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Chemical and Immunological Studies of Green Fenugreek Powder and Silkworm Powder and Their Role in Improving Immunity and Blood Characteristics in Diabetic Rats.

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KEYWORDS

Immunological effects- Chemical properties - green fenugreek and Silkworm powder - Diabetic rats.

ABSTRACT:

Immunity is the body's resistance to disease, achieved through an immune system that defends against harmful agents like pathogens. Green fenugreek powder and silkworm powder can enhance immunity by stimulating immune responses through various active components and peptides. Research indicates silkworm pupa protein and its derivatives can boost immune cell function.

Aim: This study aimed to determine the effect of green fenugreek and silkworm powder in improving the immunity and blood characteristics in diabetic rats.

Materials and Methods: Thirty male Sprague dawley rats were divided into normal (n=6) and diabetic (n=24). The second group was injected with streptozotocin (at a dose of 75 mg/ kg intraperitoneally), and those with blood glucose concentrations more than 250 mg/dL were classified as diabetic. Rats were divided into six groups, (1) non-diabetic group (n=6) received distilled water (2 mL/ day); (2) diabetic control group (n=6) received distilled water (2 mL/ day); (3) diabetic group (n=6) received basal diet +5% green fenugreek; (4) diabetic group (n=6) received basal diet +5% silkworm; (5) diabetic group (n=6) received basal diet +10% silkworm. After 28 days, blood was collected, and serum was extracted to determine Blood Parameters (RBC, Hgb, MCV, RDW, HCT, MCH , MCHC,WBC, LYM).

Results: At the end of the experiment, the best results of RBC, Hgb, HCT, MCH, WBC, LYM, MiD, and GRAN were found in group 3 (5%) green fenugreek powder. This is likely due to the high nutritional content of green fenugreek powder.

Conclusion: Green fenugreek powder and silkworm powder show potential in enhancing immunity and blood health in diabetic rats. These findings suggest that incorporating these natural supplements into diabetic treatment plans could offer a complementary approach to managing the condition. By improving immune function and blood health.

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Introduction

Immunity is the body's resistance to disease, achieved through an immune system that defends against harmful agents like pathogens. There are three main types of immunity: innate (natural defenses like skin), adaptive (developed through exposure or vaccination, creating specific antibodies), and passive (receiving antibodies from another source, providing temporary protection). A healthy lifestyle, including a balanced diet, exercise, and vaccinations, can support immune function **(Zheng *et al.,* 2020)**. Silkworm powders may support the immune system owing to their anti-inflammatory and antioxidant properties, though research is still limited. Studies suggest that the bioactive compounds in these powders can help regulate blood sugar, improve metabolic health. **(Ohnishi *et al.,* 2021)**. Silkworm powder is primarily composed of protein, with an elevated content of essential amino acids. It also contains significant amounts of fat and ash, along with various minerals like calcium, potassium, and magnesium, and a range of vitamins including riboflavin, nicotinic acid, and vitamin E. The powder includes other compounds such as fiber, antioxidants (phenolic acids and flavonoids), and polysaccharides like chitin**. (Arunkumar *et al.,* 2016).** The vast array of nutrients and bioactive substances in fenugreek is essential for enhancing general well-being and the effectiveness of biological systems. Fenugreek seeds possess a protein level of twenty-two percent to twenty-six percent, alongside a carbohydrate composition of fifty-eight percent, a fiber content of twenty-five percent, and a lipid content of 0.9 percent. The fenugreek leaves contain six percent carbohydrates, 4.4% protein, and 1.1% fiber **(Gozde et al., 2019)**. Silkworm powder possesses numerous functional qualities that can be employed in the food and pharmaceutical sectors. Current years have revealed numerous bioactive compounds in silkworm pupae with possible activities and health advantages. Silkworm proteins and hydrolyzed peptides possess multiple roles, such as enhancing immunity, exhibiting anticancer properties, and demonstrating antioxidant activity **(Sumranpath et al., 2015)**. Silkworms constitute a significant insect resource for application in medicines and other healthcare items.

Although certain active compounds in silkworm pupae have been recognized, their pharmacological effects remain ambiguous. Comprehensive study indicates that silkworm pupae possess significant potential for application in the medicinal sector. **Manosroi et al., 2020**. Fenugreek (Trigonella foenum-graecum) has been regarded as beneficial for health since ancient times. The seeds comprise numerous bioactive compounds, including nitrogenous compounds, polyphenols, vitamins, free sugars, carbohydrates, inorganic elements, proteins, mucilage, and lipids. **(Gupta et al., 2018).** Prior research has proven the advantageous effects of fenugreek extract on antidiabetic, hypolipidemic, antiplasmodic, anthelmintic, antibacterial, analgesic, and anti-inflammatory properties. It possesses antifertility, anticarcinogenic, antiulcer, antioxidant, and immunomodulatory properties, and functions as a regulator of enzymatic pathways **(Kaviarasan et al., 2006)**. Fenugreek had a comprehensive stimulatory influence on both nonspecific and specific immunological activities in mice. Stimulatory influences have been noted at a dosage of 100 milligrams/kilogram body weight and, in few cases, at 250 milligrams/kilogram **(Khalil et al., 2004).** Fenugreek powder contains vitamins (C), (A), iron, and zinc, which are crucial for a healthy immune system. Research indicates that fenugreek can stimulate both the specific (humoral) and non-specific (cellular) immune responses. It helps to regulate the body's immune functions, and its antioxidant properties protect against cellular damage that can weaken the immune system. **(Megha et al., 2012).** Animal studies show that fenugreek can increase resistance to infections and show a higher survival rate when fed a fenugreek-supplemented diet. Influences of dietary taking of fenugreek seeds on metabolic ...Additionally, fenugreek upregulated the immune-related gene expressions in kidney & liver (TNF-α and interlekin-1β) **(Zhou *et al.,* 2022).**

2- AIM OF STUDY: -

This research aimed to determine the influence of green fenugreek and silkworm powder on improving the immunity and blood characteristics in diabetic rats.

3- MATERIALS AND METHODS: -

A- Source of **silkworm powder:** Silkworm cocoons purchased from Alibaba.com, cleaned, blended, & ground into fine powder utilizing an electric grinder. To

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reduce oxidation, they were stored in dark-stoppered glass bottles until ready to be used. **(Russo, 2001).**

B- Source of **green fenugreek:** green fenugreek was purchased from Al-Baha City, KSA, local market, washed, cleaned, blended, & ground into fine powder utilizing an electric grinder. To reduce oxidation, they were stored in dark-stoppered glass bottles until ready to be used. according to **(Russo, 2001).**

C -Rats: Thirty male Sprague Dawley rats (*n*=30) weighing 150-170 g were obtained from Egypt's Ministry of Health's Animal Unit at Helwan Farm. For two weeks, the rats were housed in individual plastic cages under controlled conditions, with a temperature of 22°C and a 12-hour light/dark cycle at the Faculty of Home Economics, Menoufia University, Egypt. The rats had unrestricted access to food and water. All experiments followed the National Institute of Health's Guiding Principles for Animal Care and Use. Rats were weighed after two weeks of acclimatization and randomly allocated to one of two groups: diabetic (30rats) and normal (6 rats)

D- Induction of Diabetes (T1DM): After two weeks of acclimatization of rats, type 1 diabetes mellitus was induced by intraperitoneal injections of Streptozotocin (STZ) as described previously. The rats were injected with a dose of 75 mg/kg intraperitoneally of Streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO, USA). Following this, all rats fasted for 8 hr, and then blood samples were taken from the retro-orbital veins to determine blood glucose concentrations. The study included diabetic rats with blood glucose concentrations more than 250 mg/dL. Following the exclusion of rats with blood glucose concentrations below 250 mg/dL and deceased rats, 24 rats were included in the study and subsequently developed diabetes. In addition, diabetic rats

E- Diets:

- Basal diet: The basal diet comprises protein (10%), corn oil (10%), choline chloride (0.2%), cellulose (5%), combination of vitamin (1%), salt combination (4%) **(Hegested et al., 1941),** & corn starch (to one hundred percent). in accordance with **AIN (1993)**

F- Experimental Design

The study included all normal (6 rats) and diabetic (24 rats). In addition to the experimental procedure, all rats involved in this investigation were fed the standard diet. The proposed interventions were orally administered once per day. **AIN., (1993)** The weights of the rats were also recorded, and diabetic rats have divided into experimental groups accordingly. The following were the experimental groups:

1- The non-diabetic group (ND-Gr) consisted of six normal rats that received a daily 2 mL of distilled water orally per rat once daily.

2- The diabetic control group (DC-Gr) consisted of six diabetic rats that received a daily 2 mL of distilled water orally per rat once daily

3- The diabetic group (DC-Gr), consisting of six rats, received basal diet + 5% green fenugreek

4- The diabetic group (DC-Gr), consisting of six rats, received basal diet + 5% silkworm powder

5- The diabetic group (DC-Gr), consisting of six rats, received basal diet + 10% silkworm powder.

H- **Complete blood count (CBC) test**

The test involves RBC count, WBC count, HB, and platelet count. The outcomes of a CBC are produced by sophisticated electronic and pneumatic analyzers utilizing aperture-impedance and/or laser beam cell size and counting techniques. (**Jacobs et al., 2001).**

I- Statistical analysis:

The Student-Newman-Keuls test has been applied to separate the means following a significant main influence was discovered. The data has been examined via an entirely randomized factorial design [SAS, 1988]. Treatment variances (P0 value less than 0.05) have been deemed significant by the Costat Program. Analyses of biological outcomes have been performed utilizing one-way ANOVA. **(1967, Snedecor and Cochran)**

- **Ethical Approval**

The Science Research Ethics Committee of the Faculty of Home Economics accepted the protocol of the research #15-SREC-06-2025.

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4- RESULTS AND DISCUSSION

- **RESULTS**

4.1. Chemical composition of green fenugreek and silkworm powder:

The chemical composition of green fenugreek and silkworm powder are showed in Table 1. The outcomes have illustrated that the powder of green fenugreek contains ash, carbohydrates, fat, moisture, fiber, protein, and energy value. The dry weight (D/W) was 1.2, 50.5, 7.95, 8.6, 20.2, 12.05, and 319.75 kilocalories per one hundred grams, respectively.

Silkworm powder contains ash, carbohydrates, fat, moisture, fiber, protein, and energy value. The dry weight (D/W) was 4.39, 7.31, 13.5, 7.4, 6.1, 61.3, and 395.94 kilocalories per one hundred grams, respectively.

Table (1): Chemical composition of green fenugreek and silkworm powder

Constitutes (%)	**Green fenugreek powder**	**Silkworm powder**
Ash	1.2	4.39
Carbohydrates	50.0	7.31
Fat	7.95	13.5
Moisture	8.6	7.4
Fiber	20.2	6.1
Protein	12.05	61.3
Energy value (Kcal/l00g)	319.75	395.94

DW= Dry weight

4.2. Immunological results.

- **Blood Parameters**

Table (2) illustrates the effects of green fenugreek and silkworm powder on RBC, Hgb, MCV, RDW, HCT, MCH and MCHC in diabetic rats.

As illustrated the mean value of RBC of control positive was significantly lower (p below 0.05) when than control negative which were 1.10±0.18 and 5.30±0.15 m/ul, correspondingly Whereas the value of all the experimental groups 5% fenugreek and (5%, 10%) silkworm powder was significantly greater in comparison with control positive by mean 3.63±0.11, 1.38 ±0.03, and1.31±0.02 m/ul, respectively.

At the same time Hgb of control positive group was significantly lower (p below 0.05) as compared to control negative rats being 10.10±0.37 and 15.52±0.50 g/dl, respectively, Also the value of all the experimental groups 5% fenugreek and (5%, 10%) silkworm powder was significantly greater when compared to control positive rats by mean 16.90±1.15 16.00±1.00, and 16.50±0.62 g/dl, respectively.

As illustrated in table (2) the mean value of MCV of the control positive group was significantly greater (p below 0.05) than the control negative group, which were 65.96±4.61 and 58.62±3.16 fl, correspondingly. Also, the values of all the experimental groups 5% fenugreek and (5%, 10%) silkworm powder were significantly lower as than control positive being 40.95±3.00, 53.84±3.41, and 51.35±2.90 fl, respectively.

As illustrated in table (2) the mean value of RDW of control positive rats was significantly greater (p below 0.05) in comparison with control negative 20.42±0.52 and 13.22±0.69 %, correspondingly. Also, the values of all the experimental groups 5% fenugreek and (5%, 10%) silkworm powder were significantly reduced than control positive being 9.96±0.50 10.70±0.61, and 10.55±0.51 %, respectively.

As illustrated in table (1) results, the mean value of HCT of control positive rats was significantly lower (p below 0.05) when than the control negative group, 17.00±0.53 and 30.40±1.51 %, correspondingly. Also, the values of all the experimental groups 5% fenugreek and (5%, 10%) silkworm powder were significantly greater when than control positive group which were 29.74±1.55 20.00±1.00, and 21.00±2.11%, respectively.

The mean value of MCH of control +ve was significantly lower (p below 0.05) than control negative, which were 9.10±0.29 and 17.20±0.22 pg, correspondingly. Also, the value of all the experimental groups 5% fenugreek and (5%, 10%) silkworm powder was significantly greater when than control positive which were and 18.22±0.33 ,14.00±0.19, and 16.40±0.51 pg, respectively.

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As illustrated in table (2) the mean value of MCHC of control +ve was significantly lower (p below 0.05) than control -ve rats which were 6.40±1.22 and 12.50±0.70 g/dl, correspondingly. whereas, the value of all the experimental groups 5% fenugreek and (5%, 10%) silkworm powder was significantly greater when than control positive by mean 13.50±0.52, 14.15 ±0.72 and 14.00±0.79 gram per deciliter, respectively. It could be noticed (Table 2) that the best result of RBC, Hgb, HCT and MCH were found in group 3 (5%) green fenugreek powder. This is likely owing to the great nutritional content of green fenugreek powder, which is rich in essential minerals and vitamins like iron, folic acid, and vitamin C. These nutrients are crucial for the production and health of red blood cells, directly influencing parameters like red cell distribution width (RDW), mean corpuscular volume (MCV), hemoglobin (Hgb), hematocrit (HCT), mean corpuscular hemoglobin (MCH), The 5% concentration may provide an optimal balance, enhancing these blood markers

Table (2): Effects of green fenugreek and silkworm powder on RBC, Hgb, MCV, RDW, HCT, MCH and MCHC in diabetic rats

Group Parameter	Control (-ve)	Control (+ve)	5% green fenugreek	5% silkworm powder	10% silkworm powder
RBC (m/ul)	5.301±0.15	1.101±0.18	3.631±0.11	1.381±0.03	1.311±0.02
Hgb (g/dl)	15.521±0.50	10.101±0.37	16.901±1.15	16.001±1.00	16.501±0.62
MCV(fl)	58.621±3.16	65.961±4.61	40.951±3.00	53.841±3.41	51.351±2.90
RDW(%)	13.221±0.69	20.421±0.52	9.961±0.50	10.701±0.61	10.551±0.51
HCT(%)	30.401±1.51	17.001±0.53	29.741±1.55	20.00±1.00	21.00±2.11
MCH(pg)	17.201±0.22	9.101±0.29	18.221±0.33	14.001±0.19	16.40±0.51
MCHC(g/dl)	12.501±0.70	6.401±1.22	14.151±0.72	13.501±0.52	14.00±0.79

Variances are significant at five percent (P-value below 0.05). Control-Ve: Mice nourished on basal diet.

Control +Ve: Diabetic mice nourished on a basal diet.

-Platelets

Table (3) illustrate the influences of green fenugreek and silkworm powder on PLT and MPV in diabetic rats. As illustrated in this table, the mean value of PLT of control +ve was greater in comparison with control -ve, which were 367.10±11.19 and 110.50±3.50 k/ul, respectively. The values of all the experimental groups 5% fenugreek and (5% , 10%) silkworm powder were significantly lower (p below 0.05) when than control +ve, by mean 83.00±1.73, 80.20±2.55 and 26.30±1.45 k/ul, respectively .

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As for MPV, the mean value of control +ve was lower in comparison with control -ve, which were 2.00±0.20 and 3.85±0.13 fl, correspondingly, as well as the values of all the experimental groups 5% fenugreek and (5%, 10%) silkworm powder were greater in comparison with control positive, by mean 2.94±0.02, 3.19±0.10 and 3.92±0.02. fl, respectively. It is evident that the PLT raised while MPV decreased by hyperglycemia, while feeding with experimental diets showed the reverse. The best group was recorded was group 5 group 5 (10%) silkworm powder, concerning the red cells parameters, This This positive effect appeared when the silkworm powder percentage increased to 10%.

Table (3): Effects of green fenugreek and silkworm powder on PLT and MPV in diabetic rats.

Group Parameter	Control (-ve)	Control (+ve)	5% green fenugreek	5% silkworm powder	10% silkworm powder
PLT(k/ul)	110.50±3.50	367.10±11.19	83.001±1.73	80.201±2.55	26.301±1.45
MPV(fl)	3.85±0.13	2.00±0.20	2.941±0.02	3.191±0.10	3.921±0.02

* Variances are significant at five percent (P below 0.05). Control -Ve: Rats nourished on basal diet. Control +Ve: Diabetic rats nourished on basal diet.

-Cells related to immunity

Table (4) illustrate the effects of green fenugreek and silkworm powder on WBC, LYM, MID and GRAN in rats pretreated with Alloxan. The mean value of WBC of control positive was significantly lower (p below 0.05) than control -ve, which were 5.30±0.26 and 6.13±0.06 k/ul, correspondingly. Also, the values of all the experimental groups 5% fenugreek and (5%, 10%) silkworm powder were significantly greater when than control +ve by means 8.82±0.28, 9.47±0.06 and 10.40±0.25 k/ul, respectively.

As for LYM, the mean value of control +ve was lower in comparison with control -ve, which were 3.94±0.15 and 5.00±0.17 %L, correspondingly. while the values of all the experimental groups (5% fenugreek and (5%, 10%) silkworm powder was higher than control positive which were 7.42±0.41, 8.48±0.43 and 9.28±0.26 %L respectively.

The same table demonstrated that the mean value of MID of control +ve was significantly lower (p below 0.05) in comparison with control -ve, which were 0.52±0.017 and 0.70±0.010 % M, correspondingly, The values of all the experimental groups (5% fenugreek and (5% , 10%) silkworm powder were higher than control positive, which were 0.84±0.035, 0.95±0.034 and 1.36±0.051% M .respectively.

As for GRAN, the mean value of control +ve was significantly lower (p below 0.05) in comparison with control -ve, which were 0.29±0.019 and 0.42±0.013 %G, correspondingly. additionally, the values of all the experimental groups (5% fenugreek and (5%, 10%) silkworm powder were greater than control +ve, by mean 0.60±0.017, 0.66±0.013 and 0.82±0.020 % G, respectively. It evident that best group for WBC, LYM, MID & GRAN was that of the group 5 (10%) silkworm powder.

Table (4): Effects of green fenugreek and silkworm powder on WBC, LYM, MiD and GRAN in diabetic rats.

Group Parameter	Control (-ve)	Control (+ve)	5% green fenugreek	5% silkworm powder	10% silkworm powder

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WBC(k/ul)	6.13±0.06	5.3±00.26	8.821±0.28	9.471±0.06	10.401±0.25
LYM(%L)	5.00±0.17	3.941±0.15	7.421±0.41	8.481±0.43	9.281±0.26
MID(%M)	0.70±0.010	0.521±0.017	0.841±0.035	0.951±0.034	1.361±0.051
GRAN(%G)	0.42±0.013	0.29±0.019	0.601±0.017	0.661±0.013	0.821±0.020

DISCUSSION

Fenugreek and silkworm powders contain bioactive compounds that exhibit antioxidant and anti-inflammatory properties. These compounds help modulate the immune system through enhancing the activity of immune cells and reducing oxidative stress, which is often elevated in diabetes. Additionally, silkworm powders improve blood characteristics by regulating glucose levels and lipid profiles, thereby supporting overall metabolic health in diabetic rats. **(Hu et al., 2023).** Green fenugreek leaves are primarily composed of moisture, carbohydrates, protein, fiber, and fats, similar to many green leafy vegetables. Key components include carbohydrates, dietary fiber (both soluble and insoluble), protein, moisture, and fats. They are also rich in specific micronutrients like vitamin C, iron, calcium, zinc, and other beneficial compounds such as carotene and phenols. The leaves contain a significant amount of total phenolic content, which contributes to their antioxidant properties. (**Mohammed et al., 2018).** Silkworm powders are rich in protein, with crude protein content ranging from 51% to over 70%, and also contain high levels of lipids (fat) and ash. They are a good source of essential amino acids, various minerals like potassium and magnesium, vitamins (involving C, K, E, and B12), and beneficial bioactive compounds like phenolic acids and flavonoids. Contains beneficial compounds such as phenolic acids (like ferulic and cinnamic acid) and flavonoids (like quercetin and myricetin). (**Li et al., 2017).** Green fenugreek can improve several hematological parameters in diabetic rats, including significantly increasing RBC, Hgb, HCT, and MCV at certain doses. It may also decrease MCHC and other markers in some cases. (**Jacob et al., 2017).** Studies show fenugreek extract at doses of 300 and 400 milligrams/kilogram/day significantly increased RBC counts in diabetic rats compared to control groups. This increase in RBCs is often associated with a decrease in oxidative stress and inflammation caused by diabetes. Administration of fenugreek seed extract, particularly at a dose of 400 milligrams/kilogram/day, significantly increased hemoglobin concentrations in diabetic rats. (**Gaddam *et al.*,2015)**. High doses of fenugreek extract (400 milligrams/kilogram/day) have been shown to significantly increase (MCV) in diabetic rats. However, one study reported a significant decrease in (MCV) at this dose, showing a dose-dependent and complex effect. **(Nagulapalli et al ., 2017)** Silkworm powder treatment in diabetic rats generally does not affect RBC parameters like RBC count, Hgb, and MCV, but it can improve other related values like creatinine, blood urea nitrogen, and alkaline phosphatase. Some studies have found that silkworm powder can help improve some hematological parameters that are negatively affected by diabetes (**Quan *et al.*,2021)**. Silkworm powder can have positive effects on white blood cell (WBC) counts and their subsets in diabetic rats, though specific impacts on LYM, MiD, and GRAN require more detailed study. Research indicates that these supplements can help restore hematological parameters to near-normal levels by reducing oxidative stress, and some studies have shown that they can increase WBC counts while other studies show they can help to reduce the increase of WBC caused by diabetes. **(Raghavendra *et al.* 2020)**. Green fenugreek may have varied effects on WBC and LYM, with some studies showing it can decrease WBCs and lymphocytes,

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especially when inflammation is high, while other studies indicate that high doses can increase them. The effect depends on the context and experimental conditions, such as whether the individual is on a high-fat diet or has inflammation. **(Naicker *et al*. 2016**).

5. Conclusion

In conclusion, green fenugreek powder and silkworm powder show potential in enhancing immunity and blood health in diabetic rats. These findings suggest that incorporating these natural supplements into diabetic treatment plans could offer a complementary approach to managing the condition. By improving immune function and blood health, such supplements may help mitigate some of the complications associated with diabetes. Further research in human trials is necessary to fully understand their efficacy and safety for broader clinical use.

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