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The Role of Allicin in Regulating Insulin and Glycemic Level in White Mice with Induced Insulin Resistance

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Abstract

The current study was carried out from January 2020 to April 2020, to examine the effect of allicin extracted from garlic on some functional variables of rats affected by induced insulin resistance, the results of the current study showed , at the tenth week when subjected to a nutritional system rich with glucose, a marked elevation in fasting serum glucose level, glycated hemoglobin (HbA_{1C}) and insulin hormone, in addition to the presence of noticeable changes in lipid profile represented by significant elevation in the level of serum cholesterol and low-density lipoproteins (LDL), with a reduction in the rate of high-density lipoproteins (HDL). Administration of allicin and metformin at the twelfth week after a nutritional system rich with glucose showed significant changes represented by reduction in fasting serum glucose level, glycated hemoglobin (HbA_{1c}), insulin hormone, serum cholesterol level and LDL, and an elevation in HDL level when compared with the control group, while there were not any significant changes between allicin and metformin.

Keywords:allicin; insulin; glycemic, white mice; insulin resistance.

Introduction

Non-insulin dependent diabetes is considered the second growing health concern all over the world, characterized by metabolic disturbances and a severe elevation of blood glucose concentration higher than the normal average; due to a decrease in insulin hormone secretion or tissue sensitivity towards insulin or both **(World Health Organization, 2011;Mealey& Ocampo,2006)**.The term (insulin resistance) is often used synonymously with impairment of glucose uptake induced by insulin**(London *et al* ., 2021)**.In all the main target tissues, furthermore damage to beta-cells caused by autoimmunity is not present like the first type of diabetes, and the patients are still able to produce insulin, mostly insulin resistance is associated with several complications such as obesity, hypertension and dyslipidemia**(Eiziriki*et al* ., 2020)**, hence most type-2 patients are obese or have an elevated percentage of body fats distributed primarily in the abdomen **(Karamitri & Jockers, 2019)**.Toavoid diabetes complications modern medicine considers non-pharmacological approaches like lowering calories and increasing physical activity which are basic in treating insulin resistance.(**Anwar *et al* ., 2018) .**

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Garlic is one of the most important plants in healing power and it is one of the most used ingredients all over the world regarding its health benefits. Garlic consumption is associated with low risk of heart diseasesand atherosclerosis (**Eksi *et al* ., 2020).**It can also stimulate the immune system to resist possible diseases and maintain health. It is able to stimulate the lymphatic system to hasten the removal of wastes from the body, it also considered an active antioxidant which protects cells from free radicals' damage and can help in preventing some malignancies, heart diseases, stroke, viral infection, **(Gebreyohannes & Gebreyohannes,2013)**.In lowering blood pressure, reducing blood glucose levels, preventing blood coagulation, lowering cholesterol and lipids, resisting several stresses and antiaging effects**(Naderi *et al* ., 2019)**.

Garlic contains no less than 33 sulfur compounds and a number of enzymes and metals such as germanium, magnesium, selenium and zinc, in addition to vitamins A, B_1 and C and fibers, water and 17 amino acids**(Qiu*et al* ., 2020)** .Garlic contains a higher level of sulfur compound compared to other species in the same genus, which are responsible for its pungent smell and sharp taste and they have a medical importance**(Mostafa,2020)**. One of the most important biologically active compounds present in garlic is allicin (diallyl-thiosulfate) **(Doganturk *et al* ., 2019)** . which is not present in raw garlic, but it is produced rapidly by the effect of CS-lyase (allinase) on alliine, the former is considered the main sulfur compound in all raw and powdered garlic, allinase is activated by crushing or chopping garlic clovesthus raw crushed garlic contains a high level of allicin**(Osman *et al* ., 2020)**.

Allicin is an antioxidant that is active in controlling free radicals and supporting the role of enzymatic antioxidants, in addition it is an effective antibiotic that inhibits microorganism growth such as bacteria and fungi **(El-Saber Batiha *et al* ., 2020)** . It also lowers cholesterol, triglycerides and glucose levels in blood, it prevents platelet aggregation and hypotension and so does insulin **(Karagodin *et al* ., 2016).** which encourages the approach of combining nonpharmacological method and pharmacognosy.(**Ashish Jain , 2017) .**Current study was carried out to identify the efficacy of allicin in adjusting tissue sensitivity toward insulin and to treat insulin resistance.

Materials and procedure

Allicin was extracted as a natural product from the reaction of garlic allinase enzyme with synthetic preparation of alliine [(+)S-2-propenyl L-cysteine S-oxide] identical in chemical properties to natural alliine. As stated in method 18 **(Mirelman *etal*., 1997)**. Allicin purity and concentration were determined using high performance liquid chromatography (HPLC) technology. **(Rabinkov *et al* 1994& Rabinkov *et al* ., 1998**)

Allicin solution concentration was 2.0 – 2.4 mg/mL dissolved in phosphate buffer solution 50 mmol/L which was stable chemically during the period of the experiment with a pH of 6.8, allicin solution was preserved at 4^0C until the beginning of the experiment.

In the current study, 24 male white rats with a mean weight of 250g were used, they were fed a nutritional system rich with glucose for 12 weeks, and then were divided randomly to the three groups as follows: group (1) control group was not treated, group (2) was treated with 8mg/kg/d allicin using oral dosing in the last two weeks, group (3) was treated with metformin at a concentration of 8mg/kg/d using oral dosing in the last two weeks.

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Blood samples were taken from the ear at the beginning of the experiment and after 10 weeks under the influence of weak anesthesia, and also at the end of the experiment using direct heart stab after the dissection of the animal and pancreatectomy, and preserving the pancreas in a 10% formalin fixate from all the mice after 5 hours of fasting. Centrifugation was performed to the samples, and they were preserved in special tube at 4^0C until the beginning of measurement of fasting serum glucose, glycated hemoglobin (HbA_{1c}), insulin hormone, C-peptide and lipid profile.The (**Bancroft and Gamble, 2008)**method was used to prepare the histological sections

Results

The nutrition dependent on nutritional system rich with glucose for ten weeks led to increasing levels of fasting serum glucose, glycated hemoglobin (HbA_{1c}) and insulin hormone as shown in table (1), as well as the presence of marked changes in lipid profile represented by a significant increase in the level of serum cholesterol and low-density lipoproteins and a decrease in high-density lipoprotein as shown in table (2).

Administration of allicin and metformin separately in the twelfth week after a nutritional system rich with glucose showed significant changes represented by a decrease in fasting serum glucose, glycated hemoglobin (HbA_{1c}) and insulin hormone as shown in table (1), in addition to a reduction in serum cholesterol and low-density lipoproteins and an elevation in high-density lipoproteins compared to the control group as shown in table (2), while the results fluctuated in the activity of allicin compared to the metformin drug between significant and insignificant changes at a probability level of ($P \leq 0.05$), despite that both agents were active in lowering negative effects associated with insulin resistance.

Table (1) shows the effect of treatment with allicin and metformin on Insulin,RBS, and Hba1c.

Hba1c(%)			**RBS mmol/L**			**Insulin ng/ml**			**Parameter**
Week12	Week10	Week1	Week12	Week10	Week1	Week12	Week10	Week1	**Group**
6.75	5.56	4.34	11.30	9.82	4.79	10.86	10.53	6.90	**G1(Control)**
4.28	5.33	3.58	6.72	8.89	4.55	8.12	9.96	7.34	**G2(Allicin)**
4.13	5.83	4.21	5.69	7.97	5.10	7.52	9.78	7.02	**G3(Metformin)**
0.15			1.3			0.6			**LSD**

Table (2) shows the effect of treatment with allicin and metformin on lipid profile levels

Cholesterol Mg/dl			**LDH Mg/dl**			**LDL Mg/dl**			**Parameter**
Week12	Week10	Week1	Week12	Week10	Week1	Week12	Week10	Week1	**Group**

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84.20	80.63	78.52	18.89	21.58	25.42	52.46	48.42	32.46	**G1(Control)**
78.87	83.35	71.62	22.34	20.48	24.88	37.52	45.50	34.12	**G2(Allicin)**
74.12	82.44	70.76	23.82	22.16	25.56	35.23	43.81	33.80	**G3(Metformin)**
4.75			1.48			2.29			**LSD**

The histological study

The results of the microscopic examination showed that the pancreatic tissue consists of two secretory parts, one of them is Exocrine portion(EP) (Alveolar acinar gland) and the other part is the endocrine gland (represented by Langerhans islets) (LI). In both, there were no significant histopathological changes as a result of treatment with allicin and metformin, and this result does not raise concerns in people with diabetes or insulin resistance from the use of allicin as an alternative regulator or support for metformin.

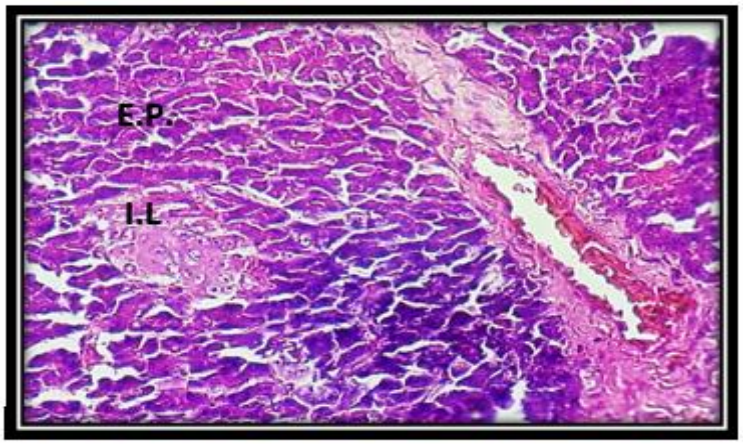

Figure 1 cross-section of the pancreatic tissue of the control group showing the exocrine portion and the islets of Langerhans

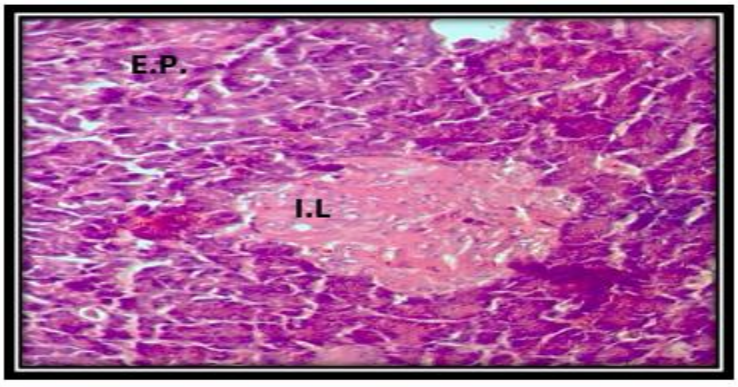

Figure 2 cross-section of the pancreatic tissue of the group treated with allicin showing the exocrine portion and the islets of Langerhans

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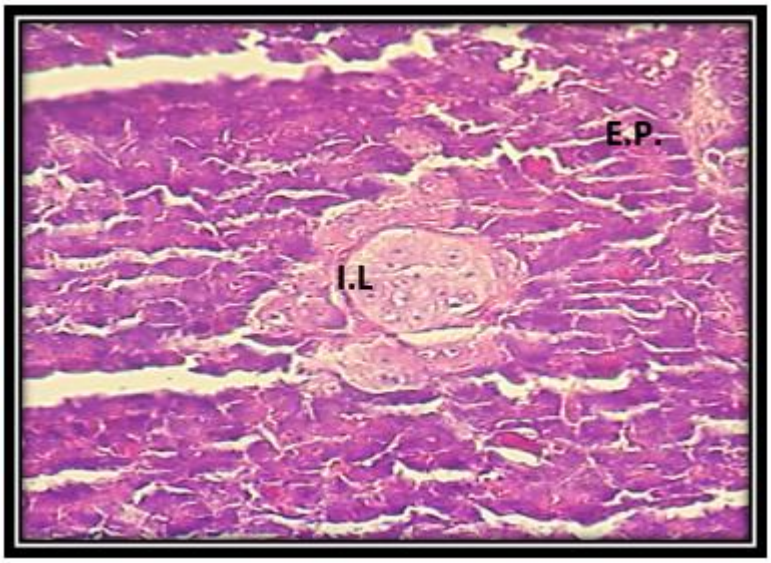

Figure 3 cross-section of the pancreatic tissue of the group treated with metformin showing the exocrine portion and the islets of Langerhans

Statistical Analysis

The results were analyzed statistically by using statistical analysis program SPSS Version 16 using ANOVA analysis and by extracting the least significant difference L.S.D.

Discussion

Hyperglycemia occurs in type-2 diabetes patients, it is possible that they suffer elevated glucose levels in blood with the case developing in a matter of weeks because of the depending on meals rich with glucose, and in contrast to diabetic ketoacidosis that occurs in type-1 diabetes, the ketones are not present in urine in this case, the reason behind that is that people with type-2 diabetes are able to secrete insulin, so the ketonic acid is not produced. This elevation is mostly due to the presence of insulin resistance in target cells **(WHO and IDF, 2006)** which leads to hyperglycemia **(Bluestone, 2010; Michael et al 2000)**, and when glucose levels are elevated in blood, the body responds by secreting insulin. Insulin is a vital hormone that ensures that the sugar is stored in our bodies to be used as energy source between meals, the storage form of cholesterol is known as bad cholesterol (LDL), so if insulin levels are elevated, cholesterol levels are elevated too, whereas the non-storage form of cholesterol is the good cholesterol (HLD), whose levels are reduced with insulin levels elevation.

The attachment between glucose and hemoglobin is anirreversiblechemicalinteraction, thus it persists the whole life of mature red blood cells (approximately 120 days), and since the diabetic patients have high levels of glucose in blood, a higher number of glucose molecules will attach to hemoglobin by the process of (glycation) leading to an elevation of glycated hemoglobin (A_{1c}) in blood**. (Xie *et al*., 2007).**

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