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The *Gryllus Bimaculatus* Extract Normalized FBG Levels, Kidney and Liver Functions in the Streptozotocin-Induced Diabetic Type 2 Mice

Rawaa Ali Rahmat[1], Maan Abdul Azeez Shafeeq[2], and Jamela Jouda[3*]
[1,2,3] *Department of Biology, College of Science, Mustansiriyah University, Bagdad, Iraq*
**E.mail: jamela.jouda@uomustansiriyah.edu.iq*

Abstract :
This study was carried out to elucidate the antidiabetic effects of hot aqueous *Gryllus bimaculatus* extract and some its complications in a streptozotocin (STZ)-induced diabetic type 2 mice. The insects were collected, sterilized with 70% alcohol, dried, ground, and sterilized with the autoclave at a temperature 121°C for 30 minutes. Some chemical tests were done for the hot aqueous of this powder to determine the active compounds of this extract. Diabetes type 2 was induced in the 40 mice by injected them with STZ 50µg/kg body weight (ip) only one induce diabetes while 20 mice were left without injection. The STZ-induced diabetic mice were divided into 4 groups (n=10). The first group consumed highly concentrated of insect extract, while the second consumed low concentration of insect extract. However, the third group consumed Metformin (Glucophage), and the fourth left without treatment. these treatments were administered orally and daily. One group of healthy was consumed highly concentrated of insect extract while the other was left without. During the experiment, FBG and body weight of mice were measured weekly. At the end of experiments, mice were sacrifices after 28 days and blood samples were collected to use in determine FBG, insulin, Urea, creatinin, GPT, ALP, TC, TG, and HDL levels. Then, the pancreases were collected in the 10% formalin to use in the histologic procedure. Administration of the insect extract significantly rescued representative diabetes marker and liver and kidney function markers in STZ-induced diabetic mice. Collectively, our results suggest that *Gryllus bimaculatus* contributes to the maintenance of liver, kidney and pancreatic β-cell function against a diabetic state through the regulations against apoptosis and anabolic metabolism as well as against the Insulin resistant.

Keywords: *Gryllus bimaculatus*, liver function, kidney function, FBG, and insulin

Introduction:

Diabetes Mellitus (DM 2), as a chronic disease, is a group of metabolic diseases with a hyperglycemia characteristic that occurs due to abnormalities in insulin secretion, insulin action, or both (1), and it's correlated to an insulin resistance-like diagnosis. Other organs have recently been identified as playing a key role in the pathogenesis of hyperglycemia in DM2, and it is now known that not only dysfunction of the pancreas, but also the liver, adipose tissue, gut, kidneys, and central nervous system may contribute to this hyperglycemic condition(2).

Research indicates that there is relationship between DM and liver abnormalities. Hyperglycemia can destroy liver cells and increase morbidity and mortality among diabetics that may cause a number of liver abnormalities (3). On the other hand, fat accumulation in liver may cause resistance of insulin and lead to dysfunction in metabolism (4). The biochemical changes that commonly occur in DM are similar to liver disease, from the abnormal liver enzymes secretion to the development of cancer stem cells or even liver failure in the final stages (5).

Hyperlipidemia (6) is another consequence of diabetes that occurs in tandem with hyperglycemia. It's indicated by elevated cholesterol, triglycerides, and phospholipids, as

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well as alterations in lipoproteins (7). The increased frequency of atherosclerotic disease, which is a primary cause of premature death in diabetic patients, has piqued researchers' interest in studying plasma lipids in diabetes (8).

Moreover, Diabetic nephropathy, ischemia damage from vascular disease and hypertension, and other renal disorders unrelated to diabetes can all be seen in persons with diabetes. According to certain research, half of all diabetics with severe kidney function impairment do not have albuminuria. These findings show that testing for albuminuria alone may not be enough to detect all diabetics with renal disease. To identify patients with kidney disorders other than diabetic nephropathy, measurements of urine albumin excretion must be used with estimates of kidney function and urinalyses (9).

Because of these complications linked with the diabetes, the controlling of DM is a very urgent need in light of the available chemical treatments which has several drawbacks (10). Thus, modern medicine turned to alternative treatments, which included treatments from a plant or animal source (11). Insects like crickets are ranked fourth among the extremely nutritious functional food sources worldwide. When compared to beef (40%), pork(55%), and poultry (55%), cricket production efficiency is relatively high (80%). Furthermore, Insects are also becoming more acceptable as animal protein source (12). The cricket *Gryllus bimaculatus*, according to Ahn *et al.,* (13), contains unsaturated fatty acids that can be utilized as a nutrition as well as a treatment for fever, diarrhea, kidney stones, and hypertension. Furthermore, investigations claim that the ethanol extract of *Gryllus bimaculatus* is not toxic to humans (14). As a result, this investigation was carried out to see if ingesting *Gryllus bimaculatus* powder might normalize the physiological parameters associated with diabetes and its complications in a STZ-induced rat model of T2D as an antidiabetic condition.

Material and Method:

The collected insects (*Gryllus bimaculatus*) were sent to the Natural History Research Center and Museum / University of Baghdad, for the purpose of diagnosis. Their type was confirmed as shown in the book numbered (276). These insects were washed with 70% alcohol and dried for 48 hours at room temperature. After sterilization and drying, the dried insect body was taken and ground by an electric mill into a powder, kept in a closed glass container, and sterilized in an autoclave at a temperature 121°C for 30 minutes and then the powder was kept until it was used.

In a 1:1 ration, 10 ml of organic solvents mixture made up of hexane and ethyl acetate were added to 0.1 g of insect powder. The liquid had been filtered. The resulting filtrate was used to measure FTIR spectra using a Shimadzu FTIR-8400S FTIR spectrum analyzer and gas chromatography-mass (GC-mass) with a Shimadzu GCMS-QP2010 Ultra Gas chromatography–mass spectrometry (15).

About 90 mg of insect powder was dissolved in 250 ml of distilled water in volumetric flasks, as a hot solution, it was placed in the Shaker incubator at 50°C for 48 hours. This mixture was then filtered using Wattman 1 filter paper and centrifuged for 6 minutes at 4000 rpm.

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The filtrate was divided into two parts, 150 ml was taken from the filtrate and placed in a glass vial without any dilution and served as high concentration, and the remaining 100 ml was diluted with 100 ml of hot distilled water and placed in another glass vial and served as low concentration (16).

About 57 mg of metformin (Company Merck, Germany origin) was prepared by dissolving in 10 ml of distilled water. 100µl from this concentration for mice that has average body weight 25g is equivalent to 2000 mg for an adult human, which is the average dose for people with diabetes, where doses range from 1000 to 3000 mg per day (17).

Sixty BALB/c mice were used in this study, from the Iraqi Center for Cancer Research / Mustansiriyah University and placed in the same place where the experiment was conducted under climate control conditions of the animal house with a temperature of 22-25, 60% humidity, a 12-h dark cycle, and free access to food and water. These mice aged about 8-9 weeks and weighted ranges from 20 to 27 grams. Forty mice were randomly injected inter peritoneum (IP) with 100µl streptozocin (STZ) in final concentration 50µg/g body weight, according to (18), to induce diabetic disease while 20 mice were left without injection and served as healthy group. After that, food was prevented from mice for 12h. Then, the fasting blood glucose (FBG) level was measured in mice using "On Call Plus"ACON company USA origin. Mice that had FBG level more than 150 mg/dl served as diabetic mice.
The diabetic mice were divided into 4 groups (n=10) depend on their treatment which administered orally and daily in the rate of (100µl): high dosage of insect extract (G.E.H) low dosage of insect extract (G.E.L), Metformin (G.M), and without treatment (G.C.STZ). The healthy group was also divided to two groups: one group was consumed high dosage of insect extract (G.C.E) and the other without (G.C). During the experiment, body weight of mice were calculate and FBG level were determined using "On Call Plus" ACON/ USA every week. In the end of experiment, the mice were scarified and blood was collected and centrifuged 3000 rpm for 5 min. The serum was collected and used to determine quantitative of (FBG) by Enzymatic method using kit from Biosystem company, insulin (INS) concentrations by ELISA Kit from cusabio company, urea and creatinine concentrations by enzymatic colorimetric method according to Linear chemical company kit, Alkaline phosphatase (ALP) using p-nitrophenyl phosphate (pNPP)as a phosphatase substrate by Abnova Company , (GPT) using Activity Assay by GenWay Biotech company, Total Cholesterol (TC) by biolabo, TG and HDL concentration by enzymatic colorimetric method using Linear chemical Reagents and instruments. The pancreases were also collected and put in 10% formalin and used in histology process according (19).

Results are expressed as mean ± standard error and analyzed by one-way analysis of variance followed by Fisher's test for multiple comparisons, using Statview version 5.0. Differences were considered significant when $p<0.05$.

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Results:

Through the analysis of spectroscopy using the technique of gas chromatography–mass spectrometry and FTIR spectrum , there are more than 12 compounds in the organic extract of the *Gryllus bimaculatus* (Table 1 and Table 2).

Oleic Acid (C18H34O2) occupy the largest percentage of the insect extract componnds with a value (42.92%) and Octadecanoic acid (C18H36O2) occupy the lowest value (9.78%) , while each of Linoelaidic acid (C18H32O2) and Myristic acid (C14H28O2) have a similar proportions value (23.88%; 23.42% respectively) (Figure 1).

Table (1): GC-mass results of *Gryllus bimaculatus* extract

Peak NO.	Name	Chemical Formula	Molecular weight (g/mol)	Area of Retention	Area (%)	Retention time (min.)
1	Myristic acid n-Hexadecanoic acid	($C_{14}H_{28}O_2$)	228.38	268033915	23.42	44.277
2	Linoelaidic acid 9,12-Octadecadienoic acid (Z,Z)-	($C_{18}H_{32}O_2$)	280.45	273361443	23.88	48.364
3	Oleic Acid 9-Octadecenoic acid (Z)- 9-Octadecenoic acid (E)-	($C_{18}H_{34}O_2$)	282.47	491302317	42.92	48.518
4	Octadecanoic acid 1-Heptadecanecarboxylic acid	($C_{18}H_{36}O_2$)	284.48	111988419	9.78	48.975

Figure (1): GC-mass results of *Gryllus bimaculatus* extract

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FTIR spectrum analysis was used to detect the functional groups of the compounds and their bonds in order to be able to distinguish the type of compounds present in the insect extract. Functional groups can be associated with characteristic infrared absorption bands, which correspond to the fundamental vibrations of the functional groups (20), and as a result of electron vibrations between the bonds, and by the active groups, peaks appear in different places within the areas of measurement of the spectrum (Figure 2).

Table (2): FTIR spectroscopy results and the group and its frequencies for the insect extract *Gryllus bimaculatus*

Type of Compound			Functional Group	Wave number (cm^{-1})	Vibration	Transmittance (%T)
Hydroxyl Group			O-H	3899.90-3283.67	Broad	69.04 – 63.71
Aromatic Rings			C-H	2853.77-3008.11	Strong	63.20 - 52.49
Sulfhydryl group	S-H	2338.12-2353.25	Medium	65.80		
Carbonyl group	C=O	1512.67-1754.66	Broad	63.96 – 56.09		
Carboxylic acid	COOH	1076.18-1319.64	Medium	60.05 – 62.44		
Carbonyl	C-O	749.90 – 487.14	Medium	68.21 – 69.90		

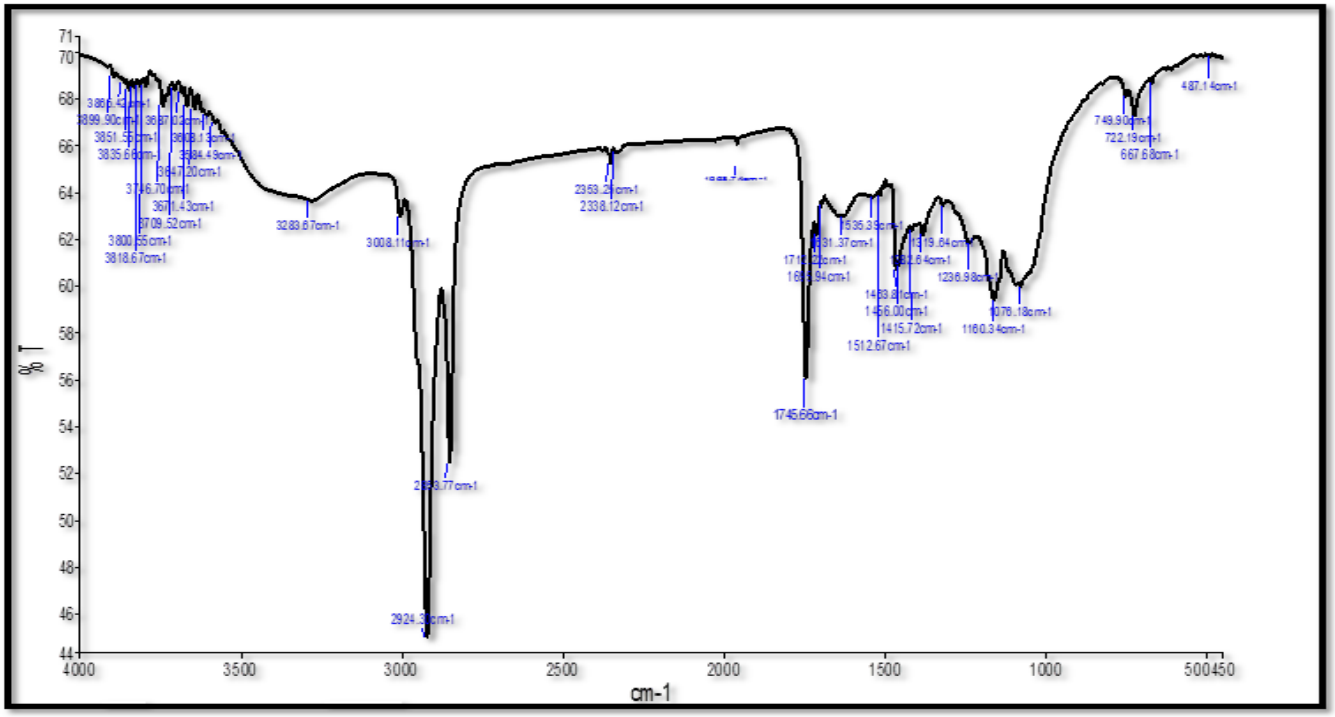

Figure (2): FTIR spectroscopy results for the *Gryllus bimaculatus*

It is well established that what distinguishes type 1 diabetes from type 2 is primarily a significant defect in the islets of Langerhans, especially the beta cells that produce insulin by an autoimmune attack on the β-cells and caused an insulin deficient state; although a small number of functioning β-cells may remain (21). In this study, the results of histological study shows that a very small number of islet cells of Langerhans were injured that could not cause type1 diabetes (figure 3), in addition to a significant increase in insulin hormone level of

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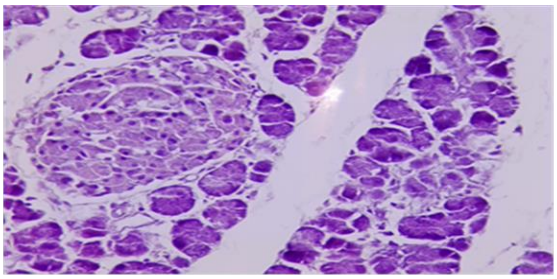
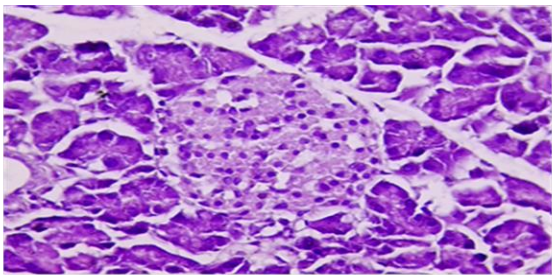
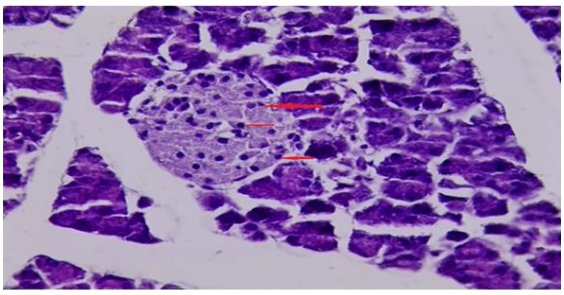
STZ-induced compared to the control (figure 3A). Thus, these evidences could prove that the type of diabetes which was induced in the mice used in this study was type 2 not type 1. On the other hand, (figure 3C) shows that even this small defect in the cells of Langerhans was disappeared completely when the treatment with the insect extract was used compared to metformin.



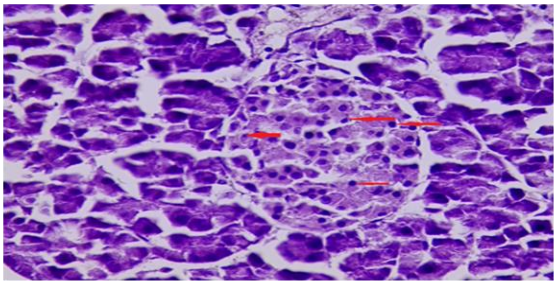
(A) Histological examination of pancreas section of negative control group reveals the normal structure and shape of endocrine cells.



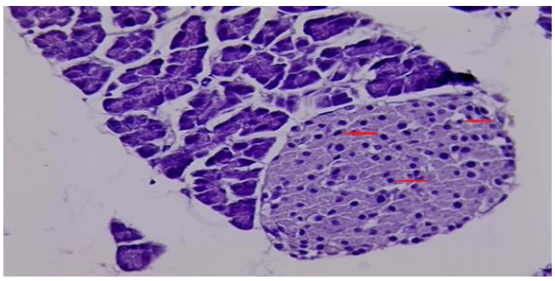
(B) Histological examination of pancreas sections of diabetic mice were left without treatment reveals shows rare apoptotic endocrine cells.



(C) Pancreas sections of diabetic mice were treated with 100µl Metformin (G.M) shows section of Islet of Langerhans there was atrophy of islet with certain apoptotic cells.



(D) Pancreas sections of diabetic mice were treated with 100µl low concentration of insect extract shows dispersed apoptotic cells.



(E) Pancreas section of diabetic mice were treated with 100µl highly concentrated of insect extract shows dispersed apoptotic cells of endocrine cell.

Figure (3): Histological sections of pancreas of all experimental groups (X40) (H & E).

As well as in the case of insulin levels (nIU/ml), where its levels decreased very significantly in the mice treated with low or high dose of insect extract and metformin (8.1 ± 0.6, 9.1 ± 0.9, and 7.9 ± 1.3 (nIU/ml), respectively), approaching its level in the negative control group (4.8 ± 1.1 nIU/ml) compared to diabetic mice without treatment (24.2 ± 3.6 nIU/ml) (figure 4).

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The changes in the FBG levels and body weight of all studied mice every week for 4 weeks were measured and then the relative changes of FBG and body weight were calculated by the following equation and shown in the table 3 and 4:

Relative change % = [(mice value after 4weeks - mice value in the zero time) / mice value in the zero time] x 100

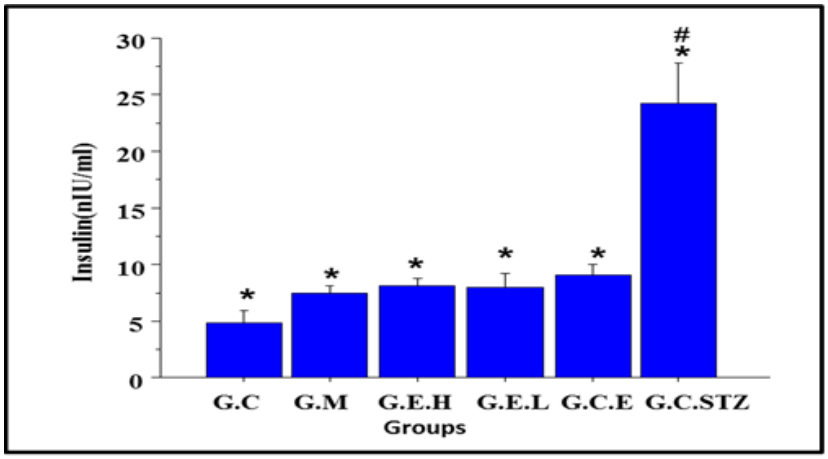

Figure (4): Insulin levels in all groups at the end of experiment. *the significant difference between negative control group (C.G) v.s. other groups. #Significant difference between positive control group (C.STZ.G) v.s. other treated groups.

In the zero time, the mice were not on their diet instead they were drinking 10% sucrose while in the next weeks; they were put on their diet 1day before the test. Thus the FBG levels were significantly higher ($P<0.05$) in the zero time of all mice groups compared to other weeks even though healthy control group without treatment (G.C) (95.4 ± 4.5mg/dl) and treated with high concentration of insect extract (G.C.E) (89.0 ± 6.2 mg/dl) although were much lower than diabetic mice groups without treatment (G.C.STZ) (170.2 ± 3.6 mg/dl), treated with metformin (G.M), high (G.E.H) and low concentrations of insect extract (G.E.L) (178.1 ± 4.4, 187.0 ± 7.6 mg/dl, and 196.2 ± 7.8 mg/dl, respectively). In all time points except in zero time, the significantly highest ($P<0.05$) FBG levels was in G.C.STZ group (186 ± 6.0, 182.7 ± 9.2, 180.5 ± 5.6, and 169.2 ± 6.4 mg/dl, respectively with time) compared to G.C (68.1 ± 4.3, 75.4 ± 5.0, 74.7 ± 5.0, and 75.4 ± 5.3 mg/dl, respectively with the time), and G.C.E (59.4 ± 4.7, 61 ± 5.0, 69.6 ± 5.8, and 70.0 ± 5.8 mg/dl, respectively with the time) and it was significantly lowest ($P<0.05$) in the G.E.L group (111.2 ± 9.0, 90.7 ± 4.4, 82.3 ± 3.8, and 80.5 ± 5.2 mg/dl, respectively with the time). In the end of experiment, the closest value of FBG level to the healthy control group (75.4 ± 5.3 mg/dl) was in diabetic mice groups treated with high and low concentrations of insect extract (80.5 ± 5.2, 94.7 ± 4 mg/dl, respectively) compared to metformin (102.7 ± 7.2 mg/dl). Importantly, there was no significantly difference between FBG levels of healthy mice treated with high concentration of insect extract compared to healthy control group without any treatment. The relative change of FBG% in the untreated diabetic mice group was positively (+2.1%) which means FBG levels get higher with time while in the other groups; it was negatively which means FBG level get lower with time. The best relative change of FBG% was in the diabetic mice

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treated with low concentration of insect extract which was -60% compared to -48% in G.E.H and -40% in G.M. (table 3).

The mice were randomly distributed to the groups, so the mean of body weight was non-significantly (P>0.05) differ among these groups. Normally, the body weights of the mice get higher with the time in all groups except in diabetic mice without treatment which was almost a same in all time points (23.8 ± 0.8, 22.5 ± 1.0, 23.7 ± 1.1, 23.7 ± 1.1, and 23.5 ± 0.8 mg/dl, respectively with the time). These differences were significant (P<0.05) only in 4 and 5 weeks (27.2 ± 0.8 and 28.4 ± 0.6 mg/dl, respectively in G.C; 26.6 ± 0.5 and 27.4 ± 0.9 mg/dl, respectively in G.C.E; 24.9 ± 0.6 and 25.3 ± 0.6 mg/dl, respectively in G.M; 26.2 ± 0.5and 26.5 ± 0.7 mg/dl, respectively in G.E.H; and 26.5 ± 0.6 and 27.0 ± 0.7 mg/dl, respectively in G.E.L) compared to zero-time (25.7 ± 0.9, 25.2 ± 0.4, 23.6 ± 0.8, 24.5 ± 0.9, and 24.2 ± 1.0 mg/dl, respectively). The relative change of body weight% was only 3% in the diabetic mice without treatment while was high in the treated diabetics mice with metformin, high and low concentrations of insect extract (13.4, 10.7, and 12,6%, respectively) but it still significantly lower than it in the healthy control (16.1%) (Table 4).

Table (3): FBG levels in all time point and Relative Change of FBG

Groups	FBG (mg/dl) Mean ± SE					Relative Change of FBG%
	Zero-time	1-week	2-week	3-week	4-week	
G.C	95.4 ± 4.5Ca	68.1 ± 4.3Aa	75.4 ± 5.0Bb	74.7 ± 5.0Ba	75.4 ± 5.3Ba	-19.3 c
G.C.E	89.0 ± 6.2Da	59.4 ± 4.7Aa	61 ± 5.0Ba	69.6 ± 5.8Ca	70.0 ± 5.8Ca	- 18.8 c
G.C.STZ	170.2 ± 3.6Ab	186 ± 6.0Bd	182.7 ± 9.2Be	180.5 ± 5.6Bd	169.2 ± 6.4Ae	2.1 d
G.M	178.1 ± 4.4Ab	96.1 ± 3.7Cb	98.3 ± 6.5Cc	93.3 ± 4.9Bc	102.7 ± 7.2Dd	- 40.8 b
G.E.H	187.0 ± 7.6Dc	118 ± 5.6Cc	105.5 ± 5.8Bd	97.5 ± 5.3Ac	94.7 ± 4.1Ac	- 48.6 b
G.E.L	196.2 ± 7.8Dd	111.2 ± 9.0Cc	90.7 ± 4.4Bc	82.3 ± 3.8Ab	80.5 ± 5.2Ab	- 60.6 a
The small letters explain the significant differences among mice groups. The large letters explain the significant differences among time points. Significant value is P<0.05						

Table (4): Body Weight and Relative Change in Weeks.

Groups	Body weight (g) Mean ± SE					Relative Change of Body weight%
	Zero-time	1-week	2-week	3-week	4-week	
G.C	25.7 ± 0.9Aa	26.7 ± 0.9Ac	27.5 ± 0.6Bc	27.2 ± 0.8Bb	28.4 ± 0.6Bd	16.1 ± 1.9c
G.C.E	25.2 ± 0.4Aa	25.8 ± 0.4Ab	26.4 ± 0.5Ac	26.6 ± 0.5Bb	27.4 ± 0.9Bc	10.1 ± 0.7b
G.C.STZ	23.8 ± 0.8Ab	22.5 ± 1.0Aa	23.7 ± 1.1Aa	23.7 ± 1.1Aa	23.5 ± 0.8Aa	3.5 ± 0.7a
G.M	23.6 ± 0.8Ab	23.3 ± 0.7Aa	24.1 ± 0.6Aa	24.9 ± 0.6Ba	25.3 ± 0.6Bb	13.4 ± 1.1b
G.E.H	24.5 ± 0.9Aa	24.8 ± 0.7Ab	25.3 ± 0.6Ab	26.2 ± 0.5Bb	26.5 ± 0.7Bb	10.7 ± 1.4b
G.E.L	24.2 ± 1.0Aa	24.8 ± 0.8Ab	25.8 ± 0.5Ab	26.5 ± 0.6Bb	27.0 ± 0.7Bc	12.6 ± 1.8b
The small letters explain the significant differences among mice groups. The large letters explain the significant differences among time points. Significant value is P<0.05						

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The level of blood urea (mg/dL) and serum creatinine (mg/dL) were significantly increased (P<0.05) in G.C.STZ group (145.9 ± 11.8 and 2.5 ± 0.34 mg/dL, respectively) compared to other groups. Their levels in G.M group were also significantly increased (44.3 ± 2.4 and 0.787 ± 0.03 mg/dL, respectively) in comparison with the control groups -G.C group (31.0 ± 4.4 and 0.358 ± 0.06 mg/dL, respectively) and G.C.E group (32.6 ± 0.5 and 0.586 ± 0.06 mg/dL, respectively)-, and other treatment groups (G.C.L and G.C.H) which have a comparable levels of blood urea to the G.C group (34.5 ± 1.0 and 30.9 ± 1.8 mg/dL, respectively) and significant higher serum creatinine (0.519 ± 0.05 and 0.621 ± 0.04 mg/dL, respectively) compared to control groups (table 5).

Table (5): Kidney Function in serum in all groups at the end of experiment

Groups	B.ur (mg/dL)	S.Cr(mg/dL)
G.C	31.0 ± 4.4a	0.358 ± 0.06a
G.M	44.3 ± 2.4b	0.787 ± 0.03c
G.E.H	30.9 ± 1.8a	0.621 ± 0.04b
G.E.L	34.5 ± 1.0a	0.519 ± 0.05b
G.C.E	32.6 ± 0.5a	0.586 ± 0.06b
G.C.STZ	145.9 ± 11.8c	2.5 ± 0.34d
The small letters explain the significant differences among mice groups Significant value is P<0.05		

The level of alkaline phosphatase (ALP) (U/L) and Glutamate pyruvate transaminase (GPT) (U/L) significant increase in G.C.STZ group (383.5 ± 43.1 and 138.5 ± 16.0 U/L, respectively) compared to other groups. Their levels were also significant increase in G.M group (128.5 ± 13.5 and 50.2 ± 9.2 U/L, respectively) in comparison with the control groups; G.C group (39.5 ± 7.9 and 29.1 ± 5.1 U/L, respectively) and G.C.E group (57.3 ± 8.6 and 34.7 ± 7.2 U/L, respectively); and other treatment groups. On the other hand, their levels were significantly decreased in the mice groups treated with insect extract; G.E.H group (73.0 ± 9.7 and 42.4 ± 2.8 U/L, respectively) and G.E.L group (53.8 ± 7.4 and 37.0 ± 5.9 U/L, respectively) (table 6).

Table (6): Liver Function in serum in all groups at the end of experiment

Groups	ALP(U/L)	GPT (U/L)
G.C	39.5 ± 7.9a	29.1 ± 5.1a
G.M	128.5 ± 13.5d	50.2 ± 9.2c
G.E.H	73.0 ± 9.7c	42.4 ± 2.8b
G.E.L	53.8 ± 7.4b	37.0 ± 5.9b
G.C.E	57.3 ± 8.6b	34.7 ± 7.2b
G.C.STZ	383.5 ± 43.1e	138.5 ± 16.0d
The small letters explain the significant differences among mice groups Significant value is P<0.05		

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Triglyceride level (TG) (mg/dl), and cholesterol (mg/dl), increased significantly in G.C.STZ group (282.2 ± 19.4 and 600.4 ± 62.9 mg/dl, respectively) compared with control groups - G.C group (133.9 ± 22.4 and 116.7 ± 17.5 mg/dl, respectively) and G.C.E group (129.5 ± 22.6 and 150.2 ± 9.6 mg/dl, respectively)- and treatment groups. In the treatment groups these levels were significantly decrease compared to the control groups; G.C.H group (172.5 ± 9.6 and 204.4 ± 7.9 mg/dl, respectively) and G.C.L group (145.9 ± 11.8 and 178.7 ± 15.1 mg/dl, respectively); except in G.M group, TG and levels were also increased similar to what is in G.C.STZ (292.5 ± 21.4 mg/dl) while cholesterol level was decrease (217.8 ± 8.7 mg/dl) compared to control groups. On the other hand, HDL level (mg/dl) decrease in G.C.STZ group (40.7 ± 6.2 mg/dl) compared with control groups; G.C group (88.6 ± 2.7 mg/dl) and G.C.E group (81.8 ± 4.9 mg/dl); and treatment groups. In the treatment groups, HDL level significant increase. Its level in the G.E.H and G.E.L groups were comparable to its level in the control groups (77.9 ± 4.4 and 78.7 ± 3.4 mg/dl, respectively) while its level decrease in the G.M group (61.7 ± 2.6 mg/dl) compared with other treatment groups (Table 7).

Table (7): Lipid profile in serum all groups at the end of experiment

Groups	TG(mg/dl)	Ch (mg/dl)	HDL(mg/dl)
G.C	133.9 ± 22.4a	116.7 ± 17.5a	88.6 ± 2.7b
G.M	292.5 ± 21.4c	217.8 ± 8.7c	61.7 ± 2.6c
G.E.H	172.5 ± 9.6b	204.4 ± 7.9c	77.9 ± 4.4b
G.E.L	145.9 ± 11.8a	178.7 ± 15.1b	78.7 ± 3.4b
G.C.E	129.5 ± 22.6a	150.2 ± 9.6b	81.8 ± 4.9b
G.C.STZ	282.2 ± 19.4c	600.4 ± 62.9d	40.7 ± 6.2a
The small letters explain the significant differences among mice groups Significant value is P<0.05			

Discussion:

Previous investigations have shown that T2DM is characterized by progressive β cell destruction as a result of chronic IR and the loss of cell mass and function (22). The apoptosis of β cells in all diabetes patients is the major mechanism driving the loss in β cell mass in people with T2DM (23). In patients with T2DM, pancreatic β cells are try to secrete sufficient quantities of insulin to compensate for the reduced insulin sensitivity which could cause hyperinsulinaemia, but the secreted insulin quantities are still insufficient, which is due to insulin secretion failure and a significant decrease in the number of functioning β cells (24). Compensatory hyperinsulinemia develops initially, but the first phase of insulin secretion is lost early in the disorder which leads to alteration in insulin-to-glucagon ratio in favor of glucagon, thus leading to increased hepatic glucose production and hyperglycemia (25). Similar histological effects were reported in the current study, which supports these findings in Figure (7-B) which shows apoptosis of acini in the pancreas of diabetes mice group, and similar physiological effects in figure (3) which shows that the insulin significantly increase in the diabetes mice group.

Karl *et al*., (26) found that as metformin administration was observed to aid in the restoration of normal β cell mass and the reduction of acini apoptosis. Metformin's antidiabetic effects

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may be due to a decrease in hepatic gluconeogenesis, regeneration of hepatocytes, decreased glucose absorption, and increased insulin sensitivity, as a result of an increase in glucose uptake and utilization (26). As a result, insulin concentrations may be normalized. This evidence could explain our results after treated with metformin which shows decrease in the number of apoptotic acini and decrease in insulin concentrations (Figure (7-C) and figure 3). Interestingly, our results showed that using the insect extracts in both concentrations (high and low) as a treatment decrease in the apoptosis of beta cells and normalized the insulin level (Figure (7-D&E) and figure 3). As a result, the decrease in beta cell death in the group that received insect extract treatments could be indicative of pancreatic islet regeneration. In addition, when compared to the metformin group, insect extract had a higher effect on pancreatic histology and insulin levels. This result may relate to the antioxidant effect of insect extract which were found in our chemical results. In agreement with this study, Park *et al.*,2020 (27) who studies the effect of the same insect extract on the rat-induced diabetes type 1 found a clear recovery in the STZ-induced diabetes group, implying that *Gryllus bimaculatus* powder preserved pancreatic ß - cell function and maintained pancreatic structure. However, he used an insulin tolerance test to determine insulin sensitivity in the presence of *Gryllus bimaculatus* powder and concluded that treatment with the *Gryllus bimaculatus* powder improved insulin sensitivity in the STZ-induced diabetes model and had a beneficial effect against insulin resistance.

Diabetes is becoming more common, and its consequences are becoming a critical public health concern. Furthermore, there are few treatment options for diabetic nephropathy are limited, which is becoming a global epidemic. Traditional herbal medicine and its principal components have been widely explored as complementary and alternative medicine in order to find safe and effective medications for the treatment and prevention of diabetic nephropathy (28). The current researchers focused into how insect extract protects STZ-induced diabetic mice from having impaired kidney function. Our research found that STZ-induced diabetic mice had considerably higher serum Creatinine and blood urea levels, indicating that these mice had exhibited severe kidney injury. Chronic inflammation and oxidative stress are related to the advancement of renal disease and hyperglycemia, according to Forbes *et al*. (29). The formation of reactive oxygen species (ROS) clogs kidney cells under hyperglycemic situations (30). As a result of the excessive creation of ROS, the antioxidant defenses in the kidney are depleted, resulting in the oxidation of DNA, lipids, and proteins. Furthermore, hyperglycemia impairs anti-oxidative processes by glycating scavenging enzymes including SOD and catalase (31). According to our findings, the treatment of insect extract or metformin dramatically lowered blood glucose levels in STZ-induced diabetic mice. Interestingly, as previously demonstrated our insect extract contains several antioxidant groups and may reduce ROS production.

T2D has been associated to nonalcoholic fatty liver disease (NAFLD), liver cirrhosis, and hepatocellular carcinoma, among other liver disorders (32) As a result, people with T2D are more likely to have abnormal liver function tests than healthy people who aren't diabetic (33). Because hepatic glucose production accounts for 79% of endogenous glucose synthesis in the fasting state (34) and is responsible for metabolizing the equivalent of (60–65 %) of the oral glucose load, the liver plays a role in glycemic homeostasis (35). ALP and GPT are

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significant common markers representing basic liver function status, and their variations can reflect the extent of hepatocytic damage as a series of signs (36). The levels of ALP and GPT in diabetic mice were higher in this study, which was consistent with Shrestha *et al*., (37) who found a statistically significant increase in GPT levels in diabetic patients compared to control groups, and Shaheen et al., (38) who found an elevated level of ALP in diabetic subjects. Since the formation of free radicals and a corresponding decrease in cell antioxidant capacity are the primary processes for diabetes complications (39), which can impact a variety of organs, including the liver, one of the most important organs (40). According to our data, diabetic mice treated with insect extract and metformin had considerably lower liver enzyme levels than diabetic mice that were not treated. When hepatic enzymes have high serum activity, they promote liver cell destruction, as seen in diabetic mice. The preventive impact of the insect extract, possibly via its antioxidant effect in reversing liver damage caused by diabetes, may be responsible for the lower levels of liver enzymes revealed in this study.

Hyperlipidemia is a well-known complication of diabetes mellitus (41) that coexists with hyperglycemia and is characterized by elevated cholesterol, triglycerides, and phospholipids, as well as alterations in lipoproteins (7). The well-known greater incidence of atherosclerotic disease, which is a significant cause of premature death in diabetic patients, has sparked interest in studying plasma lipids in diabetes (8). In the present study, serum triglycerides and cholesterol were significantly elevated while HDL cholesterol was significantly decreased in STZ-diabetic mice. Interestingly, the results further indicated that all these lipid and lipoprotein abnormalities were normalized by insect extract in diabetic mice compared to many other chemical treatment such as insulin did not return lipid levels to normal (42). Other research has found that glycated HDL clearance is hastened from the circulation, in contrast to glycated LDL, which has a lower catabolic rate. The accelerated clearance of HDL was seen even with mild glycation and was suggested as a contributing cause of low plasma levels of HDL in diabetic patients and therefore works as another factor underlying increased risk of atherosclerotic disease in diabetic patients (43). In the present study, elevated cholesterol, triglycerides and decreased HDL cholesterol concentrations in diabetic mice appear to be markedly altered favorably by insect extract supplementation could be partly due to the control of hyperglycemia on one hand and due to the containing of this extract on the other hand.

From this experimental data, it is evident that *Gryllus bimaculatus* extract efficiently regulated blood glucose, insulin, and pancreatic histology in diabetic mice and very efficiently ameliorated kidney, liver and lipid abnormalities associated with diabetes in STZ-diabetic mice by virtue of various essential antioxidant and antidiabetic compounds. The synergistic role played by these compounds is attributed to the protection of diabetic mice against these abnormalities.

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