تأثير الحلبة المنبتة والغير منبتة على دلائل المناعة فى فئران التجارب

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المستخلص عربى

صممت الدراسة الحالية لدراسة تأثير الحلبة المنبتة والغير منبتة على مؤشرات المناعة وغيرها من المتغيرات البيوكيميائية في الفئران . تم استخدام ثلاثين(٣٠) من ذكور الفئران البيضاء نوعها ألبينو 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 المعاملة لمده ٢٨ يوما . وفى نهايه التجربه تم وزن الفئران ثم ذبحهم وتجميع عينات الدم بعد الصيام ١٢ ساعه .تم تحديد التركيب الكيميائي ، و مضادات الأكسدة ، و الفينولات الكلية ، والكاروتين ، و التقييم البيولوجي ، إنزيمات الكبد ومؤشرات 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 وNa . كان للإنبات نسبة عالية من النشاط المضاد للأكسدة والفينول الكلي والكاروتين مقارنة بالبذور غير المنبتة . أظهر تقييم زيادة وزن الجسم ونسبة كفاءة التغذية وكمية الغذاء المتناول أن جميع المجموعات المعالجة كانت أعلى من المجموعة

الموجبة الضابطة مما أظهر فرقاً معنوياً. 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٪.

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

Abstract

The present study was investigated to study the effect of germinated and non-germinated fenugreek seeds on immune indicators and other biochemical parameters in rats. Thirty (30) male albino rats weighing (160 ± 10 g), were used. 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 category (25rats) were injected with Cyclosporine(50mg/kg/day) dissolved in olive oil subcutaneously for ten consecutive days to induce immune deficiency, 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. Chemical composition, Antioxidant activity, total phenol, Carotene, biological evaluation, liver enzymes and immune indicators were determined. The results of the obtained data showed that the percentage of protein in the germinated fenugreek increased to 40.13% when compared with non-germinated fenugreek. Also, germination caused increase in the mineral P, Cu, Ma, Ca, 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. Evaluation of body weight gain, feed efficiency ratio and feed intake showed that all treated groups were higher than control positive group showing a significant difference. The 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 has been found to have important bioactive compounds. It has a vital role to improve immunity, especially germinated fenugreek 7.5% followed by 5%.

Keywords: Cyclosporine, Fenugreek, germination, Antioxidant activity, rats, immunity.

Introduction

Immune system uses the body's own defense mechanisms to guard against damage, disease, and infections **(Childs *et al*., 2019).** Composed of various tissues, cells, and proteins, the immune system is a highly complex and multifaceted system, forming an intricate network of cells and proteins that moves throughout the human body via the lymph stream and blood stream. A healthy immune system is able to distinguish between body cells (self) and foreign materials (non-self), eliminating the latter **(Terrie, 2017)**.

Immunity decreases and morbidity increases with increasing age **(Ritz and Gardner, 2009)**, deficiency of zinc, selenium, iron, copper, or vitamins A, C, E, B6, or B9, overeating and being overweight or obese may also hamper the immune response. Moreover, chronic stress, lack of sleep, specific medical conditions, immunosuppressive agents, or immune-mediated diseases damage immune system **(Terrie, 2017)**.

Nutraceuticals obtained from plants act as the probable immunomodulating agents in such products. Phytochemicals (flavonoids, folate, polyamines, alkaloids, terpenoids) and other, essential nutrients (mainly carbohydrates, proteins, fatty acids, minerals, and vitamins) play an important role in maintaining a balance between health and disease **(Parveen *et al*., 2020)**.

The importance of including medicinal herbs in the dietary choices is well-known for maintaining the health lifestyle and preventing several types of diet-related ailments including immune Tumour and cardiovascular diseases **(Thorat and Gaikwad, 2019).**

Fenugreek is known as one of the plants having all these traits and an eminent plant crop used in human diets. Fenugreek *(Trigonella foenum-graecum, L*.) belongs to the Fabaceae family and has been used as an important spice since ancient times **(Aasim *et al*., 2018).** Fenugreek seeds and leaves contain fiber, protein, beta-carotene, vitamins, minerals, gums, alkaloids, flavonoids, steroidal sapogenins, dysgenic, trig coumarin, nicotinic acid, trim ethyl coumadin, and trigonelline **(Sarwar *et al*., 2020).**

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 biosynthesis of proteins, and play special roles in "anchoring" membrane proteins within the cell membrane as well as tryptophan is also a precursor to the neurotransmitter serotonin, the hormone melatonin, and vitamin B3 **(Tewari *et a*l., 2020).**

Nagamma *et al*., (2019), suggested that Fenugreek seed extract significantly improves level of blood counts such as MCV, MCH, MCHC, red blood cell distribution width, hemoglobin (Hb), hematocrit, and platelet count in rats with High fat diet-induced obesity. 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, it has been identified by Crop department, Faculty of Agriculture Menoufia University.

Cyclosporine was obtained from an El-Gomhoria company for Trading Drugs, Chemical and Medical Instruments, Cairo, Egypt.

Olive oil was obtained from the herbal store in Shebin El-Kom.

Thirty adult normal male albino rats Sprague Dawley strains their weighing were 160±10g and were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.

Methods:

Preparation of germinated fenugreek:

Fenugreek seeds were cleaned, graded, sorted and washed three times using potable water. Then, the seeds were soaked in potable water for 24 hrs. at room temperature (22±2C°) with a seed: water ratio of 1:5 (w/v). The unimpeded water was discarded and the soaked seeds were rinsed twice by boiled cooled water to avoid post contamination during germination. The soaked seeds were germinated in plastic sieves covered with sterilized cloth for 72 hrs. at room temperature with frequent watering. The germinated fenugreek seeds were dried in a drying oven (at the Faculty of Agriculture, Menoufia University) at 40C° for 24 h **(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).

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. **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 category (25 rats) were by the previous method to induce immune deficiency, 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.

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:

Biological evaluation of the different diets was carried out by determination of feed intake daily, body weight gain g (BWG g /day) and feed efficiency ratio (FER) according to **(Chapman *et al*., 1959) using the following equations**:

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

Chemical composition of germinated fenugreek as compared to non-germinated fenugreek seeds are 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 10.76, 40.13, 1.38, 3.74, 2.34, 41.75g/100g and 339.94 kcal respectively. These results are in the same trend of **Taraseviciene *et al.*, (2009**) who showed that the crude protein content of the fenugreek seeds ranged from 26.10 to 29.89%. The crude protein content of fenugreek seeds increased with germination time. The highest crude protein recorded with 72 h germination time and the lowest is 24 h germination time. This is because germination is a biotechnological process, in which metabolic enzymes, such as proteinases, are activated. 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. As a consequence, the nutritional quality of proteins can be enhanced, that is why the germination process is suggested as a technological procedure for improving the nutritional quality of legumes and other seeds**.** A similar trend in decrease in fat content in fenugreek seeds after soaking has been observed by **Hooda and Jood (2003)**. Total carbohydrates (44.8 %) decreased but marginally. This decrease might be attributed to enzymatic degradation of seeds during soaking **Mathur and Chaudhary (2009)**. Decrease in dietary fiber content after soaking has also been previously reported by **Hooda and Jood,2003)**. Germination of fenugreek seeds caused decrease in fat content as compared to raw seeds. Loss of fat during germination may be due to its consumption as an energy source in the process of germination **Mansour and El-Adway(1994**).**El-Aal (1986)** reported decrease in total fat content along with decrease in free fatty acids, monoglycerides and polar lipids upon germination. The protein content increased from 32.7–41.2 % after germination. This increase might be due to reduction of seed nitrates into protein or ammonium compound **Hooda and Jood (2003).** Increase in protein content of germinated seeds might be attributed to enzymatic synthesis of protein, which is in consent with the findings of **Mansour and EL-Adway (1994). Mathur and Chaudhary(2009)** and **Amankwah *et al.*, (2009)** reported that the observed decrease in ash content of fenugreek flour samples during germination might be due to leaching of minerals during steeping and washing, the removal of moisture generally increased concentrations of nutrients 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	10.76 ±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	1.74 ±0.86 [a]	2.97 ±0.54 [b]
Ash	2.34 ±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, cobber, magnesium, calcium and zinc were high in germinated seeds as compared to non-germinated seeds while germination led to decrease the mean values of potassium, sodium and iron. Gupta et al. indicated that germinated fenugreek seeds contained Ca, P and Mg in high concentrations and low level of Fe were as 0.36 g/kg in the seeds. **Duhan *et al.***, (**2002)** found that germination caused the mineral Zn and Fe content decreased whereas other minerals (Ca and P) increased significantly. Decrease in Fe content in germinated fenugreek seed flour might be due to leaching of Fe in to soaking medium. Decrease in Fe content during 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 forms complexes with essential dietary minerals such as Ca, Fe, Zn and Mg makes them biologically unavailable for absorption. The phytase activity increased on germination causing catabolism of phytic acid germinated seeds is due to leaching of Zn into 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 increasing 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 significantly had high content of the determined parameter as compared non-geminated 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 of 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 increasing was due to the endogenous enzymes of the legumes are activated and the most important enzymes are the hydrolases and polyphenol loxydases, whose activity increases during germination. Also, **Aqil *et al.***, (**2006).** reported that the extract of fenugreek seeds had 74.33±5.13 mg GAE/g total phenolics and showed 57.45±2.44 % inhibition by DPPH method. **Lopez-Amorosa *et al*., (2006)** revealed that quantitative and qualitative change of phenolic compounds during germination influence the antioxidant property. Legumes contain other bioactive compounds beside phenolic such as vitamins and carotenoids at different concentrations that might also behave as antioxidant. These compounds might also exert synergetic activities among themselves and with phenolic compounds, which could be the main reason of the observed differences in the antioxidant activities.

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 levels results showed that significant changes as compared with both of controls, the changes to negative control group were decreased while were increased to positive control expect the group with 5%. In 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 control positive was lower than control negative. Treated groups showed significant increases in mean value as compared with control positive expect 5% non-germinated fenugreek showed non-significant as compared with 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 chicken compared to the initial weight. also**, Amein *et al.*, (2019**), found that fenugreek inclusion in the broiler diets could increase feed conversion efficiency, enhance metabolic processes, and optimize nutrients utilization. This observation can be attributed not only to the availability of essential fatty acids and high-quality proteins in fenugreek, but also to the presence of steroid saponins, which activate the hypothalamus gland, enhance feed intake, and stimulate the digestive system.

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**

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (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 experiment rats. In case of IGA and IGM, the highest levels recorded for 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 7.5% germinated fenugreek group, the mean value 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 recorded the lowest value.

In 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**

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (P ≤ 0.05). IGM: Immunoglobulin M. IGA: Immunoglobulin A. TNF: Tumor Necrosis Factor.

Data presented in **Table (6)** showed the effect of germinated and non-germinated fenugreek on serum red blood cells (RBCs) and white blood cells (WBCs) levels of experiment rats. It was found that the lowest value of

RBCs levels recorded for positive control group it was 3.25 10^6 /mm^3. RBCs values significantly (P≤0.05) increased in all treated groups, where 7.5% germinated fenugreek group recorded the highest value of mean, and it was 4.05 106/mm3 comparing to other treated groups. In case of WBCs, negative control group recorded the lowest value was by 5.68 10^3/mm^3. It was evident that Cyclosporine injection for ten days without treatment raised the levels of WBCs in infected rats to the highest value of 12.88 10^3/mm3 in positive control group. Treated groups by Cyclosporine with germinated and non-germinated fenugreek resulted in a decrease in the level of WBCs. The best improvement appeared in 7.5% germinated fenugreek group was by 9.07 103 /mm3. As for lymphocytes treating immunocompromised rats with fenugreek seeds, whether germinated or not showed significant (P≤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≤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≤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, 281.88 106 /mm3, respectively. These results agreed with **Chourasiya *et al*., (2019),** who reported that fenugreek extract significantly(P≤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 RBCs, 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 RBCs and haemoglobin, an improvement in the levels of WBCs and decreased platelets levels in rats induced by AlCl3. 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**

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (P ≤ 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 positive control group recorded the highest value of serum AST, ALT and ALP

comparing to 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 with positive control group. Group 7.5% germinated fenugreek had ahigh 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 serum liver enzymes levels (AST, ALT and ALP) on immune system of experiment 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**

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (P ≤ 0.05). AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. ALP: Alkaline phosphatase.

Conclusion

It may be inferred from the present study that nutritional and therapeutic quality of fenugreek seeds can be improved through processing methods soaking and germination and roasting.

the percentage of protein, vitamin C, antioxidants, total phenols, and carotene increased than dry fenugreek seeds. Therefore, it is recommend eating the germinated fenugreek to raise immunity.

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