تأثير الحلبة المنبتة والغير منبتة على دلائل المناعة فى فئران التجارب

ا.د/ محمد مصطفى السيد
أستاذ التغذية وعلوم الأطعمة المتفرغ وعميد
كلية الأقتصاد المنزلى سابقاً
كلية الاقتصاد المنزلى – جامعة المنوفية

أ/ أميرة احمد عبد السلام
باحثة ماجستير – تخصص تغذية وعلوم الأطعمة
كلية الأقتصاد المنزلى – جامعة المنوفية

د/أميرة حمدي درويش
مدرس التغذية وعلوم الأطعمة
كلية الاقتصاد المنزلي – جامعة المنوفية

المستخلص عربى

صممت الدراسة الحالية لدراسة تأثير الحلبة المنبتة والغير منبتة على بعض مؤشرات المناعة وغيرها من المتغيرات البيوكيميائية في الفئران . تم استخدام ثلاثين(٣٠) من ذكور الفئران الألبينو وزنها (١٦٠ ± ١٠ جم) وتم تقسيمهم إلى مجموعتين رئيسيتين ،المجموعه الاولى كانت المجموعه الضابطة السالبة (٥ فئران)والمجموعه الثانية (٢٥فأرا) حقنت بالسيكلوسبورين (٥٠مللى جرام/كيلو جرام /يوم)المذاب فى زيت الزيتون تحت الجلد للاصابه بخفض المناعه ،ثم قسمت الى خمس مجموعات (٥فئران فى كل مجموعة) ، واحده منها بقيت كمجموعة ضابطة موجبة ،اما المجموعات الاربعه الباقية فقد اعطيت ٥٪, ٧.٥٪ بذور حلبه منبتة وغير منبتة واستمرت التجربة لمده ٢٨ يوما . وفى نهايتها تم وزن الفئران ثم ذبحهم وتجميع عينات الدم بعد الصيام ١٢ ساعه .تم تقدير التركيب الكيميائي ، و مضادات الأكسدة ، و الفينولات الكلية ، والكاروتين ، إنزيمات الكبد و بعض مؤشرات المناعة. أظهرت نتائج البيانات المتحصل عليها أن نسبة البروتين في الحلبة المنبتة ارتفعت إلى ٤٠.١٣٪ بالمقارنة مع الحلبة غير المنبتة . كما تسبب الإنبات في زيادة المعادن p و Cu و Mn و Ca و Zn بينما انخفضت المعادن الاخرى Fe و K و Na . كان للإنبات نسبة عالية من النشاط المضاد للأكسدة والفينول الكلي والكاروتين مقارنة بالبذور غير المنبتة . كما أظهرت النتائج أن إضافة الحلبة المنبتة وغير المنبتة لى المجموعات المعالجة ،ادت الى عوده مستويات الجلوبيولينات الى المستوى الطبيعي الذي كان قريبًا الى مستوى

المجموعة الضابطة السالبة وتحسين مستويات عامل النخر الورمى مقارنة بالمجموعة الضابطة الموجبة. خلصت الدراسة إلى أن الحلبة المنبتة تحتوي على مركبات حيوية نشطة بيولوجيًا. لها دور حيوي في تحسين الحاله الصحيه لخفض المناعة وخاصة الحلبة المنبتة بنسبه ٧.٥٪ تليها ٥٪.

الكلمات المفتاحية :السيكلوسبورين، الحلبة، الإنبات، النشاط المضاد للأكسدة، الجلوبيولينات المناعية.

Effect of Germinated and Non-Germinated Fenugreek (*Trigonella foenum-graecum*) seeds on Immune Indicators in Experimental Rats

Abstract

The current study aimed to investigate the effect of germinated and non-germinated fenugreek seeds on some immune indicators and biochemical parameters in rats. Thirty (30) male albino rats weighing (160 ± 10 g) were used. They were divided into two main groups (5 rats each), the first was fed on a basal diet as a negative control group, while the second group (25rats) was injected with cyclosporine(50mg/kg/day) dissolved in olive oil subcutaneously for ten consecutive days to induce immunosuppressed rats , then divided into five groups one of them kept as a positive control group, while the left four groups were given a basal diet with 5 %, 7.5% of germinated and non-germinated fenugreek for 28 days.Chemical composition, antioxidant activity, total phenol, carotene, liver enzymes, and some immune indicators were determined.The findings indicated that, in comparison to the non-germinated fenugreek, the level of protein in the germinated fenugreek increased to 40.13%.Also, germination caused an increase in the minerals P, Cu, Ma, Ca and Zn, whereas other minerals Fe, K and Na decreased significantly. The germinated significantly had high content of antioxidant activity, total phenol and Carotene as compared to non-germinated seeds. Results also showed that the addition of germinated and non-germinated fenugreek to treated groups, the immunoglobulin production levels began to return to the normal level, which was close to the level of the negative control group. The study concluded that fenugreek seeds have been found to have important bioactive compounds. It has a vital role in improving immunity, especially germinated fenugreek 7.5% followed by 5%.

Keywords: Cyclosporine, Fenugreek, Germination, Antioxidant activity, Immunoglobulin.

Introduction

Immune system uses the body's own defense mechanisms to guard against damage, disease, and infections (**Childs *et al*., 2019).** It is a very complex and multidimensional system made up of different tissues, cells, and proteins. It forms a complex network of cells and proteins that travels throughout the body through the blood and lymphatic systems. A robust immune system can discriminate between foreign substances (non-self) and body cells (self), removing the latter **(Terrie, 2017).** Age-related declines in immunity and increases in morbidity include deficiencies in zinc, selenium, iron, copper, or vitamins A, C, E, B6, or B9. Overeating and being overweight or obese can further impair the immunological response **(Ritz and Gardner, 2009).** Additionally, the immune system is harmed by immunosuppressive drugs, immunologically mediated disorders, chronic stress, sleep deprivation, and certain medical conditions **(Terrie, 2017).**The likely immunomodulating ingredients in these items are nutraceuticals derived from plants. The balance between health and illness is maintained in part by phytochemicals (flavonoids, folate, polyamines, alkaloids, and terpenoids) and other vital nutrients (primarily proteins, carbs, fatty acids, minerals, and vitamins) **(Parveen *et al.*, 2020).**One of the plants with all of these characteristics is fenugreek, which is a highly valued crop in human diets. Since ancient times, fenugreek (*Trigonella foenum-graecum, L.*), a member of the Fabaceae family, has been utilized as a significant spice **(Aasim *et al.*, 2018).** According to **Sarwar *et al*. (2020)**, fenugreek seeds, and leaves contain fiber, protein, beta-carotene, vitamins, minerals, gums, alkaloids, flavonoids, steroidal sapogenins, dysgenic, trig coumarin, nicotinic acid, trimethyl coumadin, and trigonelline.**Ojha *et al*., (2018),** reported that germinated fenugreek possesses more health potential compared to non-germinated fenugreek seeds as it led to a significant change in bioactive components and antioxidant activity. Germinated seeds are a good source of important amino acids particularly leucine, lysine and tryptophan that used in the biosynthesis of proteins, and play special roles in "anchoring" membrane proteins within the cell membrane, as well as,which tryptophan is also a precursor to the neurotransmitter serotonin, the hormone melatonin, and vitamin B3 **(Tewari *et a*l., 2020).**By reducing oxidative damage, the beneficial substances generated during germination, like polyphenols, promote immunological function. Germination also raises the antioxidant content of fenugreek seeds, which aids in lowering oxidative stress and inflammation indicators in the body **(Khan, 2018)**.So,the aim of this study was to investigate the effect of germinated and non-germinated fenugreek on the immune system of experimental rats.

Material and Methods

Materials

Fenugreek seeds were obtained from the herbal store in Shebin El-Kom, Egypt, and identification was done by the Agricultural Plant Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt.

Cyclosporine was obtained from an El-Gomhoria company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.
Olive oil was obtained from the herbal store in Shebin El-Kom.
The Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt, provided thirty adult normal male albino rats with Sprague Dawley strains, weighing 160±10g.

Methods:

Preparation of germinated fenugreek:

Three times of washing with potable water were used to clean, grade, sort, and wash the fenugreek seeds. With a seed:water ratio of 1:5 (w/v), the seeds were then steeped in drinkable water for twenty-four hours at room temperature (22±2C°). The soaked seeds were rinsed twice with heated and cooled water to prevent post-contamination during germination, and the unobstructed water was disposed of. In plastic sieves covered with sterile cloth, the wet seeds were allowed to sprout for 72 hours at room temperature while being frequently watered. The fenugreek seeds that had germinated were dried for 24 hours at 40°C in a drying oven located at Menoufia University's Faculty of Agriculture **(Shalini and Sudesh, 2004).**

Determination of chemical composition:

Moisture, Crude protein, Fat and Ash content were determined according to the method recommended by **A.O.A.C. (2010).**
Crude fiber was determined according the method of **Pearson (1971).**
The carbohydrates were calculated by the difference as follows:
% Carbohydrates =100 - (% moisture + % protein + % fat + % ash+ % fiber).
Test Methods (Carotene, Total Phenols, Antioxidant Activity and Vit C)

by **Ivanova *et al*., (2010) and Lu *et al.,* (2017).**

Induction of Immunity disorder:

Thirty (30) male albino rats weighing (160 ± 10 g) were injected with cyclosporine (50 mg/kg/day) subcutaneously in olive oil for ten days according to **Couteaux *et al*., (1988).**

Experimental Designs and Animal Groups: -

After one week of convalescence rats were housed in environmentally controlled atmosphere and were feed on basal diet according to (AIN-93) guide lines (**Reeves *et al*.,1993**) in animal laboratory in the Faculty of Home Economics Menoufia University. Then, rats were distributed into 6 groups each of 5 rats in which means of rat's weight for all groups were nearly equal. All the groups of rats were housed in wire cages and fed on the experimental diet for 28 days .They were divided into two main group (5 rats each), the first was fed on basal diet as a negative control group, while the second group (25 rats) were treated by the previous method to induce immunosuppressed , then divided into five groups one of them kept as positive control group , while the left four groups were given basal diet with 5 %, 7.5% of germinated and non-germinated fenugreek for 28 days, respectively. Rats were weighed at the beginning of the experimental then weekly and the end of experiment. Animals were starved for 12 hours and then scarified at the end of 28 day.

Biological Indices Calculation:

The following formulae were used to determine the feed intake daily, body weight gain g (BWG g/day), and feed efficiency ratio (FER) in accordance with **Chapman *et al*. (1959)** in order to conduct a biological evaluation of the various diets:

Body Weight Gain = Final weight (g) - Initial Weight (g)

Feed efficiency ratio (FER) = Gain in body weight(g)/Feed intake(g).

Biochemical analysis:

Blood samples were taken from the portal vein and placed in dry, clean centrifuge tubes for serum separation. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. Serum samples were kept frozen at -20 °C until chemical analysis according to **Schermer** (**1967**).

Complete blood count (CBC) determined according to **Jacobs *et al*. (2001).** The serum was used to determine the following**:** Serum immunoglobulins (IgA and IgM) were estimated according to **Burlingame and Rubin, (1990).** Serum TNF was determined according to **Maury (1986).** The activity of aspartate aminotransferases (AST), alanine aminotransferases (ALT) and alkaline phosphates (ALP) enzymes were assigned by the method of **Yound, (1975); Tietz, (1976**) and (**Belfied and Goldbery,1971**), respectively.

Statistical Analysis:
The data were statically using a computerized costat program by one-way ANOVA. The results are presented as mean ± SD. Differences between treatments at $p \leq 0.05$ were considered significant according to **SAS, (2010**).

Results and Discussion

The chemical composition of germinated fenugreek as compared to non-germinated fenugreek seeds is shown in Table (1). The mean values of moisture, protein, fat, fiber, ash, carbohydrates and total calories were 8.06, 28.91, 9.52, 2.97, 2.47, 48.07 g/100g and 393.6 kcal., respectively for non-germinated fenugreek while for germinated seeds, the mean values were 10.70, 40.13, 3.38, 1.73, 2.31, 41.75g/100g and 339.94 kcal., respectively. These results are in the same trend of **Taraseviciene *et al.*, (2009**) who showed thatthe fenugreek seeds' crude protein concentration varied between 26.10 and 29.89%. As the germination time of fenugreek seeds grew, so did their crude protein content. The longest documented germination period was 72 hours, while the shortest was 24 hours. The reason for this is that germination is a biotechnological process that activates metabolic enzymes like proteinases. As a result of this process, some amino acids and peptides can be released, and the synthesis or utilization of others, to form new proteins, can occur. The germination process is recommended as a technological approach for enhancing the nutritional quality of legumes and other seeds because it can improve the nutritional content of proteins.After germination, the protein content increased from 32.7% to 41.2%. The decrease of seed nitrates into protein or ammonium compounds may be the cause of this rise(**Hooda and Jood, 2003).** Enzymatic protein synthesis could be the reason of the increased protein content of germinated seeds, which is in line with Mansour and EL-Adway's (1994) . **Hooda and Jood, (2003)** found a similar pattern of fenugreek seeds' lipid content decreasing following soaking.Total carbohydrates (44.8 %) decreased but marginally. This decrease might be attributed to enzymatic degradation of seeds during soaking **Mathur and Chaudhary (2009)**. Furthermore, soaking has been shown to reduce the amount of dietary fiber as reported by **Hooda and Jood,(2003)**. Germination of fenugreek seeds caused decrease in fat content as compared to raw seeds.**Mathur and Chaudhary(2009)** and **Amankwah *et al.*, (2009)** indicated that the leaching of minerals during steeping and washing may be the cause of the observed decrease in ash content of fenugreek flour samples during germination; in general, the elimination of moisture increases nutrient concentrations and can make some nutrients more available.

Table (1): Chemical composition of germinated and non-germinated fenugreek seeds.

Constituents	Germinated seeds	Non germinated seeds
Moisture	10.70 ±0.95 [a]	8.06 ±0.33[b]
Protein	40.13 ±4.65[a]	28.91 ±3.98 [b]
Fat	3.38 ±0.91 [b]	9.52 ±2.84 [a]
Fiber	1.73 ±0.86[b]	2.97 ±0.54 [a]
Ash	2.31 ±0.002 [a]	2.47±0.32 [a]
Carbohydrates	41.75 ±3.16[b]	48.07 ±5.97 [a]
Total calories	375.94 ±8.33[b]	393.6 ±6.02[a]

The results for mineral compositions of examined germinated and non-germinated fenugreek seeds were presented in Table (2). Phosphorus, copper, magnesium, calcium, and zinc were high in germinated seeds as compared to non-germinated seeds while germination led to in a decrease the mean values of potassium, sodium and iron. **Duhan *et al.***, (**2002)** found that the content of Zn and Fe minerals decreased as a result of germination, but that of Ca and P minerals significantly increased . Leaching of iron into the soaking media may be the cause of the decrease in iron concentration in flour made from germinated fenugreek seeds. A decrease in Fe content during the germination of fenugreek seeds was reported by **El-Shimi *et al*., (1984).** Increase in Ca and P might be due to decrease in phytates, tannins, and other anti-nutritional factors that bind the minerals as reported by **El- Mahdy and El- Sebaiy (1982).** While **Lestienne *et al.*, (2005)** was in contrast with the obtained results who reported that reduction in Zn content in soaked and **Shakuntala *et al.*, (2011)** showed that germination improved the availability of iron and zinc. This led to phytic acid in plant foods forming complexes with essential dietary minerals such as Ca, Fe, Zn, and Mg making them biologically unavailable for absorption. The phytase activity increased on germination causing the catabolism of phytic acid germinated seeds due to the leaching of Zn into the soaking medium. Whereas, Phytases, or myo-inositol hex phosphate phosphohydrolases, are enzymes that hydrolyze myo-inositol 1,2,3,4,5,6, -hexakis (dihydrogen phosphate) to myo-inositol and inorganic phosphate and thereby increase the in vitro availability of divalent minerals.

Table (2): Minerals composition(mg/100g) of germinated and non-germinated fenugreek seeds.

Constituents	Germinated seeds	Non- germinated seeds
P	240.91 ±7.88 [a]	223.55 ±6.44 [b]
K	543.11 ±5.07 [b]	770.08 ±10.32 [a]
Na	72.08 ±4.91 [b]	97.06 ±6.43 [a]
Cu	65.76 ±3.41[a]	57.60 ±4.03 [b]
Ma	157.94 ±9.61 [a]	73.72 ±2.29 [b]
Ca	223.87±5.76[a]	176.65±7.03 [b]
Fe	25.87±2.01 [b]	33.45±1.04 [a]
Zn	3.07±0.64 [a]	2.54±0.07 [b]

In the present study, the total phenol, carotene, vitamin C, and DPPH content were different in germinated and non-germinated fenugreek seeds (Table 3). The germinated seeds significantly had a high content of the determined parameter as compared to non-germinated seeds. These results agreed with **Ojha *et al*., (2018)**, who proved that the germination process of fenugreek led to a significant changes in antioxidant activity. Test methods **Ivanova *et al*., (2010)** and **Lu *et al*., (2017)** the germination process causes various changes in the phenolic compounds and modifies the antioxidant activity. The results of **Naidu *et al*., *(*2012)** were in the same line as the obtained results, they found that germinated fenugreek seed parts had 65.81-88.88 mg GAE/g total polyphenols and exhibited good free-radical scavenging activities from 50 to 70 % inhibition and the increase was due to the endogenous enzymes of the legumes being activated, and the most important enzymes are the hydrolases and polyphenol oxidases, whose activity increases during germination.In addition, according to **Aqil *et al.* (2006)**, the DPPH technique reported 57.45±2.44% inhibition and the fenugreek seed extract had 74.33±5.13 mg GAE/g total phenolics. The antioxidant property is influenced by the quantitative and qualitative changes in phenolic compounds during germination, as stated by **Lopez-Amorosa *et al.* (2006)**. Aside from phenolic, other bioactive compounds found in varying amounts in legumes include vitamins and carotenoids, which may also have antioxidant properties. The primary cause of the observed variations in the antioxidant activity may be the synergistic effects of these compounds both with phenolic compounds and among themselves.

Table (3): Antioxidant activities of germinated and non- germinated fenugreek seeds.

Antioxidant activities	Germinated seeds	Non- germinated seeds
Total phenols mg /100g	87.66 ±7.88 [a]	70.09 ±6.44 [b]
Carotene mg/100g	54.11 ±5.07 [a]	40.08 ±10.32 [b]
Vitamin C mg/100g	96.08 ±4.91 [a]	78.06 ±6.43 [b]
DPPH%	65.76 ±3.41[a]	57.60 ±4.03 [b]

Data presented in **Table (4**) showed the effect of germinated and non-germinated fenugreek seeds on body weight gain, feed intake and feed efficiency ratio. For body weight gain, it was observed that the highest result was detected in the group of 7.5% followed by 5% germinated seeds. All level results showed that significant changes as compared with both of the controls, the changes to negative control group were decreased while they were increased to the positive control expect for the group with 5%. In the case of feed intake, it was showed that the highest effect on level 7.5% germinated fenugreek. Concerning feed efficiency ratio, it was observed that the mean value of FER of the control positive was lower than the control negative. Treated groups showed significant increases in mean value as compared with control positive expect for 5% non-germinated fenugreek showed non-significant as compared with the positive control group. These results are congruent with the findings of **Yang *et al.*, (2022)**, who reported that fenugreek seeds increased body weight of broiler chickens compared to the initial weight. Also**, Amein *et al.*, (2019**) revealed that adding fenugreek to broiler diets may improve metabolic processes, promote nutrient use, and raise feed conversion efficiency. This observation can be explained by the presence of steroid saponins, which stimulate the digestive system, increase feed intake, and activate the hypothalamus gland, in addition to the important fatty acids and high-quality proteins found in fenugreek.

Table (4): Effect of germinated and non-germinated fenugreek seeds on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) on immune system of experiment rats.

Parameter Groups	Body weight gain (g/28 day)	Feed intake (g/day)	FER
G_1: Control (-)	38.69±3.07[a]	14.86±0.96[a]	0.093±0.002[a]
G_2: Control (+)	10.21±1.45[d]	4.97±0.04[d]	0.074±0.03[d]
G_3: 5% non-germinated fenugreek	10.73±0.87[d]	5.11±0.35[d]	0.075±0.002[d]
G_4: 5% Germinated fenugreek	17.56±2.86[c]	7.94±0.21[c]	0.079±0.005[c]
G_5: 7.5% non-germinated fenugreek	14.34±2.09[c]	6.65±0.65[c]	0.077±0.012[c]
G_6: 7.5% Germinated fenugreek	23.49±1.77[b]	10.11±0.23[b]	0.083±0.001[b]
LSD	**3.76**	**1.65**	**0.003**

The average ± standard deviation is represented by each number. Significant differences exist between the means under the same column with different superscript letters (P ≤ 0.05).

Data presented in **Table (5**) showed the effect of germinated and non-germinated fenugreek seeds on serum IGM, IGA and TNF levels of experimental rats. In the case of IGA and IGM, the highest levels were recorded for the negative control group, while positive control group recorded the lowest value as a result of injection by cyclosporine (50mg /kg / day) with significant(P≤0.05) differences. The mean values of IGM were 114.35 and 53.93 mg/dl**,** respectively**.** The mean values of IGA were 172.06 and 82.73 mg/dl**,** respectively.By adding germinated and non-germinated fenugreek to treated groups, the IGM and IGA levels began to return to the normal level, which was close to the level of the negative control group. The best result recorded for the 7.5% germinated fenugreek group, the mean values of IGM and IGA were 108.56 and 144.66 mg/dl,respectively. Followed by 5% germinated fenugreek, 7.5% non-germinated fenugreek and 5% non-germinated fenugreek which were recorded the lowest value. In the case of TNF, it was found that the highest value recorded for positive control group, while the lowest value recorded for negative control group with significant (P≤0.05) differences. The mean values were 172.30 and 76.06 mg/dl, respectively. Treating induced-cyclosporine rats by germinated and non-germinated fenugreek improved TNF levels compared to positive control group. The highest value of treated groups recorded for 5% non-germinated fenugreek group, while the lowest value recorded for 7.5% germinated fenugreek group with significant (P≤0.05) differences. The mean values were 162.29 and 127.35 mg/dl, respectively. It is also noticeable that the best results recorded for germinated groups compared to non-germinated, and this is what proved by **Ojha *et al.*, (2018)**, who reported that germinated fenugreek possess more health potential compared to non-germinated fenugreek seeds as it led to a significant change in bioactive components and anti-oxidant activity. These results had the same trend of **Alsieni *et al.*, (2021)**, who reported that treating diabetic rats with the aqueous extract of fenugreek restored IGA and IGM to its normal levels. **Bafadam *et al.*, (2021)**, explained that fenugreek seeds could inhibit the production of induc ed inflammatory cytokines such as TNF-α. In the same context a study conducted by **Abdrabouh (2022)**, who evaluated the role of fenugreek in reducing damage caused by inhaling gasoline fumes in rats, which had a significant effect in reducing the level of TNF compared to the group exposed to gasoline fumes.

Table (5): Effect of germinated and non-germinated fenugreek seeds on serum IGM, IGA and TNF levels on immune system of experiment rats.

Parameter / Groups	IGM mg/dl	IGA mg/dl	TNF mg/dl
G_1: Control (-)	114.35 ^{a} ± 0.77	172.06 ^{a} ± 0.46	76.06 ^{f} ± 0.97
G_2: Control (+)	53.93 ^{f} ± 0.23	82.73 ^{f} ± 0.90	172.30 ^{a} ± 0.14
G_3:5%non-germinated fenugreek	74.76 ^{e} ± 0.41	114.00 ^{e} ± 0.53	162.29 ^{b} ± 0.69
G_4:5%Germinated fenugreek	88.93 ^{c} ± 0.45	131.63 ^{c} ± 0.12	146.65 ^{d} ± 0.94
G_5:7.5%non-germinated fenugreek	87.50 ^{d} ± 0.80	123.40 ^{d} ± 0.75	153.35 ^{c} ± 0.48
G_6:7.5%Germinated fenugreek	108.56 ^{b} ± 0.25	144.66 ^{b} ± 0.82	127.35 ^{e} ± 0.80
LSD	**0.95**	**1.16**	**1.30**

The average ± standard deviation is represented by each number. Significant differences exist between the means under the same column with different superscript letters ($P \leq 0.05$).

Data presented in **Table (6)** showed the effect of germinated and non-germinated fenugreek on serum red blood cells (RBC) and white blood cells (WBC) levels of experiment rats. It was found that the lowest value of RBC levels recorded for positive control group it was 3.25 10^6 /mm^3. RBC values significantly ($P \leq 0.05$) increased in all treated groups, where 7.5% germinated fenugreek group recorded the highest value of mean, and it was 4.05 10^6 //mm^3 comparing to other treated groups. In the case of WBC, the negative control group recorded the lowest value which, was by 5.68 10^3 /mm^3. It was evident that cyclosporine injection for ten days without treatment raised the levels of WBC in infected rats to the highest value of 12.88 10^3//mm^3 in positive control group. Treated groups with cyclosporine with germinated and non- germinated fenugreek resulted in a decrease in the level of WBC. The best improvement appeared in the 7.5% germinated fenugreek group was 9.07 10^3//mm^3. As for lymphocytes treating immunocompromised rats with fenugreek seeds, whether germinated or not showed significant ($P \leq 0.05$) increase lymphocytes levels. The best result recorded for group six, a concentration of 7.5% germinated fenugreek seeds, which exceeded the level of lymphocytes in the negative control group with a significant difference ($P \leq 0.05$).It is clear to notice that adding different concentrations (5 and 7.5%) of germinated and non-germinated fenugreek to the basal diet, the haemoglobin returned to its normal level. The highest value of haemoglobin for treated groups recorded for 7.5% germinated fenugreek group, while the lowest value recorded for 5% non-germinated fenugreek group with significant ($P \leq 0.05$) differences. There were no significant ($P > 0.05$) differences between 5% germinated and 7.5% non-germinated fenugreek groups. It is noticeable that the effect of the germinated fenugreek is stronger than the non-germinated fenugreek.

Data also indicated that rats injected by cyclosporine (50mg/ kg) had abnormal levels of platelets comparing to negative control group which recorded the lowest value, on the other hand the highest value of for positive control group with significant differences. All treated groups showed a significant improvement in platelets levels compared to positive control group. Descending 5% non-germinated fenugreek, followed by 7.5% non-germinated fenugreek, then 5% germinated fenugreek, and finally the group closest to the normal level 7.5% germinated fenugreek. The mean values were 310.98, 303.06, 300.39, 281.88 10^6//mm^3, respectively. These results agreed with **Chourasiya *et al*., (2019),** who reported that fenugreek extract significantly($P \leq 0.05$) improved RBC and WBC count at a dose of 400 mg/kg body weight against phenyl hydrazine induced anaemic rat model.

Elghazaly *et al*., (2019), reported that fenugreek seeds may be improving immunity because they play a role in increasing RBC, as well fenugreek contains iron and it can improve anemia conditions. Also**, Algridi and Azab, (2021)** recommended the use of fenugreek seed powder is by humans to reduce hemato toxicity. Where they proved that the treatment of toxicity in male rabbits caused by aluminium chloride with fenugreek seed powder led to a significant increase in the levels of RBC and haemoglobin, an improvement in the levels of WBC and decreased platelets levels in rats induced by $AlCl_3$. Also, **Abdrabouh (2022),** reported that fenugreek seeds improved the level of haemoglobin and blood platelets, which were damaged by gasoline fumes**.**

Table (6): Effect of germinated fenugreek and non-germinated fenugreek seeds on serum red blood cells (RBCs), white blood cells (WBCs), lymphocytes, haemoglobin and platelets levels on immune system of experiment rats.

Parameter / Groups	RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	Lymphocytes	Haemoglobin g/dl	Platelets 106/mm3
G_1: Control (-)	4.75 ^{a} ± 0.08	5.68 ^{e} ± 0.28	85.00 ^{d} ± 0.52	12.21 ^{a} ± 0.09	244.80 ^{f} ± 0.94
G_2: Control (+)	3.25 ^{f} ± 0.015	12.88 ^{a} ± 0.21	83.48 ^{e} ± 0.11	9.33 ^{e} ± 0.18	321.06 ^{a} ± 0.61
G_3:5%Non-germinated fenugreek	3.55 ^{e} ± 0.015	11.18 ^{b} ± 0.21	86.57 ^{c} ± 0.41	10.00 ^{d} ± 0.06	321.06 ^{a} ± 0.61
G_4:5%Germinated fenugreek	3.85 ^{c} ± 0.015	10.08 ^{c} ± 0.15	88.14 ^{b} ± 0.37	10.36 ^{c} ± 0.13	300.39 ^{d} ± 0.67
G_5:7.5%Non-germinated fenugreek	3.65 ^{d} ± 0.015	10.15 ^{c} ± 0.10	88.13 ^{b} ± 0.38	10.33 ^{c} ± 0.18	303.06 ^{c} ± 0.53
G_6:7.5% Germinated fenugreek	4.05 ^{b} ± 0.015	9.07 ^{d} ± 0.18	90.18 ^{a} ± 0.33	11.29 ^{b} ± 0.03	281.88 ^{e} ± 1.60
LSD	**0.063**	**0.35**	**0.66**	**0.22**	**1.71**

The average ± standard deviation is represented by each number. Significant differences exist between the means under the same column with different superscript letters ($P \le 0.05$).*RBCs: Red Blood Cells. WBCs: White Blood Cells. Lymphocytes. Haemoglobin. Platelets*.

Data presented in **Table (7**) showed the effect of germinated and non-germinated fenugreek seeds on liver enzymes. It was found that the positive control group recorded the highest values of serum AST, ALT and ALP compared to the negative control group. Treated groups with the different levels of germinated and non-germinated fenugreek caused significant reduction in AST, ALT and ALP levels when compared to the positive control group. Group 7.5% germinated fenugreek had a high effect on liver enzymes. According to **Nagamma *et al.* (2019)**, fenugreek seed extract dramatically raises blood counts in rats with high-fat diet-induced obesity, including MCV, MCH, MCHC, red blood cell distribution width, hemoglobin (Hb), hematocrit, and platelet count.These findings agreed with **Almalki (2022**), confirmed that aqueous extract of germinated fenugreek seeds decreased levels of serum ALT and AST in rats with hepatorenal-toxicity by lead. Also, **EL Hak *et al*., (2022)**, recommended fenugreek seed supplementation as a regular nutrient for liver protection against aflatoxin B1 toxicity by improving liver functions. **Mehram *et al*., (2022)**, indicated that, water extract of fenugreek (raw, germinated and green leaves) caused a decrease in serum ALT and AST of malnourished rats.

Table (7): Effect of germinated fenugreek and non-germinated fenugreek seeds on serum liver enzymes levels (AST, ALT and ALP) on immune system of experiment rats.

Groups \ Parameter	AST U/L	ALT U/L	ALP U/L
G_1: Control (-)	35.56±4.87^{d}	34.88±4.08^{e}	76.39±5.76^{e}
G_2: Control (+)	82.99±3.01^{a}	68.39±3.89^{a}	172.89±7.94^{a}
G_3:5% non-germinated fenugreek	77.66±2.64^{a}	61.58±4.03^{b}	162.48±4.92^{b}
G_4: 5% Germinated fenugreek	68.44±3.06^{b}	51.54±3.81^{c}	146.82±5.01^{c}
G_5:7.5% non-germinated fenugreek	63.69±2.88^{b}	53.99±2.85^{c}	153.49±4.71^{c}
G_6: 7.5% Germinated fenugreek	51.48±3.71^{c}	42.66±1.03^{d}	127.44±3.52^{d}
LSD	**6.06**	**5.65**	**6.56**

The average ± standard deviation is represented by each number. Significant differences exist between the means under the same column with different superscript letters ($P \leq 0.05$).*AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. ALP: Alkaline phosphatase.*

Conclusion

The study demonstrated that germination improves fenugreek seed nutrition, functionality, and immunological aspects to a great extent. Improvement in the protein content of germinated seeds was remarkable along with mineral bioavailability, especially calcium, phosphorus, magnesium, and antioxidants which also included total phenols, carotene, vitamin C, and DPPH activity.Moreover, supplementation of rat diets with germinated fenugreek seeds resulted in improved weight gain, increased feed efficiency, enhanced RBCs, hemoglobin levels, and immunoglobulins IgA and IgM. It also showed positive effects on inflammatory TNF-α and WBC count by reducing these markers, indicating potential immune-strengthening and anti-inflammatory properties. It's clear that these findings prove enhanced health benefit aspects for germinated fenugreek seeds compared to non-germinated ones.As discussed, germination is a low-cost, efficient biotechnological method to use for improving the nutritional value overall.

References.

A. O.A.C (Association of Official Agricultural Chemists) (2010): Official Methods of the Association of Official Analytical Chemists. 15th ed. AOAC 2200 Wilson boulevard arling, Virginia, 22201, U.S.A.

Aasim, M.; Baloch, F. S.; Nadeem, M. A.; Bakhsh, A.; Sameeullah, M. and Day, S. (2018): Fenugreek (*Trigonella foenum-graecum, L.*): An underutilized edible plant of modern world. Global perspectives on underutilized crops, 381-408.

Abdrabouh, A. E. (2022): Inflammatory and proapoptotic effects of inhaling gasoline fumes on the lung and ameliorative effects of fenugreek seeds. Scientific Reports, 12(1), 14446.

Algridi, M. A. and Azab, A. E. (2021): Ameliorating Effects of Fenugreek Seeds Powder against Hemato toxicity Induced by Aluminum Chloride in Male Rabbits. J. Biotechnology and Bioprocessing, 2(4), 2766-2314.

Almalki, D. A. (2022): Hepatorenal Protective Effect of Fenugreek Aqueous Extract against Lead Toxicity in Experimental Rats. In Doklady Biochemistry and Biophysics, 507(1), 318-325.

Alsieni MA, El Rabey HA, Al-Sieni AI and Al-Seeni M.N(2021): Comparison between the Antioxidant and Antidiabetic Activity of Fenugreek and Buckthorn in Streptozotocin-Induced Diabetic Male Rats. Biomed Res Int. 2021 Aug 27; 2021:7202447. doi: 10.1155/2021/7202447.

Amankwah EA, Barimah J, Nuamah AKM, Oldham JH. and Nnaji CO. (2009): Formulation of weaning food from fermented maize, rice, soybean and fishmeal. Pak. J. Nutr., 8(11): 1747-1752.

Amein, S. M.; Mosaad, G. M. and Hussein, M. K. (2019): Effect of some medicinal plants as feed additives on growth performance, blood constituents and carcass characteristics of broilers. Journal of Advanced Veterinary Research, 9(4):170-177.

Aqil, F.; Ahmad,I. and Mehmood Z (2006): "Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants," Turkish Journal of Biology: Vol. 30: No. 3, Article 11. Available at: https://journals.tubitak.gov.tr/biology/vol30/iss3/11.

Bafadam, S.; Mahmoudabady, M.; Niazmand, S.; Rezaee, S. A. and Soukhtanloo, M. (2021): Cardioprotective effects of Fenugreek (*Trigonellafoenum-graceum*) seed extract in streptozotocin induced diabetic rats. Journal of Cardiovascular and Thoracic Research, 13(1): 28.

Belfied, A. and Goldberg,D.(1971):Alkaline phosphatase colorimetric method. J. Enzyme.,4: 561-570.

Burlingame, R. W.and Rubin, R. L. (1990): Subnucleosome structures as substrates in enzyme-linked immunosorbent assays. Journal of Immunological Methods, 134(2):187-199.

Chapman, D., Castilla,R.and Campbell, J.(1959): Evaluation of protein in food.I.A.method for the determination of protein efficiency ratiocan J. Biochem.Phesiol.,37: 679-686.

Childs, C. E.; Calder, P. C. and Miles, E. A. (2019): Diet and immune function. Nutrients, 11(8): 193.

Chourasiya, A.; Sahu, R. K. and Khan, M. A. (2019): Anti-Anemic and haemopoietic evaluation of *Trigonella foenum-graecum* (Fenugreek) in rodent model. Journal of Drug Delivery and Therapeutics, 9(4-s): 332-337.

Couteaux, R., Mira, J. C., and D'Albis, A. (1988); Regeneration of muscles after cardiotoxin injury. I. Cytological aspects. Biol. Cell 62, 171–182.

Duhan A, Khetarpaul N and Bishnoi S. (2002); Content of phytic acid and HCL- extractability of calcium, phosphorus and iron as affected by various domestic processing and cooking methods. *Food Chem.* 78:9–14.

EL Hak, H. N. G.; Metawea, S. I. and Nabil, Z. I. (2022): Fenugreek (*Trigonella foenumgraecum, L.*) supplementation safeguards male mice from aflatoxin B1-induced liver and kidney damage. Comparative Clinical Pathology, 1-18.

El- Mahdy, A, and El- Sebaiy,A, (**1982)**: Changes in phytate and minerals during germination and cooking of fenugreek seeds. *Food Chem.;* 9:149–158.

Elghazaly, N. A.; Zaatout, H. H.; Radwan, E. H.; Elghazaly, M. M. and Elsheikha, E. A. (2019): Trigonella Foenum-Graecum Extract Benefits on Haemato logical, Biochemical and Male Reproductive system as a complementary therapy with glimepiride in treating streptozotocin induced diabetic rats System as A Complementary Therapy with Glimepiride in Treating Streptozotocin Induced Diabetic Rats. Journal of Bioinformatics and Diabetes, 1(3): 45.

El-Shimi, N, M, Damir ,A.A and Ragab, M. (1984); Changes in some nutrients of fenugreek seeds during germination. *Food Chem.* 14:11–19.

Gombart, A. F.; Pierre, A. and Maggini, S. (2020): A review of micronutrients and the immune system–working in harmony to reduce the risk of infection. Nutrients, 12(1): 236.

Hooda S, Jood S. (2003); Effect of soaking and germination on nutrient and anti-nutrient contents of fenugreek (*Trigonella foenum- graecum*). *J Food Biochem.*, 27:165–176.

Ivanova, J. I., Birnbaum, H. G., Kidolezi, Y., Subramanian, G., Khan, S. A., & Jacobs, S., Oxley,K. and Demott,W.(2010): Laboratory Test Handbook. Lexi-Comp, Inc. The Journal of Urology.,4:405-405.

Jacobs, S., Oxley,K. and Demott,W.(2001): Laboratory Test Handbook.Lexi-Comp, Inc. The Journal of Urology.,4:405-405.

Khan, F. (2018): Effect of sprouted fenugreek seeds on various diseases: a review. Journal of Diabetes, Metabolic Disorders & Control, 5(4), 119–125. https://doi.org/10.15406/JDMDC.2018.05.00149

Lestienne, I.; Christele, I.V.and Claire M. (2005); Effect of soaking whole and legume seeds on iron, zinc and phytate contents. Food Chem. 89:421–4

Lopez-Amoros, M.L., Hernandez, T. and Estrella, I. 2006. Effect of germination on legume phenolic compounds nd their antioxidant activity. Journal Food Composition and Analysis 19: 277-283.

Lu, X.; Li, N.; Qiao, X.; Qiu, Z. and Liu, P. (2017); Composition analysis and antioxidant properties of black garlic extract. J. Food Drug Anal., 25: 340-349.
Mathur P, and Chaudhary M. (2009): Effect of domestic processing on proximate composition of fenugreek seeds. J Food Sci Technol., 46:255–258.
Maury,C.(1986): Serum TNF determination using ELISA kits.Acta.Med. Scan.,3:220 - 387.

Mehram, E. B.; Salem, A. A.; Alanany, A. M. and Alfauomy, G. A. (2022): The Protective effect of water extract of fenugreek seeds, chicory and olive leaves on some biochemical parameters. Egyptian Journal of Nutrition and Health,17(1):35-54.
Nagamma, T.; Konuri, A.; Nayak, C. D.; Kamath, S. U.;Udupa, P. E. and Nayak, Y. (2019): Dose-dependent effects of fenugreek seed extract on the biochemical and hematological parameters in high-fat diet-fed rats. Journal of Taibah University Medical Sciences, 14(4): 383.
Naidu MM, Khanum H, Sulochanamma G, Sowbhagya HB, Hebbar, UH, Prakash M, and Srinivas P(2012) : Effect of drying methods on the quality characteristics of fenugreek (*Trigonella foenum-graecum*) greens, Dry Technol 30, 808-816.

Ojha, P.;Prajapati, P. andKarki, T. B. (2018): Soaking and germination effect on bioactive components of fenugreek seeds (*Trigonella foenum -graecum* ,L.). International Food Research Journal, 25(2): 690-694.
Parveen, B.; Parveen, A.; Parveen, R.; Ahmad, S.; Ahmad, M. and Iqbal, M. (2020): Challenges and opportunities for traditional herbal medicine today, with special reference to its status in India. Ann Phytomed, 9(2): 97-112.

Pearson, D. (1971): The chemical analysis of food, national college of food technology, University of Readings. J. And A. Churchill.,6(2):179-185.
Reeves, P.G., Nielsen, F.H., and Fahey, G.C., (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. The Journal of Nutrition, 123(11):1939-1951.
Ritz, B. W. and Gardner, E. M. (2009): Nutraceuticals and immune restoration in the elderly. Handbook on Immune senescence: Basic Understanding and Clinical Applications, 1611-1627.

Sarwar, S.; Hanif, M. A.; Ayub, M. A.; Boakye, Y. D. and Agyare, C. (2020): Fenugreek. In Medicinal Plants of South Asia (pp. 257-271). Elsevier.

SAS Institute Inc.Editors, Cary, NC.SAS, (Statistical Analysis System) (2010): SAS User's Guide: Statistics.

Schermer,S.(1967): The Blood Morphology of Laboratory Animal. langmans printed in great britain, green and co ltd.,3:320- 350.

Shakuntala S, Jarpala PN, Thangraj J, Madineni MN and Pullabhatla S. (2011): Characterization of germinated fenugreek (*Trigonella foenum-graecum L.)* seed fractions. *Int J Food Sci.* 2011; 46:2337–2343.

Shalini, H. and Sudesh, J. (2004): Nutritional Evaluation of Wheat–Fenugreek Blends for Product Making. Plant Foods for Human Nutrition 59: 149–154.

Taraseviciene, Z.; Danilčenko, H.; Jariene, E.; Paulauskienė, A. and Gajewski, M. (2009): Changes in some chemical components during germination of broccoli seeds. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37(2): 173-176.

Terrie, Y. C. (2017): Nutritional supplements marketed to boost the immune system. Pharmacy Time, 83(9).

Tewari, D.; Jóźwik, A.; Łysek-Gładysińska, M.; Grzybek, W.; Adamus-Białek, W., Bicki, J. and Atanasov, A. G. (2020): Fenugreek (*Trigonella foenum-graecum, L.*) seeds dietary supplementation regulates liver antioxidant defense systems in aging mice. Nutrients, 12(9): 2552.

Tietz,N.(1976): Fundamentals of Clinical Chemistry. Philadelphia. B.W. Standers, P.243.

Yang, L.; Chen, L.; Zheng, K.; Ma, Y. J.; He, R. X.; Arowolo, M. A. and He, J. H. (2022): Effects of fenugreek seed extracts on growth performance and intestinal health of broilers. Poultry Science, 101(7): 101939.

Yound, D. S. (1975); Determination of GOT. Clin. Chem., 22 (5): 21.