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Biological, nutritional, and microbiological studies on green fenugreek and silkworm powder and their positive effects in improving the functional status in diabetic rats

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ABSTRACT

Extracts from certain plants and insects, such as silkworms and green fenugreek leaves, are used to lower blood sugar concentrations & enhance the sensitivity of insulin. This is due to the compounds they contain, such as peptides and flavonoids. Aim: This research aimed to assess the effect of green fenugreek & silkworm on improving the functional status in hyperglycemic rats and the antibacterial activity of green fenugreek and silkworm powder on six pathogenic bacteria. 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 glucose, liver enzymes, kidney function, and blood lipids. Also antibacterial activity of green fenugreek and silkworm powder on six pathological bacteria strains were specified through the diffusion agar method. Results: At the end of the experiment, the glucose level was significantly reduced ($p<0.05$). The values of all groups were significantly lower in liver enzymes and lipid profile as compared to the control positive. Conclusion: Fenugreek and silkworm powder are rich in bioactive compounds, such as antioxidants and anti-inflammatory agents, which help regulate blood sugar levels and improve insulin sensitivity. These substances also enhance gut microbiota, which plays a role in better nutrient absorption and metabolic health.

KEYWORDS: Green Fenugreek - Silkworm Powder - Functional Status- Hyperglycemic Rats.

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INTRODUCTION

Fenugreek is extensively utilized as an adjunctive herbal remedy for diabetes mellitus. Many clinical investigations have illustrated its advantageous impact on lipid profiles & glycemic control parameters. (Zheng et al., 2020). The silkworm, scientifically designated as Bombyx mori, is an economically significant insect that consumes mulberry leaves. The silkworm is classified within the order Lepidoptera & the family of silk moths, having been domesticated from the ancestral silkworm in ancient China. It is homologous to the wild silkworm, possessing twenty-eight pairs of chromosomes, now located in China. Silkworms have historically been utilized in Korea and China for the treatment of diabetes, suggesting their therapeutic efficacy in managing the condition. Initial research suggested that crude silkworm powder reduces blood glucose levels in diabetic rats. (Sumranpath et al., 2015). An investigation including thirty-eight cases with type 2 diabetes mellitus (T2DM) managed with silkworm extract (SE) and metformin demonstrated that silkworm powder reduced blood glucose concentrations following the meal (Dandapat et al., 2014). Diabetes, recognized as the 3rd most prevalent non-infectious chronic disease globally, following cancer and cardiovascular diseases, significantly affects human health and arises from islet beta-cell failure. Historical records about diabetes therapy with silkworms are well-documented in ancient medical books (Zhou et al., 2021). The hypoglycemic & pharmacological effects of silkworm have been evidenced in contemporary studies; nevertheless, the protective mechanisms on islet function & its active constituents still ambiguous (Zhou et al., 2022). As the global occurrence of diabetes rises, the applicability of fenugreek for diabetes mellitus is being examined. A prior systematic analysis on the enhancement of glucose tolerance by medicinal foods indicated that fenugreek exerts more pronounced effects on HOMA-IR and fasting blood glucose (FBG) compared to other medicinal foods for reduced glucose tolerance. Additionally, preceding meta-analysis research found

that fenugreek enhances, HDL, TG, LDL & TC in people having T2DM. Consequently, fenugreek is recommended as a beneficial complementary & alternative treatment for regulating lipid profiles & blood glucose in peoples having type 2 diabetes mellitus & prediabetes. (AlKurd et al., 2022). Consequently, fenugreek is recommended as an efficacious alternative and complementary therapy for regulating blood glucose & lipid concentrations in persons with T2DM & prediabetes. Despite the existence of meta-analyses & systematic reviews regarding the hypocholesterolemic & anti-diabetic properties of fenugreek, only one reperch has been published by (Gong et al. 2016). Concerning the influence of fenugreek on diabetic hyperlipidemia & hyperglycemia. Nonetheless, in the past seven years following Gong's report, other clinical researches was undertaken. Consequently, the objective of our research is to update the most recent meta-analysis & systematic review regarding the safety & efficacy of fenugreek in people having type 2 diabetes mellitus & prediabetes. (Page et al ., 2020). Many reports have documented the diverse pharmacological actions of the silkworm, including anticancer, antioxidant, antiviral, antibacterial, hepatoprotective, & antihypertensive effects (Zhu et al., 2022). Silkworm powders, sericin, and fibroin from three strains had therapeutic promise for lowering plasma glucose levels. Treatments with fibroin, silkworm powder, and sericin didn't affect hematological parameters. Enhancements in creatinine, BUN, lipid profiles, and ALP were reported in the therapy groups (Alkurd et al., 2020).

AIM OF STUDY

This study aimed to determine the effect of green fenugreek and silkworm on improving the functional status in hyperglycemic rats.

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

B- Source of green fenugreek: green fenugreek were 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

G- Biological evaluation:
The biological assessment of the diverse diets has been conducted by calculating the food efficiency ratio (FIR) and body weight gain % (BWG) in accordance with Chapman et al., 1959] utilizing the following formulas:

H- Blood sampling: Initially, blood samples have been collected from the retro-orbital vein following a fasting period of twelve hours, while at the end of each experiment, they have been gathered from the hepatic portal vein. Samples of the blood have been gathered into clean, dry centrifuge glass tubes & permitted to clot in a water bath at thirty-seven degrees Celsius for twenty-eight minutes. The serum was subsequently separated by centrifuging the tubes at four thousand revolutions per minute for ten minutes. The serum has been cautiously aspirated & transferred to a clean Eppendorf tube, where it was kept at minus twenty degrees Celsius until analysis. This method was labeled by (Schermer,1967).

I- Microbiological methods:

Preparation of green fenugreek and silkworm powder samples for microbiological investigation:

Ten grams of each green fenugreek and silkworm powder samples were homogenized with 90 ml of Distilled water so as to give 0.1 dilution. Then different dilutions (1: 10-1 to 1: 10-6) were prepared to be used for microorganisms' tests.

Cultivation and enumeration media:

Molds and yeast (ICMSF, 1996):

The medium used was malt - yeast extract agar of the following composition (g / L):

Meat extract	20.0
Yeast extract	10.0
Agar	20.0

The medium was prepared and sterilized at 120 °C. for 15 min. To the cooled (50 °C.) medium add-30 mg of each penicillin G and streptomycin sulphate (to inhibit bacterial growth). Pour the cooled medium in plates and left for solidification. Inoculate the plates as mentioned above. After that the plates were incubated at 25 °C. for a period of 7 - 10 days after which colonies were counted and expressed as cfu / g sample, for bottle water and juices the filter membrane method was used.

Coliform bacterial (Oxoid):

The medium used was m - Endo agar (Millipore) of the following composition (g / L):

Yeast extract	1.500
Pancreatic digest of casein	5.000
Thiopeptone	5.000
Tryptone	10.000
Lactose	12.500
Sodium desoxycholate	0.100
Dipotassium phosphate	4.370
Monopotassium phosphate	1.375
Sodium chloride	5.000
Sodium lauryl sulfate	0.050
Sodium sulfate	2.100
Basic fuchsin	1.050
Agar	15.000

Rehydrate 48 grams of the ready-made agar medium (Millipore) in 1 L distilled water continuing 20 ml. 95 % ethanol. Heat to near boiling to dissolved agar. Cool to 45 - 50° C. and dispense the appropriate quantities into 60 mm. sterile petri dishes. Refrigerate finished medium in the dark, and discard unused agar after two weeks.

Add 1 ml. of each dilution of sample (prepared as mentioned before) on the surface of Endo agar plate, spread the inoculum by sterile spreader. At least 3 plates from each dilution were inoculated.

The plates were incubated at 35° C. for 22 - 24 h., after which the colonies appeared were observed for their color as follows:

* Typical coliform colonies appeared pink to dark red color with metallic sheen counts both typical and atypical colonies. Occasionally a typical sheen colony may be produced by non coliform organisms and atypical colonies may be cloliforms. Preferably verify all typical and atypical colonies by swobbing at least 5-10 colonies from a given plate, and the verified colonies

$$\frac{\text{Number of verified colonies}}{\text{Total coliform colonies}} \times 100$$

/ 1 gram sample or / 100 ml. water =

If coliform absent, report the coliform colonies as < 1 coliform / gram or / 100 ml.

If confluent growth occurs covering the entire plate or the entire membrane filter, or colonies are not discrete, report results as (too numerous to count TNTC) (WHO, 1988).

Staphylococcus aureus

The medium used was Baird - Parker agar (Oxoid) of the following composition (g / L):

Tryptone	10.0
Lab - Lemco powder	5.0
Yeast extract	1.0
Sodium pyruvate	10.0
Glycine	12.0

Lithium chloride	5.0
Agar	20.0

pH 6.8 ±0.2

Suspend 63 gram of the ready-made agar medium (Oxoid) in one liter of distilled water, and boil to dissolve the medium completely, sterile by autoclaving at 121° C. for 15 min. Cool to 50° C. and add aseptically 50 ml. of egg yolk tellurite emulsion SR54 (Oxoid) mix before pouring. Store the plates at 4 °C.

Inoculate the plates (as described before) and incubate at 35° C for 24 - 48 h., after which look' for Staph. aureus colonies which appear as gory - black shiny convex colonies 1 - 3 mm. Diameter with margin and surrounded by zone of clearing 2-3 mm. These colonies were picked up and tested for coogulase production (ICMSF, 1996).

Bacillus cereus

The medium used was Bacillus cereus selective agar medium with supplement SR99. This medium was of the following composition (g / L):

Peptone	1.00
Mannitol	10.00
Sodium chloride	2.00
Magnesium sulphate	0.10
Disodium hydrogen phosphate	2.50
Potasium hydrogen phosphate	0.25
Bromothymol blue	0.12
Sodium pyruvate	10.00
Agar	14.00

pH 7.2 ±0.2

J- Biochemical Analysis:

Lipid profile:

- Measurements of serum triglycerides: The serum triglycerides have been measured utilizing enzymatic methods and kits in accordance with (Young, 1975 and Fossati,1982).
- - Measurements of serum total cholesterol: Serum total cholesterol has been measured using the colorimetric technique previously explained by (Thomas,1992).

Measurements of high-density lipoprotein (HDL-c): high-density lipoprotein has been estimated in accordance with the technique established with (Fredewaid 1972 & Grodon & Amer, 1977).

- - Measurements of very low-density lipoprotein cholesterol (VLDL-c): very low-density lipoprotein cholesterol has been measured in milligrams per deciliter in accordance with (Lee & Nieman,1996).

Measurements of low-density lipoprotein cholesterol (LDL-c): LDL-c has been measured in milligrams per deciliters in accordance with (Lee and Nieman ,1996)

- Determination of atherogenic index (AI): Determination of AI = (LDL-c + very low-density lipoprotein cholesterol) (Kikuchi-Hayakawa et al., 1998).

Liver functions:

- Measurements alanine transaminase: - performed regarding the procedure of (Clinica Chimica Acta, 1980),
- Measurements aminotransferase (AST) Measurements of serum AST has been carried out in accordance with the technique of (Hafkenscheid ,1979).
- Measurements of serum globulin: Serum globulin has been determined in accordance with the method defined by (Henry, 1964).
- Serum albumin (SAlb): Serum albumin has been determined with regard to the technique described by (Doumas et al., 1971).

Kidney functions:

- Determination of serum urea: Urea has been determined by enzymatic technique in accordance with (Patton & Crouch ,1977).
- Measurements of serum creatinine: Serum creatinine has been measured in accordance with the technique expressed with (Henry ,1974).
- Measurements of serum uric a`: Serum uric a` been measured calorimetrically in accordance with the technique of (Barham & Trinder ,1972).
- Measurements of blood glucose: Enzymatic measurements of serum glucose have been performed calorimetrically in accordance with the technique of (Tinder ,1969).

J- Statistical analysis:

The student-Newman-Keuls test has been utilized to separate the means after a significant main effect has been discovered. The data was 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 were conducted using one-way ANOVA. (1967, Snedecor and Cochran)

- Ethical Approval

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

4- RESULTS AND DISCUSSION

RESULTS

4.1. Microbiological results.

Inhibitory effects of varying doses of green fenugreek and silkworm powder on specific pathogenic microorganism:

Information expressed in table (1) illustrates the inhibitory influence of various levels of green fenugreek and silkworm powder on certain pathogenic microorganisms enumerated in liquid media.

It is obvious to notice that a complete inhibition (100%) of E. coli was recorded with 1.6 % soaked of green fenugreek and silkworm powder, whereas the lowest inhibition percent was 99.87 %.

In case of Salmonella sp., the gathered outcomes indicated that a complete inhibition (100%) was recorded with all tested soaked of green fenugreek and silkworm powder concentrations (0.4 %, 0.8%, 1.2 % and 1.6 %).

In contrary, a complete inhibition (100%) of Bacillus cereus has been documented with all soaked green fenugreek & silkworm powder levels except with 0.4 percent. the value was 97.97 %.

The maximum inhibition percent of Staphylococcus aureus has been verified with 1.6 % soaked of green fenugreek and silkworm powder (100.0%), while the lowest one was recorded with 0.4 % soaked green fenugreek and silkworm powder. The value was 99.94%.

In contrast, the inhibition percent of Aspergillus niger & Candida albicans documented the maximum inhibition percent with 1.2 and 1.6 % soaked of green fenugreek and silkworm powder being 99.98 & 99.99 %, respectively. While the lowest inhibition percent has been documented with 0.4 and 0.8 % soaked green fenugreek & silkworm powder. The values were 99.85 % & 99.72 %, respectively. These conclusions are in line with (Lina Al-Timimi,2019). They showed that silkworms produce a protein called sericin, which has been shown to possess antimicrobial properties. This protein can inhibit the growth of various bacteria, making it an effective natural agent in combating bacterial infections. Additionally, sericin can enhance the healing process by promoting cellular regeneration and protecting tissues from further infection.

Table (1): Inhibitory effect of various levels of soaked green fenugreek and silkworm powder on certain pathogenic microorganisms enumerated in liquid media:

Tested organisms	Spices concentrations				
	Control	0.4%	0.8%	1.2%	1.6%
Escherichia coli 1	1.0 × 106	1.3 ×103	1.5×102	1.0 ×102	N.D.
Salmonella sp.	1.0 × 106	N.D.	N.D.	N.D.	N.D.
Bacillus cereus	1.0 ×106	3.0 × 102	N.D.	N.D.	N.D.
Staphylococcus aureus	1.0 ×106	6.0 × 102	5.0 ×102	2.0 ×102	N.D.
Aspergillus niger	1.0 ×106	1.5 ×103	4.0 ×102	1.5 ×102	1.2 ×102
Candida albicans	1.0 ×106	2.8 × 103	1.1 ×102	1.0 ×102	0.7 × 102

4.2. Biological results.

Effects of green fenugreek and silkworm powder on BWG %, feed intake, & feed efficiency ratio of diabetic rats.

Table (2) presents the effects of green fenugreek & silkworm powder over twenty- eight days on body weight gain, food intake, & feed efficiency ratio in diabetic mice. The mean value of BWG for the positive control was greater in comparison with that of the negative control, recorded at -0.36±0.0026 & 0.076±0.0017, respectively. However, the values of all groups were significantly elevated ($p<0.05$) in comparison with the positive control, with averages of 1.32±0.072, 1.36±0.051, and 1.99±0.010 g respectively.

The mean value of FI for the positive control was greater in comparison with that of the negative control, recorded at 6.50±0.050 g and 6.90±0.010 g, respectively. The values of all groups exceeded the positive control, with means of 27.90±0.008, 28.00±0.007, and 17.771±0.039 g, respectively. However, the values of all groups exceeded those of the positive control.

The FER of the positive control was significantly greater (p-value under 0.05) than that of the negative control, measuring 0.06±0.0007 and 0.011±0.005, respectively. However, the values of all groups exceeded those of the positive control, with means of 0.047±0.0019, 0.049±0.0123, and 0.112±0.0018, respectively.

Table (2): Effects of green fenugreek and silkworm powder on BWG %, feed intake, & EFR of diabetic rats.

Group Parameter	Control (-ve)	Control (+ve)	5% green fenugreek	5% silkworm powder	10% silkworm powder
BWG(g)	0.076±0.0017	0.36±0.0026	1.321±0.072	1.361±0.051	1.991±0.010

Mean±SD					
Fi(g) Mean±SD	6.90±0.010	6.50±0.050	27.901±0.008	28.001±0.007	17.771±0.039
FER Mean±SD	0.011±0.005	0.06±0.0007	0.0471±0.0019	0.0491±0.0123	0.1121±0.0018

Differences are significant at five percent (P-value under 0.05). Control-Ve: Rats nourished on standard diet.
Control +Ve: Diabetic mice nourished on basal diet.

4.3. Biochemical results

Effects of green fenugreek and silkworm powder on serum glucose of diabetic mice.

The table indicates that the mean serum glucose concentration of the Control (+ve) group was greater compared to that of control (-) group, recorded at 169.00±3.46 and 75.00±1.80 milligrams per deciliter, correspondingly. The values for the five percent fenugreek group and the five percent and ten percent silkworm powder groups were significantly lower ($p<0.05$) in comparison with the positive control, with means of 131.20±2.03, 127.00±1.87, and 125±1.23 mg/dl , respectively. The findings indicate a significant distinction between 5 and 10% silkworm powder. 10% silkworm powder is the optimal treatment.

Table (3): Effects of green fenugreek and silkworm powder on serum glucose of diabetic rats.

Group

Group **Parameter**	**Control (-ve)**	**Control (+ve)**	**5% green fenugreek**	**5% silkworm powder**	**10% silkworm powder**
Glucose (mg/dl) Mean±SD	75.00±1.80	169.00±3.46	131.20±1.61	127.00±1.82	125±1.50

* Values are represented as mean ± SD.** Values which don't have the same letter in each column are significantly dissimilar at -value under 0.05.

Serum liver enzymes

Table (4) demonstrates the effect of green fenugreek & silkworm powder on serum ALT, AST, ALP, & the AST/ALT ratio in diabetic rat groups. The mean AST value for the control (-) was significantly elevated ($p<0.05$) in comparison with the negative control, with values of 145.00±4.58 and 42.00±2.05 Units per Liter, correspondingly. The values of all groups were significantly reduced in comparison with the positive control, which was 66.00±1.32, 41.50±1.51, and 30.00±2.45 units per liter, respectively.

The ALT levels in the control positive group were significantly elevated ($p<0.05$) in comparison with the control negative mice, measuring 40.00±2.65 & 18.00±0.87 units per liter, respectively. The values of all groups were significantly reduced in comparison with the control positive rats, which were 22.50±1.50, 14.80±0.72, and 14.80±0.75 unite per liter, respectively.

Table 3 demonstrates that the mean AST/ALT ratio in the positive control group was significantly elevated ($p<0.05$) compared to the negative control group, with values of 3.63±0.092 and 2.33±0.061, correspondingly. The values of all groups were significantly reduced in comparison with those of the positive control group, measuring 2.93±0.061, 2.80±0.045, and 2.03±0.058, respectively.

Table (4): Effects of green fenugreek and silkworm powder on ALT, AST, ALP, and AST/ALT ratio on diabetic rats.

Group

Group **Parameter**	**Control (-ve)**	**Control (+ve)**	**5% green fenugreek**	**5% silkworm powder**	**10% silkworm powder**
AST (U/L) Mean±SD	42.00±2.05	145.00±4.58	66.001±1.32	41.50±1.51	30.00±2.45
ALT (U/L) Mean±SD	18.00±0.87	40.00±2.65	22.501±1.50	14.801±0.72	14.80±0.75
AST/ALT Ratio Mean±SD	2.33±0.061	3.63±0.092	2.931±0.061	2.801±0.045	2.031± 0 058

Kidney Function

Information in table (5) results illustrate the effects of green fenugreek and silkworm powder on serum creatinine, uric acid, and urea in diabetic groups.

The data indicate that the mean creatinine level in the positive group (+) was elevated in comparison with that in the control (-) group, measuring 1.20±0.044 and 0.70±0.032 mg/dl, correspondingly. Furthermore, the results for all groups were reduced to the positive control, measuring 0.90±0.026, 0.80±0.021, and 0.63±0.019 mg/dl, respectively.

The serum urea concentration in the positive control group was significantly greater in comparison with that in the negative control group, measuring 162.00±7.21 milligrams per deciliter and 37.00±2.45 milligrams per deciliter, respectively. Furthermore, the readings for all groups were inferior to the positive control, with means of 130.00±4.36, 119.00±5.29, and 94.00±1.73 mg/dl, respectively.

The outcomes indicated that the mean uric a` level in the positive control group was elevated compared to that in the negative control group, measuring 3.50±0.22 and 2.00±0.11 mg/dl, respectively. Furthermore, the results for all groups were inferior to the positive control, measuring 2.60±0.07, 1.45±0.08, and 1.01±0.03 mg/dl, respectively.

Table (5): Effects of green fenugreek and silkworm powder on serum creatinine, urea, and uric acid in diabetic groups.
Group

Group **Parameter**	**Control (-ve)**	**Control (+ve)**	**5% green fenugreek**	**5% silkworm powder**	**10% silkworm powder**
Creatinine (mg/dl) Mean± standard deviation	0.70±0.032	1.201±0.044	0.901±0.026	0.801±0.021	0.631±0.019
Uric a` (mg/dl) Mean± standard deviation	2.001±0.11	3.501±0.22	130.001±4.36	119.001±5.29	94.001±1.73
Urea (mg/dl) Mean± standard deviation	2.33+0.061	3.63±0.092	2.601±0.07	1.451±0.08	1.011±0.03

lipid profile

The information in table (6) demonstrates the effects of green fenugreek and silkworm powder on serum TG, TC, LDLc, HDLc, A.I. and VLDLc ratio in diabetic mice.

The table indicates that the mean total cholesterol (TC) value for the control (+) group was greater in comparison with that of the control (-) group, recorded at 191.00±3.61 and 142.00±2.64 milligrams per deciliter, respectively. Furthermore, the values for all experimental groups were significantly lower ($p<0.05$) in comparison with the control (+), with means of 160.00±2.65, 131.00±3.61, and 130.00±4.88 mg/dl, respectively.

The triglyceride levels of the control (+) group were elevated compared to the control (-) group, measuring 188.00±6.08 mg/dl and 121.00±3.41 mg/dl, respectively. Furthermore, the results for all other groups were inferior to that of the positive control, recorded at 174.00±5.29, 159.00±3.60, and 119.00±3.46 mg/dl, correspondingly.

The mean value of HDLc in the control positive group has been reduced compared to that in the control negative group, recorded at 68.00±1.73 mg/dl deciliter & 110.00±4.35 mg/dl , respectively. Furthermore, the values for all groups exceeded that of the control positive, with means of 90.70±3.76, 93.30±1.54, and 102.00±2.65 mg/dl , respectively.

The table demonstrated that the LDL concentrations of the control (+) were elevated compared to those of the control (-) , measuring 85.40±2.51 and 7.80±0.26 milligrams per deciliter, respectively. The values of all groups have been reduced in comparison with the positive control, with means of 34.50±2.18, 6.10±0.55, and 4.20±0.26 mg/dl , respectively.

The outcomes illustrated that the mean value of VLDLc in the control (-) group was elevated than that in the negative control group, measuring 37.60±2.42 & 24.20±0.72 mg/dl , respectively. Moreover, the readings for all groups were reduced to the positive control, with the mean of 34.80±1.59, 31.60±1.44, and 23.80±0.55 mg/dl , respectively.

The table indicated that the A.I. ratio for control positive was superior to that of control negative, measuring 1.81±0.025 and 0.29±0.017, respectively. In contrast, the values for all groups were reduced in comparison with control positive, by means 0.76±0.026, 0.40±0.017, and 0.27±0.015, respectively.

Table (6) Effects of green fenugreek and silkworm powder on serum TC, TG, HDLc, LDLc, VLDLc & A.I. ratio in diabetic mice

Group Parameter	Control (-ve)	Control (+ve)	5% green fenugreek	5% silkworm powder	10% silkworm powder
TC (mg/dl) Mean±SD	142.00±2.64	191.00±3.61	160.001±2.65	131.001±3.61	130.001±4.88
TG (mg/dl) Mean±SD	121.00±3.41	188.001±6.08	174.001±5.29	159.0013.60	119.00±3.46
HDL (mg/dl) Mean±SD	110.001±4.35	68.00±1.73	90.701±3.76	93.301±1.54	102,00±2.65
LDL (mc/dl) Mean±SD	7.80±0.26	85.401±2.51	34.501±2.18	6.101±0.55	4.201±0.26
VLDL (mg/dl)	24.20±0.72	37.601±2.42	34.801±1.59	31.60±1.44	23.801±0.55
Atherogenic index (A.I. Ratio	0.29±0.017	1.81±0.025	0.76±0.026	0.401±0.017	0.27±0.015

DISCUSSION

The study results suggest that incorporating green fenugreek and silkworm powder with foods could potentially offer a dual benefit for managing hyperglycemia and combating bacterial infections. This indicates a promising avenue for developing natural and multi-functional therapeutic agents. Additional research is required to explore the mechanisms behind these effects and their possible applicability in human health interventions (QI et al., 2020). Silkworm powder improves functional status in diabetic rats through reducing blood glucose concentrations, enhancing glucose tolerance, protecting pancreatic islet cells, and exhibiting antioxidant & anti-inflammatory effects. These positive effects are primarily attributed to the major component, 1-deoxynojirimycin (1-DNJ), and other bioactive compounds like sericin, which collectively inhibit glucose-elevating enzymes, modulate glucose metabolism, & reduce oxidative stress. (WEI et al., 2022). The ability of fenugreek to lessen glycemia is because of its abundant content of the viscous dietary fire galactomannan. (Mathur et al., 2020). Dietary fiber from fenugreek reduces postprandial glucose concentrations. The mechanisms underlying these influences are insufficiently clarified. Fenugreek seeds comprise 45.4 percent dietary fiber, comprising thirty-two percent insoluble fiber & 13.3 percent soluble fiber, while the gum is composed of mannose & galactose. The specified compounds are related to a diminished glycemic effect. The hypoglycemic effect of fenugreek was specifically evidenced in humans & animals having type 2 & type 1 diabetes mellitus. (Gaddam et al.,2015). Components in silkworm powder, particularly silk sericin, have been illustrated to improve insulin resistance & enhance sensitivity to insulin in diabetic models. Silkworm extracts can protect pancreatic β-cells, which are responsible for producing insulin, by reducing inflammatory markers and oxidative damage. (LIU et al ., 2023) silkworm powder contains antioxidants like SOD & other compounds that reduce pro-inflammatory cytokines (like TNF-α) & enhance antioxidant capacity in diabetic rats. Studies also examine the beneficial effects of silkworm powder, such as its positive influence on gut microbial diversity, the production of short-chain fatty acids, & potential applications in improving metabolic health (LI et al.,2020). The research examined the effect of fenugreek-enriched therapeutic food on the blood sugar concentrations in twenty-four cases with non-insulin dependent diabetes mellitus. A therapeutic meal was made from legumes, specifically green gram, bengal gram, dry peas, horse gram, & fenugreek seeds. An amount of thirty grams of the product was administered over about one month, resulting in a considerable reduction in both postprandial & fasting blood glucose concentrations. The efficacy of a high-fiber fenugreek diet in diabetes treatment was concluded. (Luo et al. 2023). Fenugreek may reduce fat levels due to its saponin content, which is converted into sapogenins in the gastrointestinal system. Fenugreek seeds comprise twenty-five percent fiber, which can impede the rate of glucose absorption following meal. This could be a 2ry mechanism for its hypoglycemic impact. Fenugreek seed extract, utilizing ethanol, methanol, acetone, dichloromethane, hexane, & ethyl acetate, exhibits radical scavenging action. Additionally, fenugreek demonstrates a protective impact against lipid peroxidation & enhances enzymatic antioxidants. (Tiwari et al. 2023). Fenugreek seeds exhibit several pharmacological activities, involving anti-inflammatory, antioxidant, hypoglycemic, hypocholesterolemic, & antiulcerogenic properties. Fenugreek has been utilized in traditional medicine for decades to address many different diseases, including digestive illness & skin inflammation. Steroidal saponins, including diosgenin & gitogenin, are identified as the primary active compounds responsible for its therapeutic benefits. Fenugreek has demonstrated the ability to suppress cancer cell proliferation, induce apoptosis, & affect gut microbiota homeostasis in chickens, underscoring its supplementary health advantages. (Mehrzadi et al ., 2021). , .

CONCLUSION

The research highlights the potential of green fenugreek and silkworm in improving hyperglycemic conditions and combating bacterial infections. This research may lead to new treatment options for diabetes management. Where fenugreek and silkworm powder are rich in bioactive compounds, like anti-inflammatory and antioxidants agents, which aid regulate blood sugar concentrations and enhance sensitivity to insulin. These substances also enhance gut microbiota, which has a role in better nutrient absorption and metabolic health.

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