)	37.97	24.31	24.88	24.00	25.14
Fat (g)	3.33	3.79	3.34	3.80	3.37
Ash (g)	3.63	2.23	2.40	3.63	2.81
Fiber (g)	2.40	6.79	8.20	6.97	8.25
Carbohydrates (g)	52.67	61.88	61.18	61.60	60.43
Energy (Kcal)	393	379	374	377	373
Immune elements for veggie burger					
Fe (mg/100g)	7.08	9.80	8.68	10.46	9.36
Zn (mg/100g)	1.02	4.17	4.10	4.14	4.07
Se (µg/100g)	6.37	8.72	8.14	8.55	7.98
Cu (mg/100g)	1.89	2.03	1.63	2.03	1.64

Total Phenols, Flavonoids and Anti -Oxidants activity (DPPH) in burger samples

The results in Table (11) showed Total Phenols, Flavonoids and Anti -Oxidants activity (DPPH) in burger samples. Generally, burger made by soybeans showed significant decreased ($p \leq 0.05$) in the mean value of DPPH as compared with other samples. While burger made by germinated and cooked lentil had a higher level of Flavonoids and DPPH than burger made by soaked and cooked lentil. On the other hand, burger made by spinach combine with germinated and cooked lentil revealed significant increased ($p \leq 0.05$) in T. Phenols and DPPH levels compared with burger made by spinach combine with soaked and cooked lentil.

These results are in agreement with **Ghasemzaeh *et al.*, (2010)** reported that the antioxidant activity by DPPH scavenging radical may be attributed to the presence of high levels of total phenolic and flavonoids which play a key role as proton donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Table (11) Total Phenols, Flavonoids and Anti -Oxidants activity (DPPH) in burger samples

Formulas	T. Phenols (mg/100g)	Flavonoids (mg/100g)	DPPH %
1	751.80±0.43 ^c	76.17±0.54 ^c	76.87±0.01 ^c
2	1079.70±0.01 ^c	83.62±0.30 ^b	86.75±0.08 ^c
3	949.64±0.33 ^d	96.14±0.31 ^a	87.46±0.01 ^b
4	1216.37±0.01 ^b	39.06±0.32 ^e	78.42±0.01 ^d
5	1263.05±0.02 ^a	58.37±1.35 ^d	89.30±0.05 ^a

*Each value in a column followed by the same letter is not significantly different at ($p \leq 0.05$).

**Sample1 control (soybean), Sample 2, Soaked and cooked lentil powder, Sample3, Germinated and cooked lentil powder, Sample4 Soaked and cooked lentil and 2g spinach powder, Sample5, Germinated and cooked lentil and 2g spinach powder.

Texture Analyzer for veggie burger samples

Textural properties result of different burger samples were determined as cycle1 (hardness) and cycle 2(hardness, cohesiveness, springiness, gumminess and chewiness values are presented in Table (12). Results showed that, veggie burger made by soybean flour had the highest of hardness, springiness, gumminess and chewiness levels and the lowest cohesiveness level. Germinated and cooked lentil burger caused to decrease in springiness and gumminess, but increase in hardness and chewiness.

Table (12) Texture Analyzed for burger samples

Formulas	Hardness		Cycle 2			
	Cycle1	Cycle2	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (mJ)
1	58.51	42.29	0.81	3.85	47.27	182.00
2	46.26	36.11	0.94	3.66	42.06	133.30
3	50.14	36.80	0.98	3.55	39.31	153.10
4	48.08	38.77	1.11	3.38	34.39	117.70
5	50.47	35.70	0.94	3.48	37.26	138.00

*Each value in a column followed by the same letter is not significantly different at ($p \leq 0.05$).

**Sample1 control (soybean), Sample 2, Soaked and cooked lentil powder, Sample3, Germinated and cooked lentil powder, Sample4 Soaked and cooked lentil and 2g spinach powder, Sample5, Germinated and cooked lentil and 2g spinach powder.

Sensory attributes of veggie burgers samples

Sensory attributes of appearance, color, taste, odor, tenderness and overall acceptability for soybeans burger formulae (as control). Then, soybean replaced by soaked and germinated lentil. Then, mixture between them and spinach make veggie burger as shown in Table (13). Generally, veggie burger made from soybean had the highest scores of all sensory attributes. Veggie burger made by soaked and cooked lentil was better than burger made by soaked and cooked lentil combine with spinach. While, burger made by germinated and cooked lentil combine with spinach was better than germinated and cooked lentil only. There were no significant differences between the control sample (soybeans) and the burger samples of taste, color, odor and appearance

It could be concluded that all samples burger were acceptable of all sensory properties, i.e appearance, color, taste, odor, tenderness and overall acceptability for the panelists.

Table (13) Sensory attributes of burgers formulas

Formulas	Appearance	Color	Taste	Odor	Tenderness	Total /50
1	8.90±0.27 ^a	8.90±0.23 ^a	8.80±0.29 ^a	9.30±0.21 ^a	9.30±0.21 ^a	45.20±0.95 ^a
2	8.50±0.22 ^a	8.70±0.30 ^a	8.10±0.40 ^a	8.90±0.31 ^a	8.60±0.47 ^{ab}	42.80±1.33 ^{ab}
3	8.20±0.29 ^a	8.50±0.34 ^a	8.10±0.40 ^a	8.80±0.32 ^a	7.50±0.22 ^b	41.10±1.25 ^b
4	8.90±0.23 ^a	8.90±0.31 ^a	8.10±0.27 ^a	8.60±0.33 ^a	8.00±0.49 ^b	42.50±1.27 ^{ab}
5	8.40±0.22 ^a	9.00±0.33 ^a	8.20±0.32 ^a	8.60±0.37 ^a	7.80±0.38 ^b	42.00±1.17 ^{ab}

*Each value in a column followed by the same letter is not significantly different at ($p \leq 0.05$).

**Sample1 control (soybean), Sample 2, Soaked and cooked lentil powder, Sample3, Germinated and cooked lentil powder, Sample4 Soaked and cooked lentil and 2g spinach powder, Sample5, Germinated and cooked lentil and 2g spinach powder.

CONCLUSION

The findings of this study show that foods fortified with spinach and lentils achieved better results in improving the indicators of the immune system in experimental rats compared to nonfortified group (control), because these sources rich of many compounds and biologically active elements. Therefore, it must be added to meals as prevention and strengthening immune system.

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تأثير التغذية بالسبانخ والعدس على بعض الصفات المناعية في ذكور فئران التجارب

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الملخص العربي

يهدف هذا البحث الى دراسة تأثير المواد النشطة حيويًا في السبانخ والعدس كعوامل مؤثرة في تعزيز جهاز المناعة في ذكور الفئران. تم دراسة تأثير الطهي بعد اجراء عمليتي النقع و الانبات على كفاءة المكونات الكيميائية للعدس. وكذلك دراسة التركيب الكيميائي و مضادات الأكسدة للمواد الخام المستخدمة في الدراسة والبرجر المعد منها. تم تقسيم عدد 30 فأر ذكور إلى مجموعتين رئيسيتين، الأولى (5 فئران) غذيت على العليقة الضابطة، كنترول (-) والثانية (25 فأر) قسمت بشكل عشوائي الى 5 مجموعات (المجموعات المعاملة، 5 فأر/ مجموعة).

سجلت النتائج أن مسحوق السبانخ يحتوي على نسبة أعلى من المركبات الفينولية مقارنة بالعدس. زادت البروتينات والألياف في العدس المنبت ولكن انخفضت الدهون والرماد والكريبوهيدرات والطاقة مقارنة بالعدس غير المعامل . زاد محتوى عناصر الحديد والزنك والنحاس والسيلينيوم والفينول في العدس المنقوع المطبوخ مقارنة بالعدس المنبت المطبوخ.

يمكن تلخيص نتائج التقييم البيولوجي أن هناك انخفاض في مستوى السكر، ونسبة LDL (معامل الخطر)، الكوليستيرول الكلى والجليسيريدات الثلاثية وانزيمات ALT و AST بينما سجلت زيادة في الجليبولينات IgG ، IgA و WBC و HDL في المجموعات المعاملة مقارنة بالمجموعة الضابطة . وأشارت نتائج التقييم الحسي أن هناك درجة قبول مرتفعة في عينات البرجر حيث حققت درجة أكبر من 80 ٪ مقارنة بالبرجر الكنترول.

من النتائج المتحصل عليها يمكن استنتاج أن تدعيم الأطعمة بالسبانخ والعدس تحقق نتائج أفضل لتحسين مؤشرات الجهاز المناعي في فئران التجارب نظرا لاحتوائهما على العديد من المركبات والعناصر النشطة حيويًا. لذا يجب اضافتهما الى الوجبات الغذائية كوقاية ولتعزيز الجهاز المناعي.