تأثير الحلبة المنبتة والغير منبتة على دلائل المناعة فى فئران التجارب

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المستخلص عربى

صممت الدراسة الحالية لدراسة تأثير بذور الحلبة المنبتة والغير منبتة على رفع المناعة فى الفئران المستحثة بواسطه السيكلوسبورين . 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 (٥٠ملى جرام/كيلو جرام /يوم)المذاب فى زيت الزيتون تحت الجلد للاصابه بخفض المناعه لمدة عشر أيام متتالية ،ثم قسمت الى خمس مجموعات فرعية (٥فئران فى كل مجموعة) ، واحده منها بقيت كمجموعة ضابطة موجبة ،اما المجموعات الفرعية الأربعة الباقية فقد اعطيت نظاما غذائيا أساسيا بالاضافه الى ٥٪, ٧.٥٪ بذور حلبه منبتة وغير منبتة على التوالى واستمرت التجربة لمده ٢٨ يوما . أظهرت نتائج البيانات المتحصل عليها أن نسبة البروتين في الحلبة المنبتة ارتفعت إلى ٤٠.١٣٪ بالمقارنة مع الحلبة غير المنبتة(٢٨.٩١٪) . 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 الى 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 المناعية وخاصة الحلبة المنبتة بنسبه ٧.٥٪ تليها ٥٪.

الكلمات المفتاحية :السيكلوسبورين، 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 aim of this study was to explore the impact of germinated and non-germinated fenugreek seeds on enhancing immunity in rats induced by cyclosporine(CsA) . 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 (BD) as a negative control group, while the second group (25rats) injected with a daily dose of CsA(٥0mg/kg/day) dissolved in olive oil subcutaneously for ten consecutive days to induce immunosuppressed rats and further subdivided into five equal subgroups, one of them kept as a positive control group, while the left four subgroups were given a basal diet with 5 % and 7.5% of germinated and non-germinated fenugreek for 28 days, respectively .The findings indicated that the protein content in germinated fenugreek increased significantly to 40.13% compared to 28.91% in non-germinated seeds.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 seeds 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, 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).**Long-term

stress and psychological strains may cause immunological deterioration, simulating how aging affects immune function (**Alsudairy *et al*.,2022)**.Additionally, the immune system is harmed by immunosuppressive drugs, immunologically mediated disorders, chronic stress, sleep deprivation, and certain medical conditions **(Terrie, 2017). c**yclosporine(CsA)therapy may suggest attacking T cells, dendritic cells, neutrophils, and macrophages all at the same time. Additionally, the calcineurin-NFAT pathway influences the course of immunological responses by promoting the expression of genes related to inflammation and homeostasis. In innate immune cells, it is active. Furthermore, the formation of danger-associated molecular patterns by the mitochondria, which trigger several innate immunological signaling pathways, can be inhibited by CsA **(Liddicoat and Lavelle, 2019).** Food and nutrition are vital for preserving a long-term health and averting chronic illnesses. A healthy immune system depends on nutrition, and both prolonged malnutrition and overfeeding can impair immunological activation (**Lange,2021).**The likely immunomodulating ingredients in these items are nutraceuticals derived from plants. **(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),**determined that germination greatly impacted the bioactive components and antioxidant activity, fenugreek seeds offer greater health potential than non-germinated ones. In addition, tryptophan is a precursor to the neurotransmitter serotonin, the hormone melatonin, and vitamin B3. Germinated seeds are a good source of essential amino acids, in particular leucine, lysine, and tryptophan, which are used in the biosynthesis of proteins and have unique functions in "anchoring" membrane proteins within the cell membrane **(Tewari *et al*., 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 some immune indicator 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.CsAwas 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.The Institutional Animal Care and Use Committee (IACUC) of Menoufia University provided ethical consideration for this study (Reg. No., MUFHE /F/ NFS/27 /23).

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 Immunosuppression:

Thirty (30) male albino rats weighing (160±10 g) were received cyclosporine (50 mg/kg/day) subcutaneously in olive oil for ten days according to **(Elsayed *et al.*,2016) .**

Experimental Designs :

After a week of recuperation, rats were kept in a controlled environment and fed a basal diet according to AIN-93 guidelines **(Reeves *et al.*, 1993)** in an animal laboratory at Menoufia University's Faculty of Home Economics. Rats were then divided into six groups, each of 5 rats, with weight means that were almost identical for each group.The experimental diet was supplied to each group of rats for 28 days while they were kept in wire cages.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 byCsA(50mg/kg/day)for ten days to induce immunosuppressed , then divided into five subgroups one of them kept as positive control group , while the left four subgroups were given basal diet with 5 % and 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:

To perform a biological evaluation of the different diets, the following formulae were used to calculate the feed intake daily, body weight gain(BWG), and feed efficiency ratio (FER) according to **Chapman *et al.* (1959)**:Body Weight Gain(g) = Final weight (g) - Initial Weight (g)

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

Biochemical analysis:

Samples of blood were collected from the portal vein and put into sterile, dry centrifuge tubes in order to separate the serum. The serum was separated from the blood samples by rotating them for 10 minutes at 3000 rpm. Serum samples were refrigerated at -20 °C until chemical analysis 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-α 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:

A computerized costat program was used to statically analyze the data using a one-way ANOVA. The results are displayed as mean ± SD.Treatment differences were considered significant when they were $p < 0.05$ **(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, 41.75 g/100g and 339.94 kcal., respectively. These results are in the same trend as **Taraseviciene *et al.*, (2009**) who showed that the 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. This is because 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. 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 theserise (**Hooda and Jood, 2003).** Also, **Bewley *et al.*,(2013)** reported that during germination, seed storage proteins are broken down into amino acids, which are used to build new proteins needed for seedling growth. This process, along with the synthesis of new proteins, increases the protein content of germinated seeds. However, the extent of this rise varies depending on the stage and duration of germination. **Hooda and Jood, (2003)** found a similar pattern of fenugreek seeds' lipid content decreasing following soaking so germination of fenugreek seeds caused decrease in fat content as compared to raw seeds.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)**. **Mathur and Chaudhary(2009); 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 mean ± standard deviation represents the values.Values with different superscript letters in the same row are statistically different ($p \leq 0.05$).

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.**El-Shimi *et al.* (1984)** showed a decrease in iron content during fenugreek seed germination. According to **El-Mahdy and El-Sebaiy (1982)**, a decrease in phytates, tannins, and other anti-nutritional factors that bind the minerals may be the cause of an increase in Ca and P.**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.As a result of zinc leaking into the soaking solution, phytase activity elevated during germination, reducing phytic acid in germinated seeds. Phytases, also known as myo-inositol hexaphosphate phosphohydrolases, are enzymes that increase the availability of divalent minerals in vitro by hydrolyzing myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) into myo-inositol and inorganic phosphate

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]

The mean ± standard deviation represents the values. Values with different superscript letters in the same row are statistically different ($p \leq 0.05$).

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 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.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]

The mean ± standard deviation represents the values. Values with different superscript letters in the same row are statistically different ($p \leq 0.05$).

Data presented in **Table (4**) showed the effect of germinated and non-germinated fenugreek seeds on BWG, FI and FER. It was noticed that Immunosuppression rats had a significant decrease in the BWG, FI and FER as compared to the normal rats.Studies suggested that immunosuppressive drugs like cyclosporine may not significantly alter the morphology or cell density of hypothalamic nuclei involved in appetite regulation**(Schuh *et al.*, 2022).**However, the overall impact on feeding behavior remains significant, as these drugs can still influence appetite through neurochemical pathways, including those involving proinflammatory cytokines that induce anorexia**(Aou, 2001).**Administration with fenugreek in all groups expect group fed on 5% non- germenated fenugreek significantly ($P \leq 0.05$) showed a significant increase in FI, FER and BWG compared to 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 BWG, FI and FER of experimental rats.

Parameter / Groups	BWG (g// 28 day)	FI (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 ($P \leq 0.05$).BWG stands for body weight gain , FI : feed intake, FER :feed efficiency ratio.

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, It was noticed that immunosuppression rats had a significant decrease in IGA and IGM as compared to the normal rats . Germinated fenugreek showed a dose-dependent improvement in inflammation and immunological indicators among the treatment groups. IgM (108.56 mg/dl) and IgA (144.66 mg/dl) levels were close to normal in the group that received 7.5% germinated fenugreek (G6), whereas TNF-α (127.35 mg/dl) was significantly lower than in the positive control group. Interestingly, this group showed the best recovery pattern, confirming that germinated fenugreek has superior immunomodulatory and anti-inflammatory properties. That mean TNF-α had the opposite trend. This is matching with that a 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-***α*** compared to the group exposed to gasoline fumes.

Table (5): Effect of germinated and non-germinated fenugreek seeds on serum IGM, IGA and TNF-*α* levels of experimental rats.

Parameter / Groups	IGM mg/dl	IGA mg/dl	*TNF-α* 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 ($P \leq 0.05$).Ig A: Immunoglobulin A, Ig M: Immunoglobulin M, ;TNF-α: Tumor necrosis factor –α.

Data presented in **Table (6)** showed the effect of germinated and non-germinated fenugreek on RBC , WBCs, Lymphocytes ,HB and platelets levels of experimental rats.It was noticed that immunosuppression rats had a significant decrease in lymphocytes, RBCs and HB as compared to the normal rats. This decline results from cyclosporine's ability to suppress the immune system by inhibiting lymphocytes and other hematological parameters. Intervention with fenugreek seed formulations, particularly in germinated form, showed a dose-dependent ameliorative effect on these parameters. Rats receiving 7.5% germinated fenugreek displayed the most pronounced improvement, with red blood cell and hemoglobin levels approaching normal, reduced leukocyte and platelet counts, and a restoration of lymphocyte percentages. These findings suggest that germination enhances the bioactive profile of fenugreek seeds, increasing their efficacy in modulating hematopoietic and immune responses.In case of WBCs and PLT, It was noticed that immunosuppression rats had a significant increase in WBCs and PLT as compared to the normal rats.**Thomason *et al*.,(2012)** indicated that CsA, especially at immunosuppressive doses, changes the platelet plasma membrane and promotes the synthesis of thromboxane in dogs, much like what is seen in people.Administration with germinated and non germinated fenugreek significantly ($P \leq 0.05$) showed a significant decrease in WBCs and PLT compared to the positive control group.The best result showed in group (6) immunosuppression rats fed on basal diet

with 7.5% germinated fenugreek. 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.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 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 RBCs, WBCs,HB, Lymphocytes and PLT levels of experimental rats.

Parameter / Groups	RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	Lymphocytes	HB g/dl	PLT 10^6/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^{e}± 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%Nongerminated 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^{c} ± 0.67
G_5:7.5%Nongerminated fenugreek	3.65 ^{d} ± 0.015	10.15 ^{c} ± 0.10	88.13 ^{b} ± 0.38	10.33 ^{c} ± 0.18	303.06^{b} ± 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^{d} ± 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 ($P \leq 0.05$).RBCs: Red Blood Cells. WBCs: White Blood Cells, Haemoglobin.: HB, Platelets: PLT.

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.The present study's CsA-treated groups displayed significantly higher serum levels of the hepatic enzymes AST, ALT, and ALP. This could be because these enzymes are released from the cytoplasm into the bloodstream quickly after plasma membrane rupture and parenchymal cellular damage **(Aladaileh *et al*., 2020)**.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. .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 AST, ALT and ALP enzymes of experimental rats.

Parameter / Groups	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 significantly enhances the nutritional and health-promoting properties of fenugreek seeds by increasing protein, mineral bioavailability, especially calcium, phosphorus, magnesium and antioxidants. In our study, germinated seeds improved body weigh gain, blood parameters, and immune response while reducing inflammation. This highlights germination as a simple, low-cost efficient biotechnological method to use for improving the nutritional value overall.

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