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Effect of garlic and ginger extracts on the levels of glucose and Peptide-c in diabetic mice

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Abstract: This study aimed to investigate the effect of the alcoholic extract of garlic and ginger together on the levels of glucose, peptide -c and body weight in diabetic white mice.

The study included 40 male white mice, Balb/c strain, which were divided into four experimental groups (10 mice in each group). The first group was a physiological control that was injected with physiological saline (0.9%) until the end of the experiment. As for the second group, diabetes was induced with a dose of 200 mg/kg of Alloxan hydrate weight of the mouse only, while the third group developed diabetes, and then it was treated with alcoholic extract of garlic and ginger together at a dose of 500 mg/kg of mouse weight for 10 days. While the fourth group developed diabetes and was treated with Glibenclamide. At the end of the experiment, the animals were anesthetized and blood was drawn from them by cardiocentesis.

The results showed the effectiveness of garlic and ginger extracts in reducing blood glucose concentration by 35.75% and returning Peptide-c levels to their normal levels, equivalent to Glibenclamide (glyburide), which is known as an oral hypoglycemic agent.

Keywords: Alcoholic extract, garlic, ginger plants, diabetes, white mice, Peptide-c.

تأثير مستخلصي الثوم والزنجبيل في مستوى سكر الدم والببتيد c عند الفئران المستحدث عندها داء السكري

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المستخلص: هدفت هذه الدراسة إلى معرفة تأثير المستخلص الكحولي للثوم والزنجبيل معاً في مستويات كل من سكر الدم والببتيد c ووزن الجسم في الفئران البيضاء المستحدث عندها داء السكري.

شملت الدراسة 40 فأراً من ذكور الفئران البيضاء سلاله Balb/c والتي وزعت على أربع مجموعات تجريبية (10 فئران في كل مجموعة)، كانت المجموعة الأولى شاهدة فيزيولوجية حُقنت بمحلول فيزيولوجي ملحي (0.9)%حتى نهاية التجربة. أما المجموعة الثانية استُحدث عندها داء السكري بجرعة 200 ملغ/كغ من وزن الفأر من Alloxan hydrate فقط، بينما المجموعة الثالثة استحدث عندها داء السكري ومن ثم عولجت بالمستخلص الكحولي للثوم والزنجبيل معاً بجرعه 500ملغ/كغ من وزن الفأر ولمدة 10 أيام. في حين استحدث داء السكري عند المجموعة الرابعة وعولجت بعقار Glibenclamide. وفي نهاية التجربة خُدّرت الحيوانات وسحب الدم منها عن طريق بزل القلب.

أظهرت النتائج فعالية مستخلصي الثوم والزنجبيل في خفض تركيز سكر الدم بنسبة 35.75% وإعادة مستويات الببتيد c إلى قيمها الطبيعية بشكل مكافئ لعقار Glibenclamide المعروف كخافض سكر فموي.

الكلمات المفتاحية: مستخلص كحولي، الثوم، الزنجبيل، السكري، فئران بيضاء، الببتيد c.

1- Introduction.

The pharmaceutical industry has contributed to saving humanity from disease pests, but it has become an obsession that worries people because of its negative side effects. In recent years, interest in folk medicine has begun, and the use of medicines manufactured from natural sources (15).

International sources indicate to the significant spread of type 2 diabetes (35), so research has been conducted and is still being conducted in different regions of the world with the aim of finding a cure for this disease, as well as the lack of success of healthy pancreas transplants in the bodies of diabetic patients, in addition to high costs.

Garlic is one of these medicinal plants that contains many active substances with important nutritional and medicinal properties, so many researchers aimed to study its effects in multiple disease states, and it has been used in the form of powder, pills or oil on a large scale for certain therapeutic purposes such as lowering blood pressure and lowering fat (13).

Eidi *et al.*, (12) confirmed that oral administration of ethanolic extract of garlic significantly reduced the concentration of glucose and blood cholesterol and increased insulin production in diabetic rats induced with streptozotocin more effectively than Glibenclamide.

A study (24) showed that the two main components of garlic are S-Allyl Cysteine Sulfoxide and Diallyl Trisulfide, which are two sulfur compounds that have a role in increasing the effectiveness of insulin secreted from beta cells in the pancreas in control mice and mice with diabetes.

It was found when the rats fattened with ginger aqueous extract 2-4 weeks after the injection, a decrease in fat percentage and thus a reduced risk of high level of fat in the body (4).

Studies have also shown the important role of ginger in treating and protecting the liver against damage caused by mercury, so it is added as a food supplement for individuals who have been exposed to liver damage by mercury (14).

On the other hand, gingerol is the most effective substance in ginger to increase the sensitivity of cells to insulin secretion (3).

The pancreas secretes both the hormone insulin and Peptide-c in equal quantities, so by measuring the proportion of Peptide-c it is possible to know the amount of insulin secreted from the body because Peptide-c is characterized by a longer survival rate than insulin in the blood (19).

2- Objectives and importance of the research:

We aim for the following:

1- The effect of alcoholic extract of garlic and ginger together on the level of blood glucose, Peptide-c and body weight in white mice with diabetes
2- Comparing the effect of alcoholic extract of garlic and ginger with the the blood glucose-reducing Glibenclamide drug.

3- Material and methods.

3-1-Preparation of the alcoholic extract:

Alcoholic extract of garlic and ginger was prepared by soaking 320 g of garlic powder in 640 ml ethanol (95%) for three days and then drying the solvent in a rotary evaporator at 40 °C for 1.5 hours (32). The alcoholic extract of ginger was also prepared in the same way as before.

3-2- The experiment animals:

Male white mice of the Balb-c progeny brought from the Scientific Research Center in Barzeh (Damascus) at an age ranging from (5-4) weeks were used. The mice were acclimatized in the laboratory for a period ranging from (12-8) a week.

3-3-Experimental induction of diabetes:

Diabetes was induced in male mice by subcutaneous injection (20) with Alloxan at a dose of (200 mg/kg body weight) once.

The experimental animals were starved for 12 hours and then allowed after the injection to eat food and a 5% glucose solution to prevent a sharp drop in the concentration of glucose in the blood serum. (8) As for the healthy animals, they were injected with the physiological saline solution only, then they were given water and food normally. It was confirmed that diabetes occurred in animals prepared for the study and treated with Alloxan three days after the injection process.

3-4- Animals treatment:

The males of these mice were divided into four groups, each group included 10 mice, as follows:

- The physiological control, each of them injected with physiological saline solution (0.9%)) on a daily basis for ten days.
- The diabetic control in which diabetes was induced by Alloxan and left as such until the end of the experiment.
- The third experimental group who developed diabetes and was injected with alcoholic extract of garlic and ginger together at a dose of 5 00 mg/kg of body weight for a period of ten days.
- The fourth experimental group who developed diabetes and was treated with the drug (Glibenclamide) at a dose of 5 mg/kg of body weight for a period of ten days.

3-4- Obtaining blood:

Blood was collected from experimental mice by cardiac puncture with insulin syringes, after they were anesthetized with chloroform.

3-5- Blood serum:

Serum was obtained by centrifuging tubes at 3500 rpm for 10 minutes. The resulting serum was used to measure blood glucose and Peptide-c levels.

3-6 Body weight:

Mice were weighed before and after each stage with a sensitive scale.

3-7 Statistical analysis:

The results of the experiments were subjected to statistical analysis using the program SPSS Statistics 17.0 (Statistical Package for Social Sciences) and the arithmetic averages were extracted. Student test was conducted for independent samples, and a Paired Samples Statistics Student test was conducted. In addition to extracting the probability value p for each test and comparing it with the level of significance 0.05, where we accept the existence of a significant difference when the probability value is smaller than the level of significance.

4 - Results.

4-1- The effect of Alloxan injections:

4-1-1 Average weight of experimental animals:

The average weight of the experimental mice was measured before and after being injected with Alloxan for comparison with the physiological control sample. Its results are shown in the following table:

Table (1): Changes in average weight of mice before and after injection of Alloxan.

variable	Before	After	Average difference	T.test	P- vaue	Result
Weight	28.96	20.00	- 8.96	-3.09	0.009 **	Statistically significant

Statistical analysis of the results in Table (1) showed a significant decrease (p-value<0.05) in the average weight of mice in the group in which diabetes was induced, meaning that the injection of Alloxan reduced the weight of mice by 30.95%, illustrated in Figure (1).

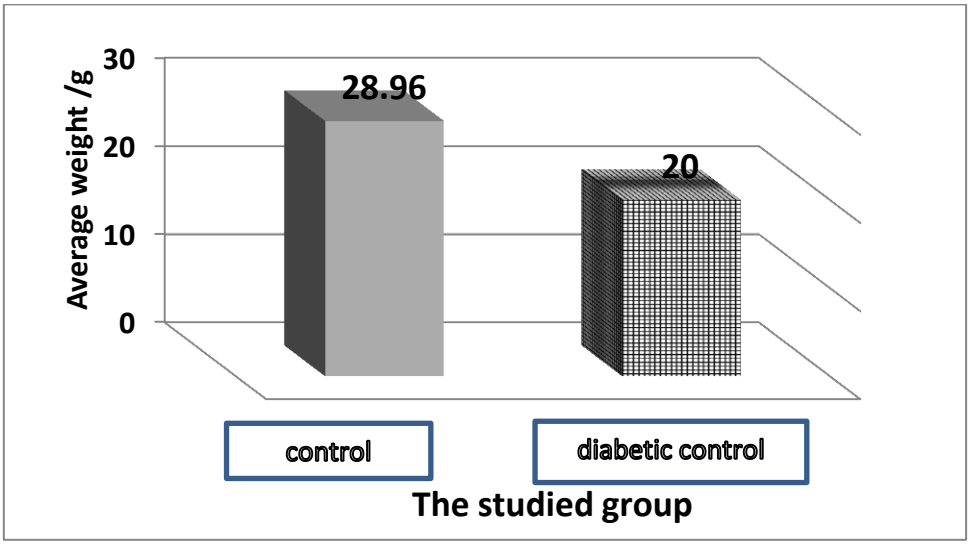

Figure (1) Changes in the level of weight in the physiological control and the group in which diabetes was induced.

4-1-2 Study of the change in blood glucose after injection of Alloxan:

The average blood glucose concentration of experimental mice was measured before and after the injection of Alloxan, as shown in the following table:

Table (2) Changes in the blood glucose level of mice before and after injection of Alloxan.

variable	Before	After	Average difference	T. test	P- vaue	Result
Glucose	141.00	395.14	254.14	6.17	00 **	Statistically significant

The results of the statistical analysis showed a significant (p-value<0.05) increase in the average glucose after the injection of Alloxan, meaning that the injection of Alloxan increased the blood glucose of the animal by 24%.180.

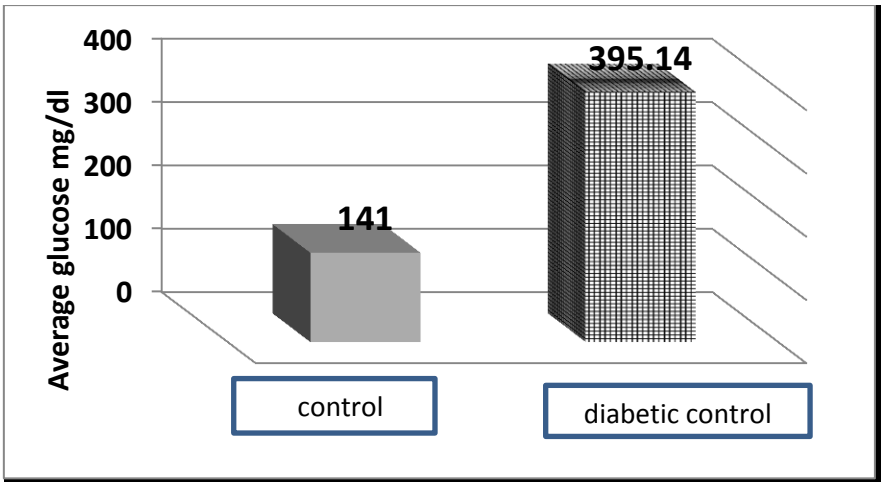

Figure 2: Changes in blood glucose level in the physiological control and the group in which diabetes was induced.

4-1-3- Study of Peptide-c change after injection with Alloxan:

The average Peptide-c in the blood of experimental mice was measured before and after the injection of Alloxan, as shown in the following table:

Table (3) Changes in the level of Peptide-c in mice before and after injection of Alloxan.

variable	Before	After	Average difference	T.test	P- vaue	Result
Peptide-c	0.20	0.63	0.43	2.73	0.017 **	Statistically significant

The results of the statistical analysis showed that there were significant, p-value<0.05, differences in the average of Peptide-c after injection with Alloxan, and that there was a significant increase in the average of Peptide-c after injection with Alloxan, meaning that the injection of Alloxan increased the proportion of Peptide-c in the animal by 215.54%, and we illustrate this in the Figure 3.

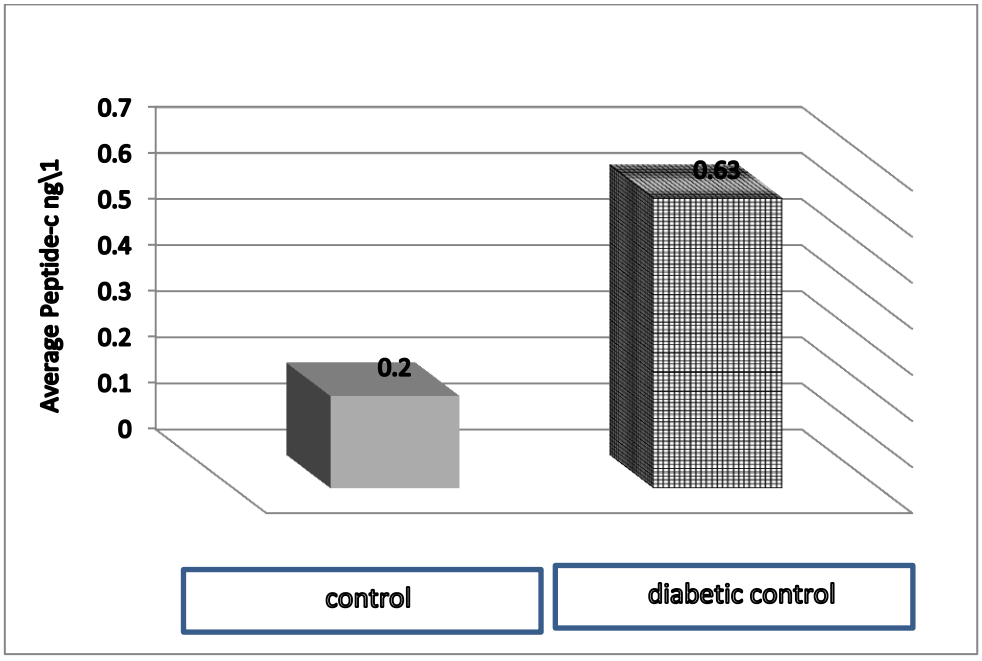

Figure (3) Changes in the level of Peptide-c in the physiological control and the group in which diabetes was introduced.

4-2- Studying the effectiveness of the alcoholic extract of garlic and ginger together:

4-2-1 On the weight of mice:

The average weight of experimental mice was measured after treatment with alcoholic extract of garlic and ginger together and compared with

The group in which diabetes was induced with the other group treated with Glibenclamide, Student's paired sample t-test was used for comparison as shown in the table(4).

Table (4) Changes in the weight of mice treated with garlic and ginger extracts together compared to those with diabetes and the group treated with Glibenclamide.

variable	Induced with diabetes	Treatment with garlic and ginger extract	Average difference	T.test	P- value	Result
Weight	20	25.80	5.80	2.69	0.018 **	Statistically significant
variable	Glibenclamide sample	Treatment with garlic and ginger extract	Average difference	T.test	P- value	Result
Weight	23.84	25.80	1.96	0.93	0.370n.s	No-tatistically significant

The results of the statistical analysis showed that there were significant statistically significant differences in the average weight after treatment with garlic and ginger extract compared with the group in which glucose was induced, and a significant increase in the average weight after treatment with garlic and ginger extract, meaning that treatment with garlic and ginger extract increased animal weight by 29% This is illustrated by Figure (4), while when comparing with Glibenclamide, it was found that there were no significant differences in the average weight and from the value of the T test, it was found that the average weight when treated with garlic and ginger extract was higher than the mean weight when treated with Glibenclamide by 8.22%. As shown in Figure (5)

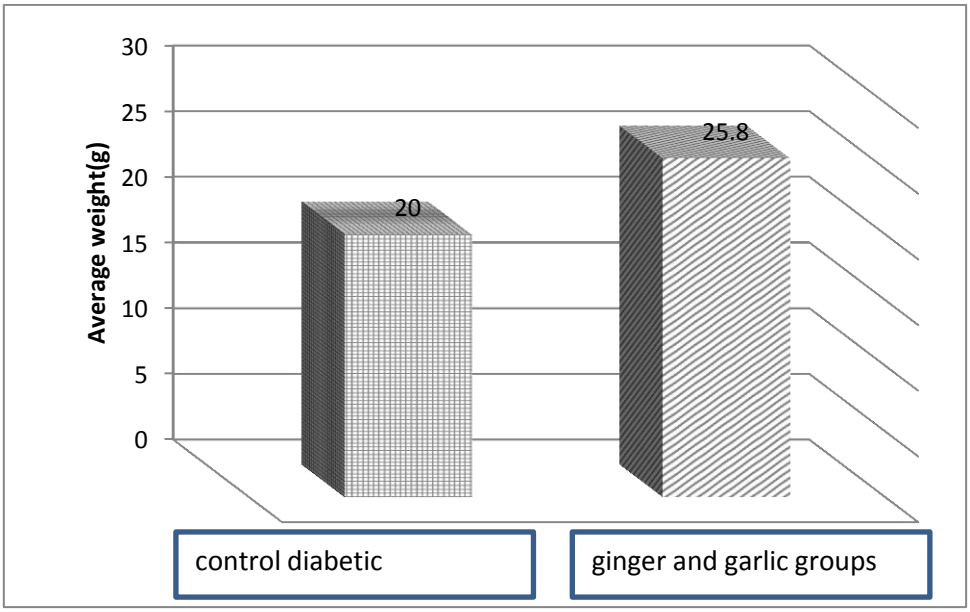

Figure (4) Changes in the average weight of mice after treatment with garlic and ginger extracts, which induce diabetes.

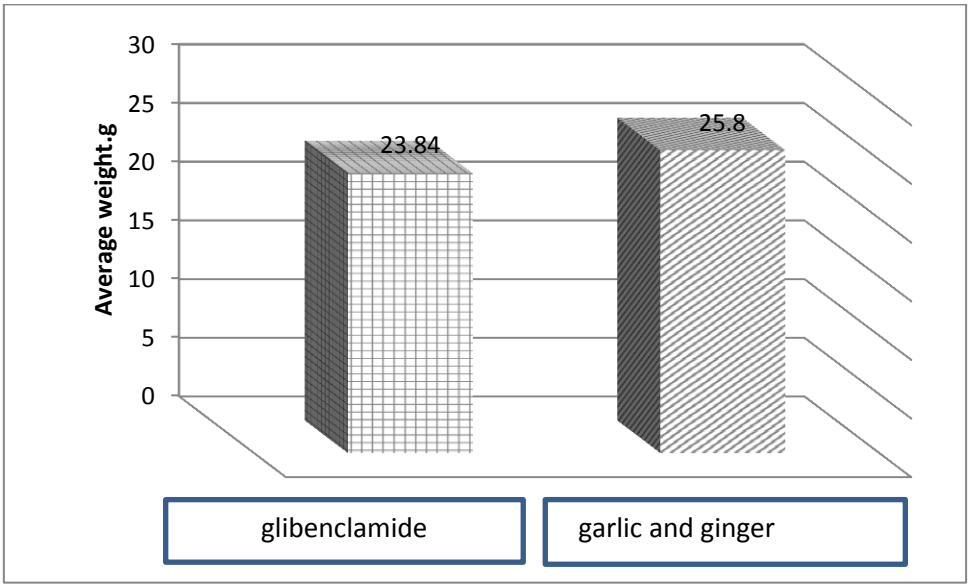

Figure (5) Changes in the average weight of mice after treatment with garlic and ginger extracts together and the group treated with Glibenclamide.

4-2-2 Average blood glucose of mice after treatment with garlic and ginger extracts:

The average blood glucose of experimental mice was measured after treatment with alcoholic extract of garlic and ginger together and compared with the group in which diabetes was induced and also compared with the other group treated with Glibenclamide, where Student's paired sample t-test was used for comparison as shown the table (5).

Table (5) Changes in blood glucose of mice treated with garlic and ginger extracts together in comparison with those with diabetes and the group treated with Glibenclamide.

variable	Induced with diabetes	Treatment with garlic and ginger extract	Average difference	T.test	P- value	Result
Glucose	395.14	253.88	-141.27	-2.90	0.012*	Statistically significant
variable	Glibenclamide sample	Treatment with garlic and ginger extract	Average difference	T.test	P- value	Result
Glucose	193.8	253.88	60.08	1.78	0.102n.s	No-tatistically significant

The results of the statistical analysis showed that there were significant statistically significant differences in the average blood glucose of rats after treatment with garlic and ginger extracts together compared to the group in which the glucose was induced, and that treatment with garlic and ginger extract reduced the blood glucose of mice by 35.75%, as shown in the figure (6), while when comparing the group treated with garlic and ginger extracts together with the drug Glibenclamide, it was found that there were no significant statistically significant differences in the average blood glucose between the two

groups, and the t-test value showed that the average glucose after treatment with garlic and ginger extract is higher than the average glucose after treatment with Glibenclamide by 31%, and this is illustrated in Figure (7).

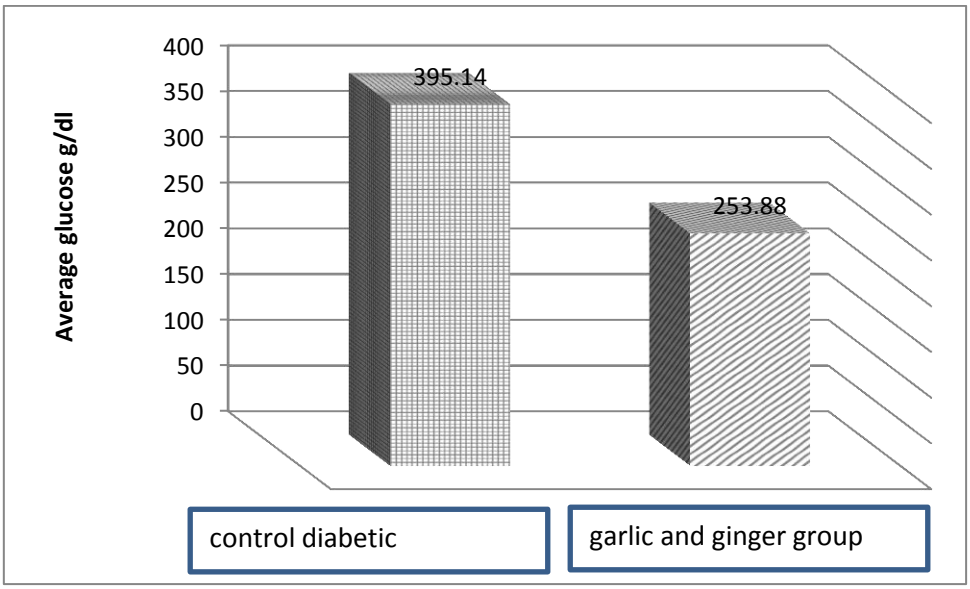

Figure (6) Changes in the average weight of mice after treatment with garlic and ginger extracts, which induce diabetes.

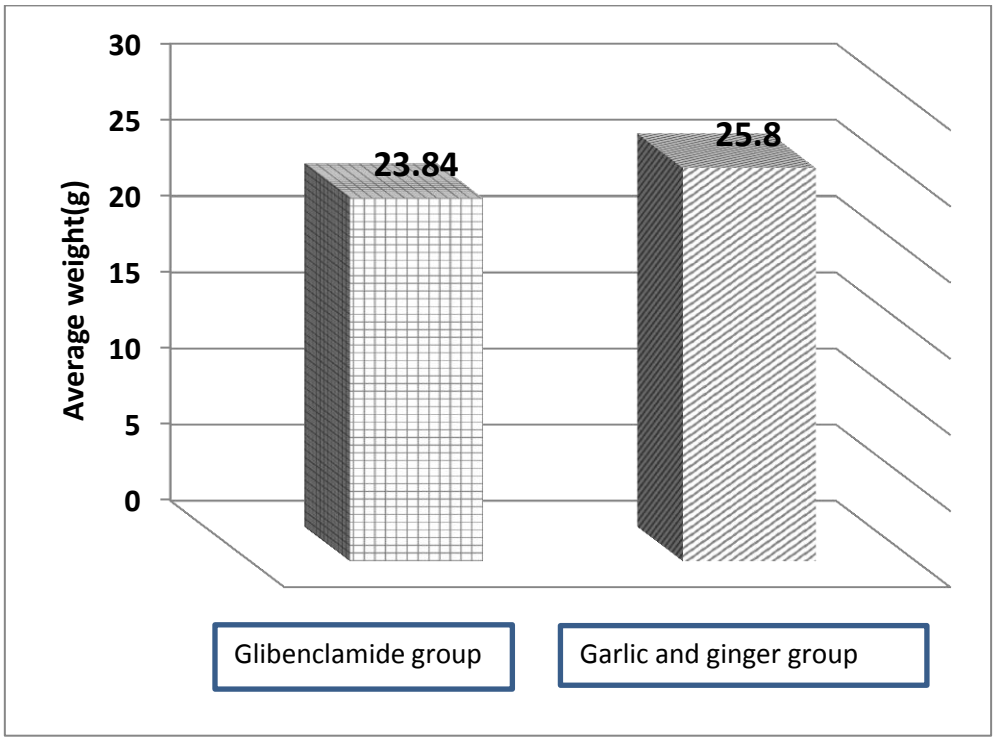

Figure (7) Changes in the average blood glucose of mice after treatment with garlic and ginger extracts together and the group treated with Glibenclamide.

4-2-3 Peptide-c:

The average concentration of Peptide-c was measured for experimental mice after treatment with alcoholic extract of garlic and ginger together and compared with the group in which diabetes was induced and with the other group treated with Glibenclamide, where Student's paired sample t-test was used for comparison as shown in the table(6) .

Table (6): Changes in the concentration of Peptide-c for mice treated with garlic and ginger extracts together compared to the diabetic and the group treated with Glibenclamide.

variable	Induced with diabetes	Treatment with garlic and ginger extract	Average difference	T.test	P- value	Result
Peptide-c	0.63	0.06	-0.57	-3.66	0.003**	Statistically significant
variable	Glibenclamide sample	Treatment with garlic and ginger extract	Average difference	T.test	P- value	Result
Peptide-c	0.25	0.06	- 0.19	-6.64	0.**	Statistically significant

The results of the statistical analysis showed, and accordingly, there were Statistically significant differences (p-(value<0.05) in the average Peptide-c after treatment with garlic and ginger extracts together, compared with the group in which glucose was introduced, and that there was a significant decrease in the average Peptide-c after treatment with this extract by 90.48%. That figure (8), and the results also showed that there were Statistically significant differences in the average of Peptide-c after treatment with both garlic and ginger extracts compared with the sample of Glibenclamide. From the t-test value it was found that the average Peptide-c after treatment with both garlic and ginger extracts is less than the average Peptide-c after Treatment with Glibenclamide by 76%, as shown in Figure (9).

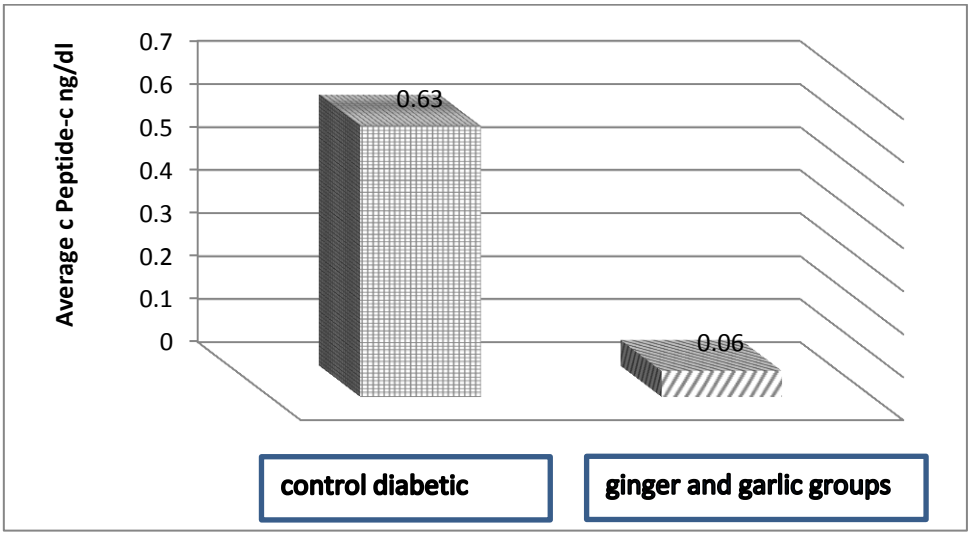

Figure (8) Changes in the concentration of Peptide-c in mice after treatment with garlic and ginger extracts together and the group in which diabetes was induced.

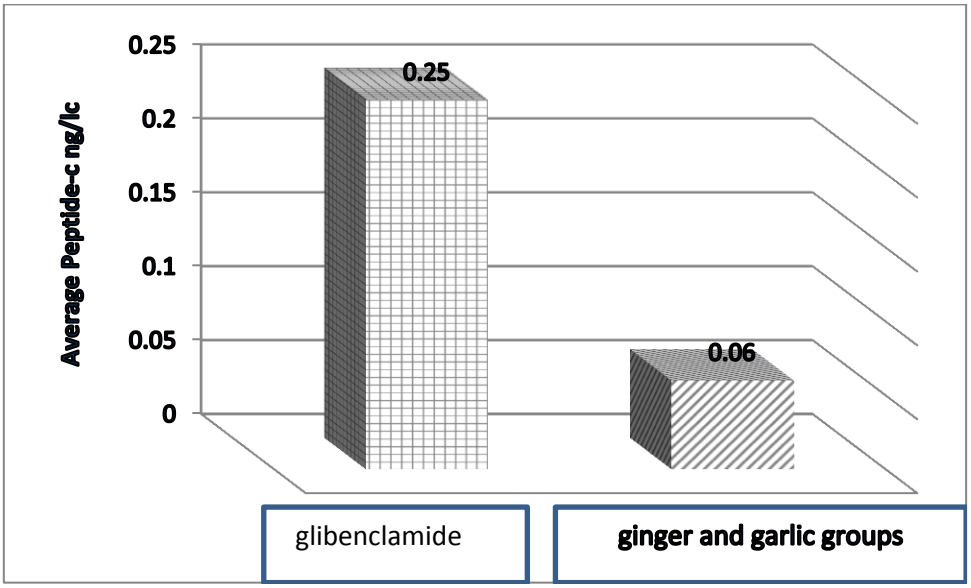

Figure (9) Changes in the concentration of Peptide-c in mice after treatment with garlic and ginger extracts together and the group treated with Glibenclamide.

5-Discussion.

The level of glucose in the blood serum after the injection of Alloxan reached (395) mg / dl, and this increase is significant compared to the concentration of blood glucose in the physiological group (141) mg / dl. These results are consistent with the findings of a number of researchers in other studies (3, 16,18,2,6)

There are several mechanisms to explain the effect of Alloxan in inducing diabetes

Interaction with the sulfodyl group (SH) [the presence of this group in beta cells is necessary for the synthesis of insulin] According to this theory, the interaction of the Alloxan with the (SH) group present in the enzyme Glucokinase (23), as the inhibition of the previous enzyme leads to the continuation of secretion Insulin and the consumption of its stock from beta cells and loss of sensitivity of these cells to the level of glucose in the blood, leading to insulin inhibition and contributing to the destruction of beta cells (27).

As for the second mechanism to explain the effect of Alloxan, it was shown that Alloxan generates oxygen water H2O2, which inhibits the event of depolarization, which is the main step that precedes the entry of calcium ions into the β-cell to release and synthesize insulin (11,33) Also, Alloxan causes an abnormality in the DNA of the cells of the islets of Langerhans in the pancreas, causing damage to the beta cells (7).

The researcher Chon et al., 1994 (9) also showed the mechanism of action of Alloxan to cause diabetes, as it converts inside the body quickly to Dialuric acid, which self-oxidizes to give the free hydroxyl radical responsible for destroying beta cells. Based on this, it can be said that the effect of Alloxan on beta cells is direct or indirect, ultimately leading to diabetes.

As the blood glucose concentration rises, the levels of Peptide-c rise, as shown in Chart (3) as an indicator of increased insulin secretion, i.e. the occurrence of insulin resistance and consequently the decrease in the number of insulin receptors and their lack of familiarity with it (26) and as a reaction to the increase in blood glucose levels, where the peptide was measured c Measures insulin-secreting activity of beta cells (19,22).

The concentration of glucose in the blood serum after treatment with alcoholic extract of garlic and ginger together reached (253.88) mg / dl, and this decrease was significant compared with the concentration of blood glucose in the control group who developed diabetes only (395) mg / dl, and when comparing its effect with the drug Glibenclamide it was found It contributes to lowering blood glucose equivalent to the drug Glibenclamide, this is consistent with the results of the researchers (21, 12), where the percentage of reduction when treated with garlic extract was (32%) while with Glibenclamide (41%), due to the reason Garlic works to lower blood glucose by increasing the formation of glycogen from excess glucose in the body by a mechanism similar to the mechanism of the effect of the drug Glibenclamide by inhibiting the glucose enzyme -6 phosphatase (17), while the drug works to encourage the entry of glucose into the body. body cells and reduce the production of the glucagon hormone (10).

However, long-term use of the drug leads to asphyxia, myocardial infarction, and stroke due to its strong affinity for S4R2A receptors as well as its selective affinity for SRR1 beta cells (Steven et al., 2007). Also, garlic and ginger, thanks to the important chemical compounds in its components, the most important of which are Allixin, S-Allyl Cysteine Sulfoxide, Diallyl Trisulfide (28,24) and the gingerol compound found in ginger extract (3) leads to an increase in Beta cells are sensitive to the level of blood glucose and the increase in insulin production in response to the high blood glucose level (this rise is for specific minutes, the half-life of insulin is 5 minutes) until finally the levels of glucose, insulin and Peptide-c return to their normal values as in the physiological control and thus protects ginger and garlic extract from Side Complications of Diabetes (14).

The reason is also that garlic extract scavenges free radicals and increases the antioxidant enzymes glutathion peroxidas- glutathion catalase- superoxid dismutase, in addition to containing large amounts of flavonoids, especially the compound Genistein- Resveratrol, which leads to the repair and restoration of beta cells and increase their doubling to increase insulin secretion (29,25).

The results of the study also showed a significant decrease ($P<0.05$) in the average weight of diabetic white mice after being treated with Alloxan, which was evident after a week of infection (Table 3), when compared with the weights of the physiological control white mice (24,5,15) The mice in the diabetic control group also appeared slender, weak and slow moving.

The reason for weight loss is due to the development of diabetes mediated by Alloxan, which works to destroy the pancreatic beta cells responsible for producing the necessary insulin hormone, to

facilitate the entry of glucose into the cells and energy production. Glucose deficiency [Inducing glucose from a non-glucose source] (15).

The reason for the decrease in the weight of diabetic mice is also due to increased urination that causes dehydration and a decrease in useful fluids for the body or to the breakdown of muscles caused by an increase in blood glucose (1).

The results of the statistical analysis when treating mice with alcoholic extract of garlic and ginger together showed a significant ($P<0.05$) increase in the weight of infected mice, this is consistent with what was stated in the researcher's study (34).

This effect may be due to the fact that garlic extract regulates blood glucose levels and returns insulin to its normal level. This hormone works to secure the necessary energy for the body through glucose instead of fats and proteins, thus increasing their structure and then increasing body weight, this is consistent with the interpretation of the researcher (5) or it may be due to the improved health in diabetic mice treated with garlic extract. This results are in agreement with the results of Thomson (31).

6- Conclusions.

1- The important role of garlic and ginger extracts in returning high levels of Peptide-c as a result of developing diabetes to their normal values as in the physiological evidence.
2- The important role of garlic and ginger extracts in lowering blood glucose equivalent to Glibenclamide.

7- Recommendations.

We recommend making use of the active ingredients of garlic and ginger in making pharmaceutical drugs that can match the role of chemical drugs with negative side effects.

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