

Compared to liver, bile duct and skeletal muscle garlic (versus normal saline) only enhances pancreas insulin m-RNA transcription that corresponds to islets cellular plasticity and local and circulating hyperinsulinemia in diabetic rats

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Abstract

Background: Garlic aqueous extract (GE) augments insulin circulating concentration in streptozotocin-induced type-1 diabetes mellitus (STZ-DM) rat model.

Objective: This study investigated at 4 stages in a time-line fashion whether modifications in insulin m-RNA transcription occur, and if they do, are they related to accumulative changes in insulin - serum and tissue concentrations and immunohistochemical (IHC) localizations and plasticities as part of the GE-induced insulinogenic mechanism(s) in the pancreas, liver, bile duct and gastrocnemius skeletal muscle (GSM) of STZ-DM rats.

Method: The body weight, food and water intake, urine output, fasting blood glucose (FBG), serum insulin (SI), in addition to tissues insulin concentrations, IHC localizations and numerical intensities and m-RNA transcriptions were investigated before (basal level = BL) and after 1, 4 and 8 weeks of oral treatment in normal rats given normal saline (NR-NS), diabetic rats given normal saline (DR-NS) and diabetic rats given GE (DR-GE). The readings collected were compared using two-way ANOVA with LSD post-hoc test (IBM SPSS - V.22) and differences were considered significant when $P < 0.05$.

Results: Compared to NR-NS, DR-NS showed typical diabetic biophysical symptoms in addition to significant accumulative increases in FBG and reductions in SI, which corresponded positively with reductions in pancreatic insulin IHC localization and numerical intensity and m-RNA transcription. Conversely, and in comparison to DR-NS, DR-GE showed amelioration of diabetic biophysical symptoms, in addition to significant accumulative decreases in FBG and increases in SI, which corresponded positively with increases in pancreatic insulin IHC localization and numerical intensity and m-RNA transcription. The liver, bile duct and GSM did not show any changes in the targeted parameters (or indicators of insulin synthesis: proinsulin or C-peptide) in response to GE treatment.

Conclusion: GE induced increases in circulating insulin concentration could have been due to increases in pancreas insulin concentrations as a result of an enhancement in islets cellular plasticity and m-RNA transcription. Accordingly, garlic insulinogenic action could be partly due to modification of pancreas genetic expression.

Introduction

Insulin, a dimer peptide, is a potent stimulator of cellular uptake of glucose, in addition to carbohydrates, lipids and protein anabolism. Through these actions and in antagonism with glucagon, insulin contributes chiefly to maintaining glucose circulating concentration within the homeostatic range of 4-6 mM [1]. As currently understood, insulin is synthesized and secreted mainly by the pancreas β -cells. Furthermore, insulin synthesis starts with the transcription of its respective gene into an m-RNA molecule, then ribosomal translation into its precursor preproinsulin, followed by conversion to insulin which is finally stored in wait for release principally in response to acute hyperglycemia [2]. Insulin release oscillates with a frequency of 3-6 minutes giving a circulating concentration between meals that ranges

between 57-79 pmol/l [3]. A sustained decline in insulin circulating concentration, most probably due to an autoimmune-induced β -cell hypoplasia (hypoplasticity), results in chronic hyperglycemia and subsequent array of linked biochemical and physiological abnormalities that are collectively designated as diabetes mellitus (DM) type-1 [2].

Streptozotocin (STZ) is a naturally occurring glucosamine-nitrosourea substance that has a potent antineoplastic activity and was found to be selectively toxic to the mammalian pancreas β -cells. Being structurally comparable to glucose and therefore lipophobic, STZ is selectively taken up and accumulated into active β -cells that express the glucose transporter protein GLUT2. In these initially functional β -cells, STZ causes severe DNA alkylation, then fragmentation and accordingly cellular necrosis [4]. The destruction of β -cells leads to an immediate and progressive hypoinsulinemia, which is followed by the principle classical symptom of DM, hyperglycemia. Typically, intraperitoneal administration of an adequate dose of STZ is used as a method to chemically induce DM type-1 rat model for investigative studies [4, 5]. Additionally, it is documented that STZ is rapidly cleared from the body with a half-life of 15 to 40 minutes [6].

The herb, *Allium sativum L.* (commonly known as garlic) was reported to effectively bring about euglycaemia in the STZ-induced DM type-1 rat model [7]. Several mechanisms were suggested to explain this euglycaemic action of garlic, and its derivatives, one of which is the induction of hyperinsulinemia making the peptide more readily bioavailable to its target cells [7, 8, 9]. Since STZ ablates the insulin producing pancreas β -cells, then it became a matter of interest as to where this garlic-induced hyperinsulinemia results from in this respective model of DM. One possible explanation for this garlic hyperinsulinemic action could be drawn from the findings of the following studies. It was reported that exogenous bioactive compounds found in a multitude of plants and herbs, which are part of many diets, impart function-changing molecular actions [2, 10, 11, 12]. Furthermore, and as far as garlic is concerned, many of its biologically active organosulfur molecules were also reported to have such molecular actions and at the genetic level activating and / or inactivating transcriptions of particular genes [13, 14]. Accordingly, there is a good possibility that garlic's hyperinsulinemic action could be the result of β -cells neogenesis and / or transdifferentiation [10] as stated hereafter.

As reviewed by Mansouri et al [15], many studies reported that the cells of the islets of Langerhans have the potential to renew and regenerate. More precisely, and as far as garlic is concerned, this herb was found to reinstate marked insulin localization detected by immunohistochemical targeting in the pancreas of STZ-induced diabetic rats [16, 17]. Furthermore, the liver, which as the pancreas arises from the anterior endoderm during early stages of embryonic development, has cells with the potential to switch to insulin producing cells *in vitro* as demonstrated in various models [18, 19, 20]. Furthermore, Sahu *et al.* [21] have shown the presence of insulin-producing cells in the hepatic biliary duct and the gall bladder of a diabetic adult human female. Similar findings were reported in mice where genetic lineage labelling has shown the presence of insulin and C-peptide [22] with cells expressing pancreas transcription factors in extra-hepatic bile ducts [23]. Intra-hepatic biliary epithelial cells were also induced *in vitro* to give rise to insulin-producing cells with typical functional markers such as GLUT2 [24]. Insulin

producing cells were also found in other organs of diabetic rodents. The transcription of insulin m-RNA as well as the expression of its protein were reported in a variety of tissues including adipose, bone marrow, spleen and thymus of different diabetic animal models [25]. More recently, adult muscle-derived stem cells (MDSC) were found to differentiate into insulin-producing cells co-expressing transcription factors specific to β -cells in response to a glucose challenge *in vitro* [26, 27]. These differentiated MDSC were shown to decrease the blood glucose levels 12 days post engraftment into diabetic mice [26].

Method

Aim

This study investigated at 4 stages in a time-line related fashion the effect of GE treatment on insulin m-RNA transcription in the pancreas, liver, bile duct and GSM, and related detected changes to any corresponding plasticity of insulin producing cells and serum and tissues insulin concentrations in an early STZ-DM type-1 rat model.

This study was carried in compliance with the ARRIVE guidelines.

Animals

The animals used in this study were healthy male Sprague-Dawley rats (HR, b.wt. = 160-180 g, ancestors procured from Harlan Laboratories - UK). The rats were housed in the Animal Care Facility under regular ambient conditions (temperature = 22-24 °C, humidity = 30-35%, natural light/dark cycle) and provided with standard rodent diet (Special Diet Services, UK) and tap water *ad libitum*. The rats were handled, maintained and experimented on according to the 'Instructions Guide' for 'The Care and Use of Laboratory Animals', 'National Research Council' [28].

Diabetic rats

Type-1 DM was induced chemically in the required number of HR rats by injecting intraperitoneally each rat with a single dose of STZ solution (60 mg/kg/0.5 ml 0.01M sodium citrate buffer, pH 4.5) after 2 h fast as described previously [29]. A group of normal rats was established by injecting intraperitoneally each of the required number of HR rats with only 0.5 ml of citrate buffer solution.

Fasting blood glucose

Fasting blood glucose (FBG) of all rats was measured in a drop of tail-blood using a portable glucometer (OneTouch UltraEasy – LifeScan, UK) after an overnight fast at day-6 post-injection (basal level = BL) with either STZ or citrate buffer and at the end of weeks 1, 4 and 8 of treatment. At BL, rats injected with STZ and had FBG concentration ≥ 16 mmol/l were taken as Type-1 diabetic rats (DR, n = 80), while rats that received only citrate buffer solution and had FBG concentration ≤ 7 mmol/l were taken as normal rats (NR, n = 48).

Garlic aqueous extract

Garlic (*Allium sativum L.*) aqueous extract (GE) was prepared, stored and used as previously described [30]. GC-MS analysis showed that the GE preparation composition was stable before and after different times of storage at -20°C as previously reported [31]. The garlic cloves used were purchased from the local market at Kuwait City (commercially available - no permission is required) and the species of this garlic was identified by Dr. Majed A. Alnaqeeb (Associate Professor), the Department of Biological Sciences – Kuwait University. The voucher specimen number of the garlic species used is ALNAQEEB MA/001/1998 KTUH and the sample is kept at the Kuwait University Herbarium (KTUH).

Oral treatment

A group of NR (n = 36) was treated with normal saline (NR-NS, 0.5 ml/kg). Alternatively, a group of DR (n = 72) was divided into two subgroups: one group (n = 36) was treated with normal saline (DR-NS, 0.5 ml/kg) while the other group (n = 36) was treated with GE (DR-GE, 500 mg/ml/kg). The dose of garlic used was found to be most effective and safe. Treatments with either NS or GE were delivered to each rat at mid-morning as a single-oral daily-dose and continued for each group as shown in Fig. 1. A group of NR (n = 8) and a group of DR (n = 8) did not receive any treatment and their data represented BL biophysical and biochemical measurements as shown in Fig. 1.

body weight, food and water intake and urine output

The body weight, food and water intake and urine output for each rat were measured gravimetrically and volumetrically using metabolic cages at these times: day-7 post injection [BL - no treatment was given, NR (n = 8) + DR (n = 8)] and then at the end of the following weeks of treatment: week 1 [W1, NR-NS (n = 12) + DR-NS (n = 12) + DR-GE (n = 12)], week 4 [W4, NR-NS (n = 12) + DR-NS (n = 12) + DR-GE (n = 12)] and week 8 [W8, NR-NS (n = 12) + DR-NS (n = 12) + DR-GE (n = 12)] as shown in Fig. 1.

Rats sacrifice and collection of samples

Following induction of anesthesia (0.2 ml/100 g b.wt.) with a mixture of ketamine (9 ml, 10%, Dutch farm Nedar, Host den berg - Holland) plus xylazine (1 ml, 10 %, Interchemie, Vernary – Holland) each group of rats were sacrificed at the end of the designated time of treatment as shown in Fig. 1 and blood and tissue samples were collected for the following analyses:

i- Serum insulin concentration

Blood was collected via cardiac puncture from each rat at BL and then at the end of each time of treatment (W1, W4 and W8 - as shown in Fig. 1) and allowed to stand at room temperature. Serum was collected and stored at -20 °C and later analyzed for insulin (SI) concentration using ELISA analysis kit following the manufacturer's instructions (SPI bio, bertin pharma, France).

ii- Tissue insulin m-RNA expression:

Pancreas, liver, bile duct and left gastrocnemius skeletal muscle (GSM) were collected and stored at -20 °C for later analysis of insulin m-RNA expression at BL (day-7 post injection – no treatment) and the end of W1, W4 and W8 of treatment as shown in Fig. 1. Total RNA was extracted using RNeasy Plus Universal Mini Kit (Qiagen, Germany). Subsequently, reverse transcription of m-RNA to c-DNA was performed using SuperScript IV First-Strand Synthesis System (Life Technologies, Lithuania). Insulin m-RNA expression was estimated with qRT-PCR using TaqMan gene expression assay following the manufacturers' instructions (Applied Biosystems, USA).

iii- Immunohistochemical localization and intensity numerical estimation of tissue insulin

Pancreas, liver, bile duct and GSM were collected and analyzed for tissue insulin immunohistochemical localization (IHC) and intensity numerical estimation at BL (day-7 post injection – no treatment) and the end of W1, W4 and W8 of treatment as shown in Fig. 1. Each tissue sample was washed in PBS (pH 7.4 - Sigma, USA), fixed overnight in 4% paraformaldehyde (Merck, Germany), and then carried through routine histological preparation ending with embedding into paraffin wax (Leica Histo-embedder, Germany). Four-µm thick sections were cut, dewaxed and rehydrated then permeabilized with 1% Triton X-100 (Sigma-Aldrich, Netherlands). Antigen retrieval was performed using Tris-EDTA (Sigma, USA) for 60 min. To prevent endogenous peroxidase activity, tissue sections were blocked using a peroxidase blocking solution (DAKO Envision peroxidase system, DAKO). Additional blocking with 2% blocking buffer (Roche, Germany) in PBS was carried out to inhibit non-specific binding. Different tissue sections were then incubated overnight with rabbit anti-insulin-1 antibody (AbCam, UK), rabbit anti-C-peptide antibody (Cell Signaling Technology, USA) or mouse anti-insulin/proinsulin antibody (Invitrogen, USA) diluted in blocking buffer at 4°C. After several washes with PBS, tissue sections were incubated with species specific peroxidase labelled secondary antibody. The signal was detected using the 3,3',5'-Diaminobenzidine (DAB) substrate-chromogen kit specific for mouse or rabbit (Dakocytomation Envision System/HRP, DAKO, USA) followed by counterstaining with haematoxylin then mounting. To show localization (immuno-tagging) of insulin, immune-stained tissue sections were examined using light microscope equipped with a camera (ECLIPSE Ti2-E, Nikon, Japan) and images were captured. Insulin immunolocalizations intensity was also estimated by analyzing 3 images from each of 3 slides for each of 3 rats for each time of sacrifice for each group using a special software (NIS-Elements BR4.6) and following Nikon Image Analysis procedures.

iv- Determination of tissue-insulin concentration

Pancreas samples collected and stored at -20 °C were analyzed for insulin (PI) concentration at BL (day-7 post injection - no treatment) and the end of W1, W4 and W8 of treatment as shown in Fig.1 using ELISA analysis kit following the manufacturer's instruction (SPI bio, Bertin pharma, France).

v- Data presentation and statistical analyses

The readings of body weight, food and water intake, urine output, FBG, SI and PI concentration, m-RNA and IHC numerical intensity are presented in graphs as mean ± SEM. Images of PI-IHC are collated into 3

sets of images as x4 illustrations. The readings were compared using two-way ANOVA with LSD post-hoc test (IBM SPSS - V.22) and differences were taken as significant when $P < 0.05$.

Results

Body weight, food and water intake and urine output:

During the 8 weeks of the study, the NR-NS group showed normal behavior and biophysical and biochemical characteristics at the 4 stages of measurement (BL, W1, W4 and W8). As treatment with NS continued, this group of rats demonstrated the following when compared to BL and specifically at W8: a significant continuous increase in body weight reaching a maximum gain of 130% (Fig. 2), an insignificant trend of increase in food intake (Fig. 3), a steady amount of water intake (Fig. 4) and a constant volume of urine output (Fig. 5). Conversely, the DR-NS group showed a classical diabetic behavior and their body weight decreased continuously culminating into a significant maximum loss of 44% compared to BL, which was also less by 360% than the weight of NR-NS at W8 (Fig. 2). Additionally, and at BL and when compared to NR-NS values, these control diabetic rats ate 60% more food (Fig. 3), drank 450% more water (Fig. 4) and excreted 718% more urine (Fig. 5). These elevated values of intake and output remained steady during the next 3 stages of the experiment at W1, W4 and W8. Conversely, the DR-GE group showed almost a typical healthy behavior and a trend of gradual increase in body weight which at W8 became significantly higher by 43% compared to BL and by 136% compared to DR-NS at the same stage (Fig. 2). Furthermore, the DR-GE, showed an initial non-significant trend of gradual decrease in food (Fig. 3) and water intake; however, both of which became significantly less by 29% and 32% compared to BL and DR-NS at W8, respectively, (Fig. 4). As far as the DR-GE urine output is concerned (Fig. 5), these rats' daily urine excretion was significantly less by 15 and 19% at W4 and W8, respectively, when compared to DR-NS values and decreased by 15% at W8 when compared to BL.

Fasting Blood glucose and serum insulin concentrations:

The FBG and SI concentrations of the NR-NS at averages of 7.5 mmol/l and 1.02 pg/ml, respectively, were normal and steady during the 4 stages of the experiment (Fig. 6 & 7). In comparison to the NR-NS, the DR-NS showed a significantly elevated FBG by 238% at BL. This hyperglycemia increased by an additional 38% at W8 compared to BL (Fig. 6). Conversely, the SI of the DR-NS was significantly less by 468% at BL compared to that of the NR-NS. The control diabetic rats SI decreased by a further 34% at W8 when compared to BL (Fig. 7). The FBG of the DR-GE decreased significantly and progressively as treatment with GE continued compared to the DR-NS approaching the level of NR-NS at W8 and reducing that difference to only a significant 38% at BL (Fig. 6). This hypoglycemic action of GE was paralleled by continuous elevations in SI reaching as high as 2352% compared to DR-NS at W8 and getting to as high as 409% compared to BL in the same group (Fig. 7).

Pancreas insulin m-RNA transcription and immunohistochemistry:

Treating diabetic rats for 8 weeks with GE had no effects on insulin immunohistochemical localization or m-RNA transcription (or any indicator of insulin synthesis: proinsulin and C-peptide) in the liver, bile duct or GSM. Alternatively, treatment with GE led to significant increases in pancreas insulin localization and m-RNA expression as seen in the DR-GE relative data. Accordingly, only the m-RNA data and immunohistochemical images of the pancreas are presented hereafter.

As shown in Fig. 8, the NR-NS pancreas insulin m-RNA expression was consistent during the 4 stages of the experiment with an average value of 1.0 $\Delta\Delta$ CT. Oppositely, the DR-NS pancreatic insulin m-RNA expression at BL was significantly less by 99% than in the NR-NS. The m-RNA of the DR-NS decreased by a further 70% as time and treatment with NS proceeded to W8. The DR-GE pancreas insulin m-RNA, similar to that in the DR-NS, showed an initial decline which afterwards gradually, however insignificantly, increased by 50% and 67% at W4 and W8, respectively, compared to W1 of the treatment period.

As far as the pancreas insulin IHC is concerned, the NR-NS group images revealed typical localization (Fig. 9) and numerical intensity (Fig. 10) of insulin quantity at the 4 stages of the experiment (BL, W1, W4 and W8). Conversely and in the DR-NS, the BL image showed a considerably decreased IHC localization (Fig. 9) and numerical intensity (Fig. 10) of insulin quantity which became even less toward the end of the experiment. Alternatively, in the DR-GE, although the BL image (before commencing GE treatment) showed some evidence of insulin localization and distribution as seen at the BL in the DR-NS, however as GE treatment commenced and continued, the images showed an accumulative increase in insulin localization and typical distribution (Fig. 9) and numerical intensity (Fig. 10) starting at W1 and reaching a maximum toward the end of the experiment at W8.

Pancreas insulin concentration:

Since insulin m-RNA and IHC analyses were only positive for the pancreas, insulin concentrations were assayed only for that tissue. Accordingly, in the NR-NS group the measured PI concentrations were steady at the 4 stages of the experiment with an average of 772 pg/g tissue. In comparison to the NR-NS and at BL, the PI of DR-NS was significantly less by 86%. The DR-NS pancreatic insulin decreased even further by another 55% at W8 when compared to BL. In DR-GE, the GE-induced accumulative increase in the pancreas insulin IHC and m-RNA transcription were mirrored by progressive increases in PI reaching as high as 53% compared to BL and 243% compared to DR-NS at W8 (Fig. 11).

Discussion

Type-1 DM is a metabolic abnormality that is primarily caused by a reduction in pancreas β -cell mass and function leading to a decrease in insulin circulating concentration. Management of type-1 DM involves following special diets and lifestyle and the intake of anti-diabetic medication which in advanced cases include injections of exogenous insulin. Cell replacement therapy like islet transplantation is another management approach, however this treatment maneuver has its disadvantages as it is limited by donor availability and immune rejections [32]. Therefore, many studies have investigated alternative management possibilities such as β -cell neogenesis and / or transdifferentiation [33].

Garlic has been shown to alleviate many classical overt and covert symptoms of type-1 DM [7]. As previously reported and as shown in this study oral treatment with GE for 8 weeks attenuated diabetic effects on FBG, body weight, food and water intake and urine output. These ameliorative actions of garlic were suggested to be principally mediated via increasing insulin circulating concentrations making the peptide more readily available to its target cells [9]. In agreement, and in a more detailed assessment, this study reports for the first time a progressive amelioration of some of the principle diabetic symptoms which paralleled accumulative increases in the insulin circulating concentration of diabetic rats during the 3 successive stages of oral treatment with GE. In addition to assessing the time-related hyperinsulinemic genetic mechanism of garlic action, another principle objective of this study was investigating other sources of the elevated insulin concentration in the STZ-induced DM type-1 rat model by examining insulin localization and numerical intensity, tissue concentration and m-RNA expression in several potential target tissues and organs.

Although STZ specifically ablates pancreas β -cells, this organ still represented a strong potential target organ for investigating the insulinogenic action of garlic. It is documented that pancreas self-renewal and expansion does occur during normal physiological conditions like pregnancy [34] as well as obesity [35]. In addition, pancreas cells' plasticity has been demonstrated through their ability to regenerate following injury or gene induction [36]. In this study, the timely monitoring of insulin serum concentration and pancreas generation in response to GE showed accumulative increments in the levels of insulin circulating concentrations which paralleled corresponding genetic activation as seen in the increase in tissue m-RNA transcription, localization, intensity and distribution. It is highly possible that this pancreas regenerative capability of insulin could be due to presence of immature β -cells that evaded the destructive actions of the single dose of STZ administered to induce type-1 DM and later became activated / differentiated by GE. This assumption is based on the fact that pancreas progenitor cells have been shown to exist in various areas of the pancreas like the intra-islets or exocrine tissue including areas near the ducts and found to contribute to the regeneration of β -cells [15, 37]. In addition, transdifferentiation of fully differentiated pancreas exocrine cell types like acinar, ductal, centroacinar [38] or endocrine cells like α - and δ -cells to insulin-producing cells could have also occurred [15, 33]. Van der Meulen et al. [39] have shown that islets' periphery possibly holds a neogenic niche that houses transcriptionally immature β -cells that lack the cell surface marker GLUT-2 and therefore do not have the ability to sense glucose. These "virgin" β -cells represent an intermediates phase between the transdifferentiation of α and β -cells and may be prompted, in response to the right stimulus, such as garlic as in this study, to become functionally mature. This view is supported by the observation in this study of a possible increase in pancreatic Islets of Langerhans's IHC and numerical intensity in the DR-GE group. The chances that pancreas β -cells (or other cells types) could have regenerated without GE help is very minimal as the pancreas of the diabetic rats treated only with NS showed continuous deterioration in the Islets of Langerhans morphology and insulin indicators. Figure 12 shows a schematic illustration that summarizes garlic's possible insulinogenic mechanism of action in the pancreas of STZ-induced type-1 DM rat.

Further to the pancreas, β -like cells generation from other organs was another possibility that was explored by several investigators [40]. When compared to other non-pancreatic cells, the liver and the biliary system, which are derived from the same embryonic layer as the pancreas, have the highest probability to convert into insulin-producing cells. Moreover, the liver also has the ability to regenerate after injury from a progenitor cell population or oval cells [41]. Similar to β -cells, liver cells also possess GLUT 2 transporter. In 2000, Ferber et al [42] demonstrated the possibility of converting mouse liver cells into insulin-producing cells through genetic manipulation. Since then, many studies have also managed to produce β -like cells from liver [43, 44]. In addition, it was also reported that the common bile duct of the liver may naturally harbor β -cells [45] or be induced to become insulin-producing cells [26]. Based on these possibilities, in this study both the liver and bile ducts were also extracted and investigated for indicators of insulin and insulin generation (such as proinsulin and C-peptide). None of the targeted indicators of insulin were detected in either of these two tissues. Although, this current observation is inconsistent with the findings of Kim et al. [46] and Vorobeychik et al. [47] where higher dose of STZ were used and activation of liver progenitor oval cells into β -cells was observed using other stimulators (not garlic). Thus, GE might not be a potent inducer of β -cells neogenesis / differentiation and therefore insulin generation following only 8 weeks of treatment. Stronger stimulators like 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) has been shown to induce oval cells' differentiation into β -cells [48]. Having stated the previous, there is a strong possibility that longer treatment with GE might lead to liver cell differentiation into β -cells. In a recent pioret study, we have observed that GE treatment for the same period of 8 weeks enhanced the expression of the regulatory gene *MafA* in the liver of STZ-diabetic rats [49]. Along the same line and although Vorobeychik et al. [47] reported liver cells differentiation, however only proinsulin was detected suggesting an incomplete functional maturity. The GSM were also tested for the presence of insulin. This muscle tissue was chosen since it showed *in vitro* the possibility of converting adult derived stem cells that possess an effective regenerative ability to cells of a different lineage like insulin-expressing cells [26, 27]. Specific homing of the undifferentiated adult stem cells to the damaged islets caused their differentiation to insulin-producing cells. No evidence of such response to GE were observed in the current study.

List Of Abbreviations

1. BL: basal level.
2. c-DNA: complementary deoxyribonucleic acid.
3. D&A: data and analysis.
4. DM: diabetes mellitus.
5. DNA: deoxyribonucleic acid.
6. DR: diabetic rat.
7. DR-GE: diabetic rat treated with garlic aqueous extract.
8. DR-NS: diabetic rat treated with normal saline.
9. FBG: fasting blood glucose.

10. FI: food intake.
11. GE: garlic aqueous extract.
12. GSM: gastrocnemius skeletal muscle.
13. HR: healthy rat.
14. IHC:
15. m-RNA: messenger ribonucleic acid.
16. NR: normal rat.
17. NR-NS: normal rat treated with normal saline.
18. Nrr: number of remaining rats.
19. NS: normal saline.
20. PI: pancreas insulin.
21. SI: serum insulin.
22. STZ:
23. T&W: treatment type and weeks of treatment.
24. I.: tagged insulin.
25. TI-mRNA: tissue insulin messenger ribonucleic acid.
26. TO-IHC+I: tissue insulin immunohistochemistry + intensity.
27. UO: urine output.
28. W:
29. W1: one week.
30. W4: four weeks.
31. W8: eight weeks.
32. WI: water intake.

Declarations

Consent for publication:

Not applicable.

Availability of data and materials:

Any data used to support the findings and conclusions of this study are available from the corresponding author upon reasonable request and in due time.

Competing interest:

The authors declare that there is no conflict of interest regarding any aspect related to this study and the publication of this article.

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Authors contributions:

Dr. Amani Al-Adsani and Prof. Khaled K. Al-Qattan jointly executed this experiment, led and supervised the analyses of the samples and data and wrote this article.

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Authors' information:

Any additional information about the authors is available upon request.

Ethics approval and consent to participate:

The use of animals in this study was carried out according to 'Instructions Guide' for 'The Care and Use of Laboratory Animals', 'National Research Council'. This work was also approved by the committee (co-chaired by Prof. Khaled K. Al-Qattan) for the Use of Laboratory Animals - The Department of Biological Sciences - Kuwait University.

Experimental research and field studies on plants, including the collection of plant material:

The garlic cloves used were purchased from the local market at Kuwait City (commercially available - no permission is required) and the species of this garlic was identified by Dr. Majed A. Alnaqeeb (Associate Professor), the Department of Biological Sciences – Kuwait University. The voucher specimen number of the garlic species used is ALNAQEEB MA/001/1998 KTUH and the sample is kept at the Kuwait University Herbarium (KTUH).

References

1. Röder PV, Wu B, Liu Y, Han W: Pancreatic regulation of glucose homeostasis. *Exp Mol Med* 2016,11:48(3):e219. DOI: 10.1038/emm.2016.6.
2. Fu Z, Gilbert ER, Liu D: Regulation of insulin synthesis and secretion and pancreatic beta-cell dysfunction in diabetes. *Curr Diabetes Rev* 2013, 1:91(1):25-53. PMID:
3. Hellman B, Gylfe E, Grapengiesser E, Dansk H, Salehi A: Insulin oscillations—clinically important rhythm. Antidiabetics should increase the pulsative component of the insulin release. *Lakartidningen* 2007, 104(32–33):2236–9. PMID:17822201.
4. Eleazu CO, Eleazu KC, Chukwuma S, Essien UN: Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. *J Diabetes Metab Disord* 2013, 12:60. DOI:10.1186/2251-6581-12-60.
5. WuJ, Yan L-J: Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. *Diabetes Metab Syndr Obes* 2015, 8:181–188. DOI: [2147/DMSO.S82272](https://doi.org/10.2147/DMSO.S82272)
6. Wethrow and Macewen: *Small animals' clinical oncology: Tumor biology and metastasis* 2013, 5 ed:30-50. ISBN: 978-1-4377-2362.
7. Padiya R, Banerjee SK: Garlic as an anti-diabetic agent: Recent progress and patent reviews. *Recent Pat Food Nutr Agric* 2013, 5(2):105-127. DOI: [2174/18761429113059990002](https://doi.org/10.2174/18761429113059990002).
8. Al-Qattan KK, Thomson M, Ali M: Garlic (*Allium sativum*) attenuate glomerular glycation in streptozotocin-induced diabetic rats: A possible role of insulin. *Pathophysiol* 2013, 20:147-152. DOI: [1016/j.pathophys.2013.04.001](https://doi.org/10.1016/j.pathophys.2013.04.001)
9. Augusti KT, Sheela CG: Antiperoxide effect of S-allylcysteine sulfoxide, an insulin secretagogue, in diabetic rats. *Experientia* 1996, 15;52(2):115-120. DOI: [1007/BF01923354](https://doi.org/10.1007/BF01923354)
10. Milner, JA: Molecular targets for bioactive food components. *J Nutrition* 2004, 134:2492S–2498S. DOI: [1093/jn/134.9.2492S](https://doi.org/10.1093/jn/134.9.2492S).
11. Upadhyay S, Dixit M: Role of polyphenols and other phytochemicals on molecular signaling. *Oxid Med Cell Longev* 2015, 504253. DOI: [1155/2015/504253](https://doi.org/10.1155/2015/504253).
12. Choi S-W, Friso S: Epigenetics: A new bridge between nutrition and health. *Adv Nutr* 2010, 1(1):8–16. DOI: [3945/an.110.1004](https://doi.org/10.3945/an.110.1004).
13. Moradabadi L, Kouhsari SM, Sani MF: Hypoglycemic effects of three medicinal plants in experimental diabetes: Inhibition of rat intestinal α -glucosidase and enhanced pancreatic insulin and cardiac Glut-4 mRNAs expression. *Iran J Pharm Res* 2013, 12(3):387-97. PMID: 24250646.
14. Hajrah NH, Abdul WM, Al-Garni SM, Sheikh A, Ahmed MMM, Hall N, Saini KS, Sabir JSM, Bora RS: Gene expression profiling to elucidate the pharmacological and toxicological effects of *Ricinus communis* leaf extract in mammalian cells. *Biotechnol* 2019, 33(1):397-407. <https://doi.org/10.1080/13102818.2019.1578691>.
15. Mansouri A: *Development and regeneration in the endocrine pancreas*. ISRN Endocrinol 2012, 640956. <https://doi.org/10.5402/2012/640956>.

16. Hosseini A, Shafiee-Nick R, Ghorbani A: Pancreatic beta cell protection/regeneration with phytotherapy. *Braz J Pharm Sci* 2015, *On-line version* ISSN 2175-979. <http://dx.doi.org/10.1590/S1984-82502015000100001>.
17. Kaur G, Padiya R, Adela R, Putcha UK, Reddy GS, Reddy BR, Kumar KP, Chakravarty S, Banerjee SK: Garlic and Resveratrol Attenuate Diabetic Complications, Loss of β -Cells, Pancreatic and Hepatic Oxidative Stress in Streptozotocin-Induced Diabetic Rats. *Front Pharmacol* 2016, 13(7):360. DOI: [3389/fphar.2016.00360](https://doi.org/10.3389/fphar.2016.00360).
18. Ferber S, Halkin A, Cohen H, Ber I, Einav Y, Goldberg I, Barshack I, Seiffers R, Kopolovic J, Kaiser N, Karasik A: Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. *Nat Medicine* 2000, 6(5):568-572. DOI: [10.1038/75050](https://doi.org/10.1038/75050).
19. Horb ME, Shen CN, Tosh D, Slack JMW: Experimental conversion of liver to pancreas. *Curr Biology* 2003, 13(2):105-15. DOI: [1016/s0960-9822\(02\)01434-3](https://doi.org/10.1016/s0960-9822(02)01434-3).
20. Zalzman M, Anker-Kitai L, Efrat S: Differentiation of human liver-derived, insulin-producing cells toward the beta-cell phenotype. *Diabetes* 2005, 54(9):2568-2575. <https://doi.org/10.2337/diabetes.54.9.2568>.
21. Sahu S, Joglekar MV, Dumbre R, Phadnis SM, Tosh D, Hardikar AA: Islet-like cell clusters occur naturally in human gall bladder and are retained in diabetic conditions. *J Cell Mol Med* 2009, 13(5):999-1000. DOI: [1111/j.1582-4934.2008.00572.x](https://doi.org/10.1111/j.1582-4934.2008.00572.x).
22. Minami K, Okuno M, Miyawaki K, Okumachi A, Ishizaki K, Oyama K, Kawaguchi M, Ishizuka N, Iwanaga T, Seino S: Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. *Proc Natl Acad Sci* 2005, 102(42):15116-15121. DOI: [10.1073/pnas.0507567102](https://doi.org/10.1073/pnas.0507567102).
23. Dutton JR, Chillingworth NL, Eberhard D, Brannon CR, Hornsey MA, Tosh D, Slack JMW: Beta cells occur naturally in extrahepatic bile ducts of mice. *J Cell Sci* 2007, 120(2):239-245. DOI: [1242/jcs.03330](https://doi.org/10.1242/jcs.03330).
24. Nagaya M, Katsuta H, Kaneto H, Bonner-Weir S, Weir GC: Adult mouse intrahepatic biliary epithelial cells induced in vitro to become insulin-producing cells. *J Endocrinol* 2009, 201(1):37-47. DOI: [1677/joe-08-0482](https://doi.org/10.1677/joe-08-0482).
25. Kojima H, Fujimiya M, Matsumura K, Nakahara T, Hara M, Chan L: Extrapancreatic insulin-producing cells in multiple organs in diabetes. *PNAS* 2004, 101(8):2458-2463. DOI: [1073/pnas.0308690100](https://doi.org/10.1073/pnas.0308690100).
26. Mitutsova V, Yeo WWY, Davaze R, Franckhauser C, Hani E, Abdullah S, Mollard P, Schaeffer M, Fernandez A, Lamb NJC: Adult muscle-derived stem cells engraft and differentiate into insulin-expressing cells in pancreatic islets of diabetic mice. *Stem Cell Res Ther* 2017, 8:86. DOI: [1186/s13287-017-0539-9](https://doi.org/10.1186/s13287-017-0539-9).
27. Wang J, Zhang F, Yang H, Wu H, Cui R, Zhao Y, Jiao C, Wang X, Liu X, Wu L, Li G, Wu X. [Effect of TEAD4 on multilineage differentiation of muscle-derived stem cells](#). *Am J Transl Res* 2018, 10(3):998-1011. PMID: [29636889](https://pubmed.ncbi.nlm.nih.gov/29636889/).

28. *Guide for the Care and Use of Laboratory Animals*: Institute of Laboratory Animal Resources: National Research Council: National Academy Press 1996, 8 ed. www.nap.edu
29. Thomson M, Al-Qattan KK, Divya JS, Ali M: Anti-diabetic and anti-oxidant potential of aged garlic extract (AGE) in streptozotocin-induced diabetic rats. *BMC Complement Altern Med* 2016, 16:17. DOI: [1186/s12906-016-0992-5](https://doi.org/10.1186/s12906-016-0992-5)
30. Thomson M, Al-Amin ZM, Al-Qattan KK, Shaban LH, Ali M: Anti-diabetic and hypolipidaemic properties of garlic (*Allium sativum*) in streptozotocin-induced diabetic rats. *Int J Diabetes Metab* 2007, 15:108-115. DOI: NA.
31. Thomson M, Al-Qattan KK, Divya JS, Ali M: Ameliorative actions of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on biomarkers of diabetes and diabetic nephropathy in rats: comparison to aspirin. *Int J Pharmacol* 2013, (8):501–512. <https://dx.doi.org/10.3923/ijp.2013.501.512>.
32. Rother KI, Harlan DM: [Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus](#). *J Clin Invest* 2004, 114(7):877-83. DOI: [1172/JCI23235](https://doi.org/10.1172/JCI23235).
33. Kim HS, Lee MK: [β-Cell regeneration through the transdifferentiation of pancreatic cells: Pancreatic progenitor cells in the pancreas](#). *J Diabetes Investig* 2016, 7(3):286-96. DOI: [1111/jdi.12475](https://doi.org/10.1111/jdi.12475).
34. Sorenson RL, Brelje TC: Adaptation of islets of Langerhans to pregnancy: beta-cell growth, enhanced insulin secretion and the role of lactogenic hormones. *Horm Metab Res* 1997, 29(6):301-7. DOI: [1055/s-2007-979040](https://doi.org/10.1055/s-2007-979040).
35. Butler PC, Meier JJ, Butler AE, Bhushan A: [The replication of beta cells in normal physiology, in disease and for therapy](#). *Nat Clin Pract Endocrinol Metab* 2007, 3(11):758-68. DOI: [1038/ncpendmet0647](https://doi.org/10.1038/ncpendmet0647).
36. Cito M, Pellegrini S, Piemonti L, Sordi V: [The potential and challenges of alternative sources of β cells for the cure of type 1 diabetes](#). *Endocr Connect* 2018, 7(3):R114-R125. DOI: [1530/EC-18-0012](https://doi.org/10.1530/EC-18-0012).
37. Venkatesan V, Gopurappilly R, Goteti SK, Dorisetty RK, Bhonde RR: [Pancreatic progenitors: The shortest route to restore islet cell mass](#). *Islets* 2011, 3(6):295-301. DOI: [4161/isl.3.6.17704](https://doi.org/10.4161/isl.3.6.17704).
38. Mfopou JK, Houbracken I, Wauters E, Mathijs I, Song I, Himpe E, Baldan J, Heimberg H, Bouwens L: Acinar phenotype is preserved in human exocrine pancreas cells cultured at low temperature: implications for lineage-tracing of β-cell neogenesis. *Biosci Rep* 2016, 36(3): e00329. DOI: [1042/BSR20150259](https://doi.org/10.1042/BSR20150259).
39. van der Meulen T, Mawla AM, DiGruccio MR, Adams MW, Nies V, Dölleman S, Liu S, Ackermann AM, Cáceres E, Hunter AE, Kaestner KH, Donaldson CJ, Huising MO: Virgin Beta Cells Persist throughout Life at a Neogenic Niche within Pancreatic Islets. *Cell Metab* 2017, 25(4):911-926. DOI: [1016/j.cmet.2017.03.017](https://doi.org/10.1016/j.cmet.2017.03.017).
40. Gerace D, Martiniello-Wilks R, O'Brien BA, Simpson AM: The use of β-cell transcription factors in engineering artificial β cells from non-pancreatic tissue. *Gene Ther* 2015, 22:1-8. DOI: NA.
41. Akhurst B, Croager EJ, Farley-Roche CA, Ong JK, Dumble ML, Knight B, Yeoh GC: [A modified choline-deficient, ethionine-supplemented diet protocol effectively induces oval cells in mouse liver](#). *Hepatology* 2001, 34(3):519-22. DOI: [1053/jhep.2001.26751](https://doi.org/10.1053/jhep.2001.26751).

42. Ferber S, Halkin A, Cohen H, Ber I, Einav Y, Goldberg I, Barshack I, Seiffers R, Kopolovic J, Kaiser N, Karasik A: Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. *Nat Med* 2000, 6(5):568-72. PMID: 26279745.
43. Zalzman M, Anker-Kitai L, Efrat S: Differentiation of human liver-derived, insulin-producing cells toward the beta-cell phenotype. *Diabetes* 2005, 54(9):2568-75. DOI: 2337/diabetes.54.9.2568.
44. Murakami-Kawaguchi S, Takasawa S, Onogawa T, Nata K, Itaya-Hironaka A, Sakuramoto-Tsuchida S, Yamauchi A, Ota H, Takeda M, Kato M, Okamoto H: Expression of Ins1 and Ins2 genes in mouse fetal liver. *Cell Tissue Res* 2014, 355(2):303-14. DOI: 1007/s00441-013-1741-4.
45. Dutton JR, Chillingworth NL, Eberhard D, Brannon CR, Hornsey MA, Tosh D, Slack JM: Beta cells occur naturally in extrahepatic bile ducts of mice. *J Cell Sci* 2007, 120(2):239-45. DOI: 1242/jcs.03330
46. Kim S, Shin JS, Kim HJ, Fisher RC, Lee MJ, Kim CW: Streptozotocin-induced diabetes can be reversed by hepatic oval cell activation through hepatic transdifferentiation and pancreatic islet regeneration. *Lab Invest* 2007, 87(7):702-712. DOI: 1038/labinvest.3700561.
47. Vorobeychik M, Bloch K, Zemel R, Bachmetov L, Tur-Kaspa R, Vardi P: Immunohistochemical evaluation of hepatic oval cell activation and differentiation toward pancreatic beta-cell phenotype in streptozotocin-induced diabetic mice. *Mol. Histol* 2008, 39(4):463-8. DOI: 10.1210/me.2009-0311.
48. Preisegger KH, Factor VM, Fuchsbichler A, Stumptner C, Denk H, Thorgeirsson SS: Atypical ductular proliferation and its inhibition by transforming growth factor beta1 in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine mouse model for chronic alcoholic liver disease. *Lab Invest* 1999, 79(2):103-9. PMID: 10068199.
49. Al-Qattan KK, Al-Otaibi A, Al-Adsani A, Al-Bader M, Thomson M, Divya SJ, Miranda L, Al-Bustan S: Raw Garlic Aqueous Extract (RGAE) Augments *MafA* Expression in the Liver of STZ-Induced Type-1 Diabetic Rats. *The FASEB Journal* 2017, 13. DOI: NA.

Figures

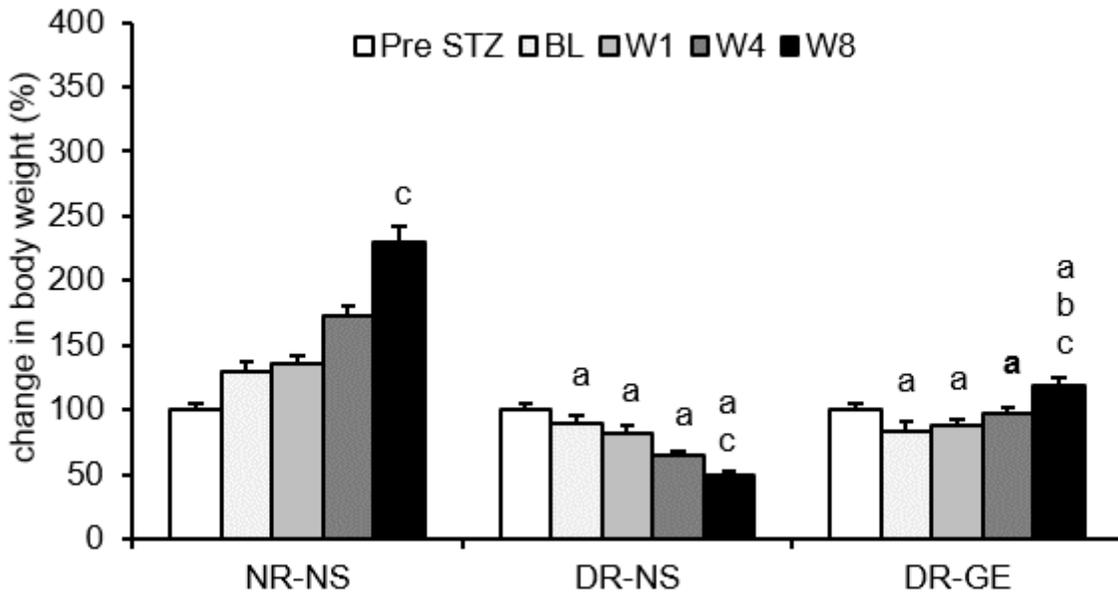


Figure 2

Effect of garlic extract on body weight. The body weight of diabetic rats treated with GE showed an initial, although insignificant, drop 7 days post injection (BL), which increased gradually thereafter until showing significant gain compared to its BL and DR-NS level at W8. Pre STZ: pre-streptozotocin injection; BL: basal level = day 7 post STZ injection = pretreatment; W1: week 1; W4: week 4; W8: week 8; NR-NS: normal rats treated with normal saline; DR-NS: diabetic rats treated with normal saline; DR-GE: diabetic rats treated with garlic extract; a: significantly different compared to NR-NS at the same stage; b: significantly different compared to DR-NS at the same stage. c: significantly different compared to BL.

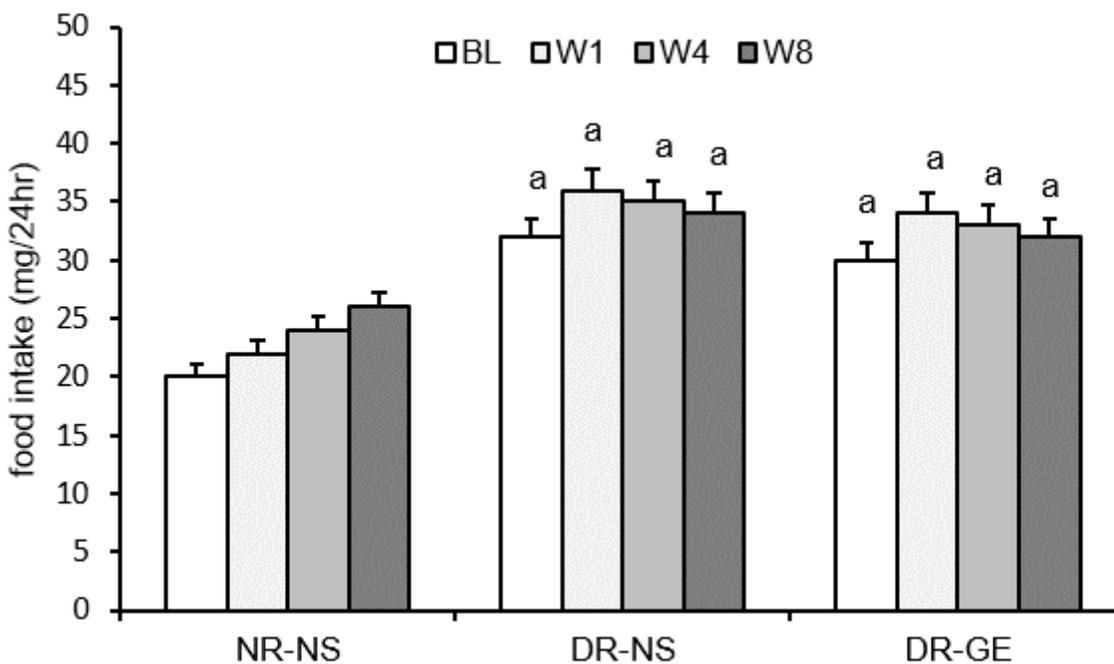


Figure 3

Effect of garlic extract on food intake. The food intake of diabetic rats treated with GE showed an overall insignificant increase which decreased gradually as treatment continued for the 8 weeks period. BL: basal level = day 7 post STZ-injection = pretreatment; W1: week 1; W4: week 4; W8: week 8; NR-NS: normal rats treated with normal saline; DR-NS: diabetic rats treated with normal saline; DR-GE: diabetic rats treated with garlic extract; a: significantly different compared to NR-NS at the same stage.

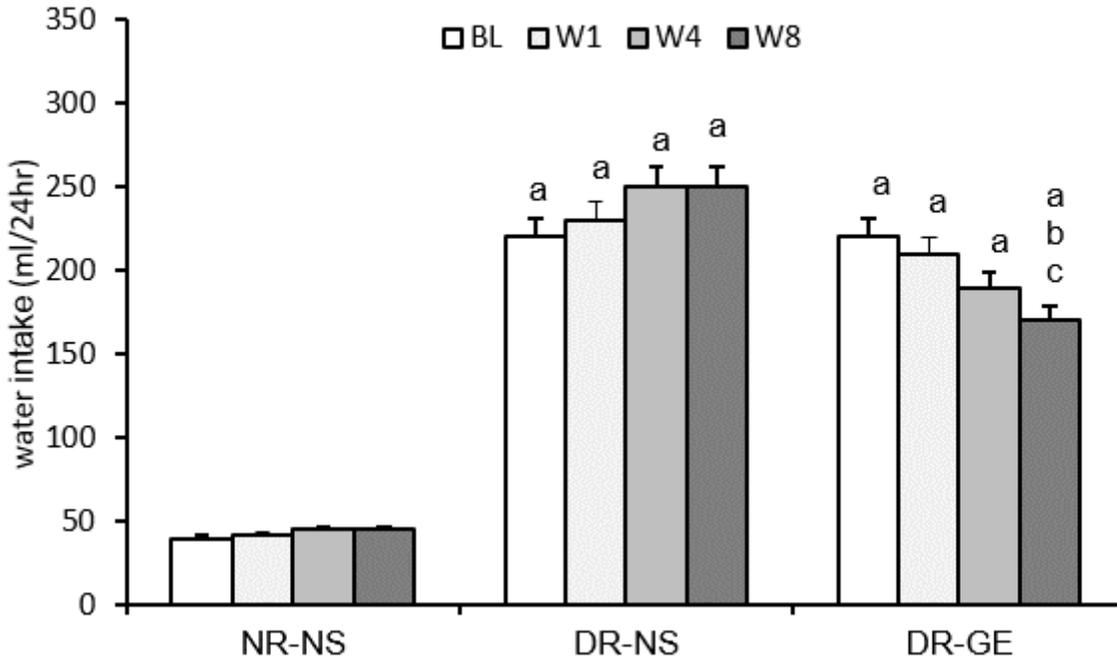


Figure 4

Effect of garlic extract on water intake. The water intake of diabetic rats treated with GE showed a continuous gradual decrease reaching a significant difference at W8 compared to its BL value and the DR-NS at the same time stage. BL: basal level = day 7 post STZ-injection = pretreatment; W1: week 1; W4: week 4; W8: week 8; NR-NS: normal rats treated with normal saline; DR-NS: diabetic rats treated with normal saline; DR-GE: diabetic rats treated with garlic extract; a: significantly different compared to NR-NS at the same stage; b: significantly different compared to DR-NS at the same stage; c: significantly different compared to BL.

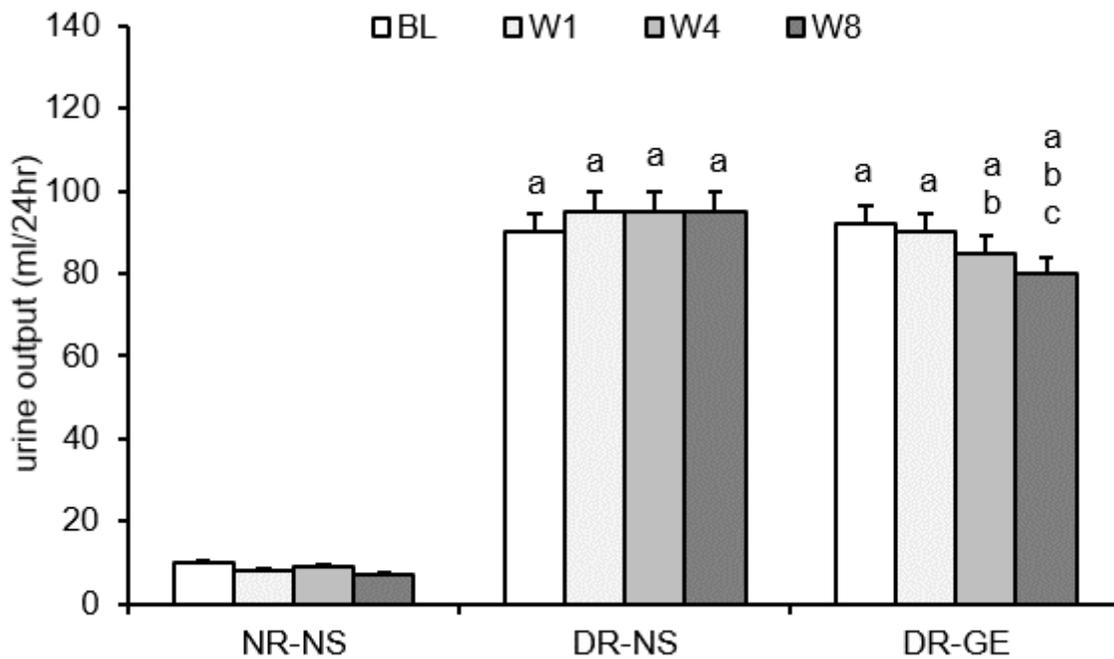


Figure 5

Effect of garlic extract on urine output. The urine output of diabetic rats treated with GE decreased continuously as treatment continued reaching a maximum decrease at W4 and W8 compared to values of DR-NS and its own BL at W8. BL: basal level = day 7 post STZ-injection = pretreatment; W1: week 1; W4: week 4; W8: week 8; NR-NS: normal rats treated with normal saline; DR-NS: diabetic rats treated with normal saline; DR-GE: diabetic rats treated with garlic extract; a: significantly different compared to NR-NS at the same stage; b: significantly different compared to DR-NS at the same stage; c: significantly different compared to BL.

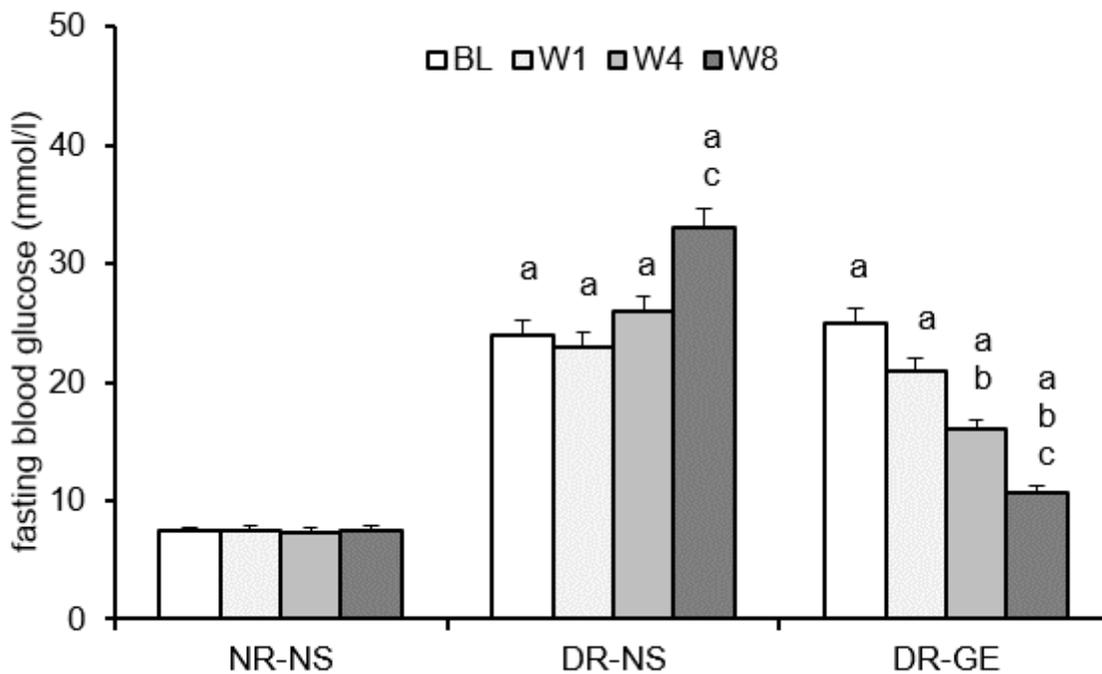


Figure 6

Effect of garlic extract on fasting blood glucose. The FBG of diabetic rats treated with GE decreased continuously reaching significant decline at W4 and W8 compared to the values of the DR-NS and its BL only at W8. BL: basal level = day 7 post STZ-injection = pretreatment; W1: week 1; W4: week 4; W8: week 8; NR-NS: normal rats treated with normal saline; DR-NS: diabetic rats treated with normal saline; DR-GE: diabetic rats treated with garlic extract; a: significantly different compared to NR-NS at the same stage; b: significantly different compared DR-NS at the same stage. c: significantly different compared to BL.

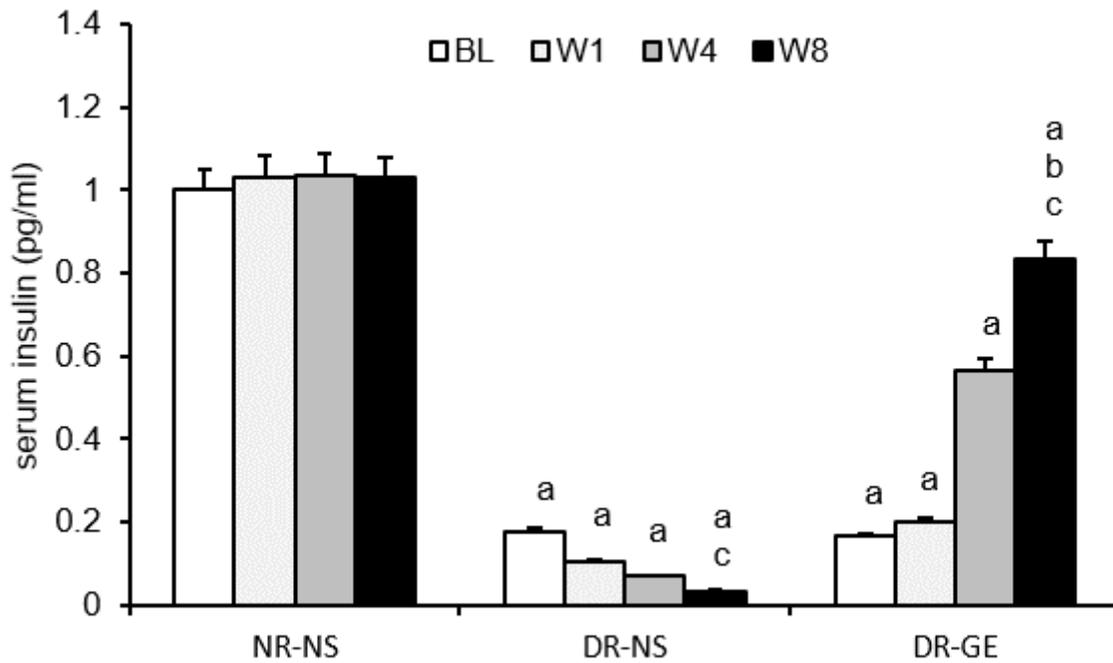


Figure 7

Effect of garlic extract on serum insulin. The serum insulin of diabetic rats treated with GE showed substantial increase at the last two stages of measurement, although it was significantly higher at W8 compared to its BL and the value of W8 for the DR-NS. BL: basal level = day 7 post STZ-injection = pretreatment; W1: week 1; W4: week 4; W8: week 8; NR-NS: normal rats treated with normal saline; DR-NS: diabetic rats treated with normal saline; DR-GE: diabetic rats treated with garlic extract; a: significantly different compared to NR-NS at the same stage; b: significantly different compared to DR-NS at the same stage; c: significantly different compared to BL.

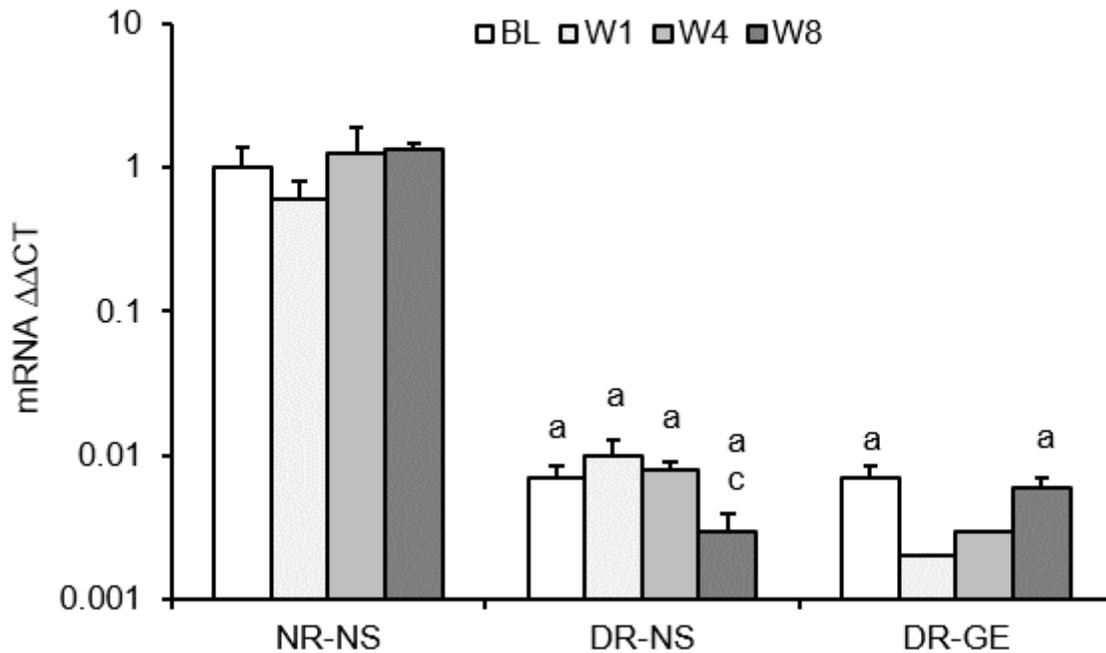


Figure 8

Effect of garlic extract on pancreas insulin m-RNA. The mRNA values of diabetic rats treated with NS showed a continuous decline reaching a significant decrease at W8. Oppositely, the mRNA values of the diabetic rats treated with GE showed a certain trend that started with an initial decrease at the BL stage and then gradually increased reaching a maximum at W8. The trend of change in the pancreas insulin mRNA of both the DR-NS and DR-GE paralleled those of pancreas insulin IHC and protein concentration and serum insulin protein concentration. BL: basal level = day 7 post STZ-injection = pretreatment; W: week = period of treatment; NR-NS: normal rats treated with normal saline; DR-NS: diabetic rats treated with normal saline; DR-GE: diabetic rats treated with garlic extract; a: significantly different compared NR-NS at the same stage; c: significantly different compared to BL.

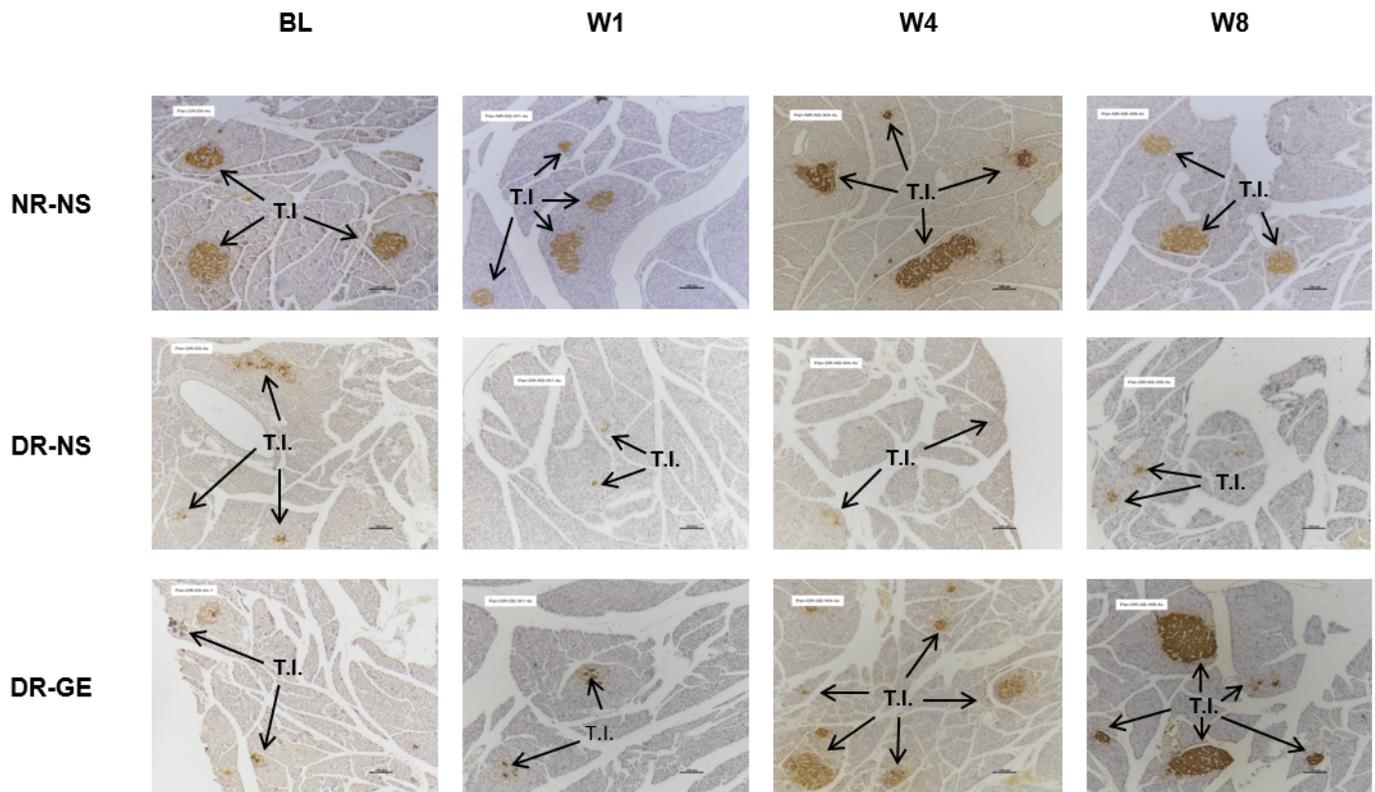


Figure 9

Effect of garlic extract on pancreas insulin immunohistochemical localization. The pancreas of normal rats treated with NS showed an evident, typical distribution of insulin tagging throughout the targeted time-stages. Alternatively, the pancreas of diabetic rats treated with NS showed considerably reduced density and scarcity in insulin tagging. In the diabetic rats treated with GE and compared to the basal level (= before commencing treatment), the pancreas showed a gradual increment in the density and distribution of insulin tagging reaching maximum at week 8. BL: basal level = day 7 post streptozotocin = pretreatment; Different periods of treatment = W1: week 1; W4: week 4; W8: week 8; NR-NS: normal rats treated with normal saline; DR-NS: diabetic rats treated with normal saline; DR-GE: diabetic rat – treated with garlic extract T.I.: tagged insulin. (magnification = 4x, scale bar = 100 µm).

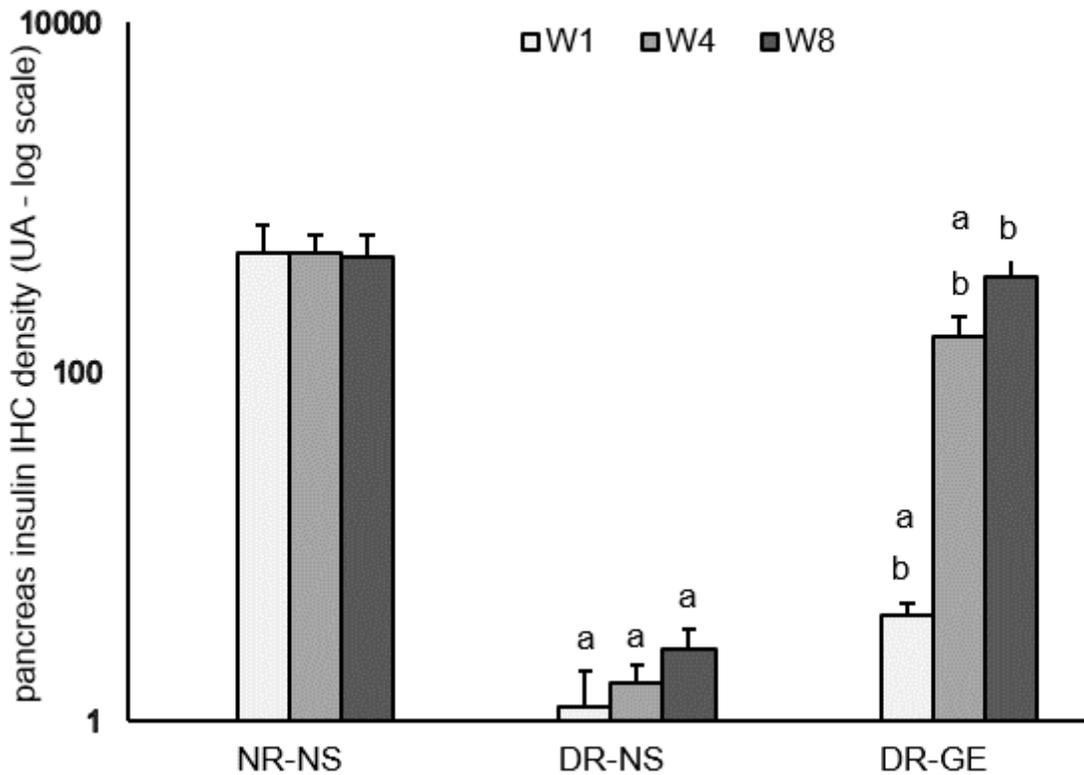


Figure 10

Effect of garlic extract on pancreas insulin immunohistochemical numerical intensity. Pancreatic insulin IHC intensity in diabetic rats treated with GE showed a significant continuous increase when compared to the values of DR-NS at all corresponding stages of treatment. Furthermore, the DR-GE although showed significant differences compared to the values of the NR-NS at the first two stages (W1 & W4), however the observed differences were decreasing until it was totally abolished at the last stage of the treatment (W8). W1: week 1; W4: week 4; W8: week 8; NR-NS: normal rats treated with normal saline; DR-NS: diabetic rats treated with normal saline; DR-GE: diabetic rats treated with garlic extract; a: significantly different compared to NR-NS at the same stage; b: significantly different compared DR-NS at the same stage.

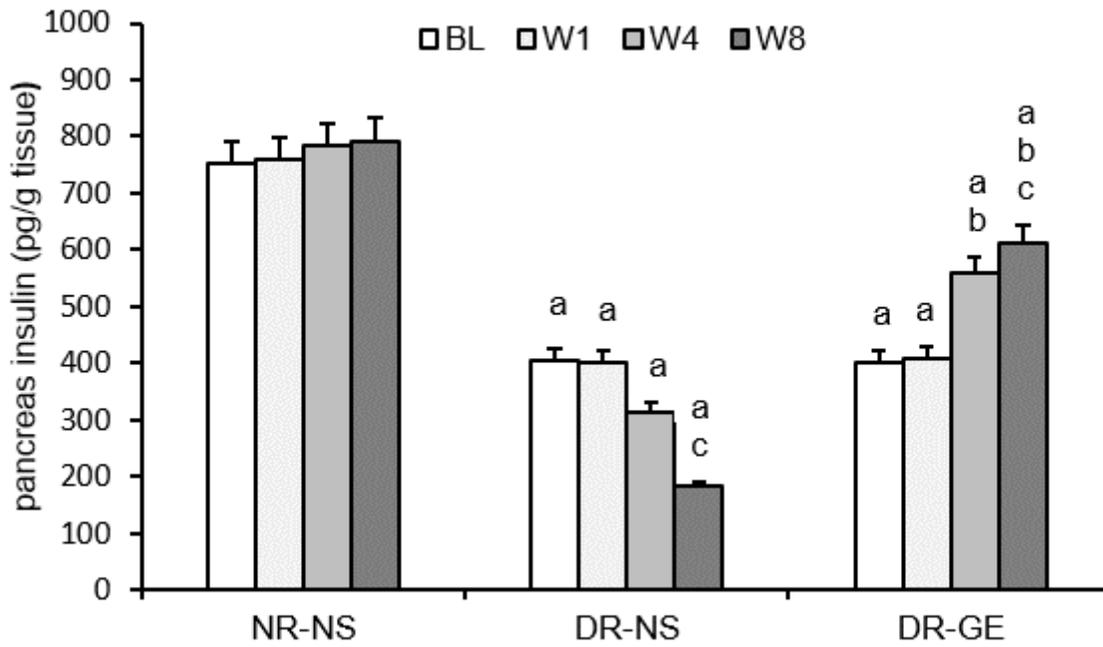


Figure 11

Effect of garlic extract on pancreas insulin. The pancreas insulin of diabetic rats treated with GE showed a significant continuous increase at W4 and W8 compared to the values of DR-NS and its own BL values at W8. BL: basal level = day 7 post STZ-injection = pretreatment; W1: week 1; W4: week 4; W8: week 8; NR-NS: normal rats treated with normal saline; DR-NS: diabetic rats treated with normal saline; DR-GE: diabetic rats treated with garlic extract; a: significantly different compared to NR-NS at the same stage; b: significantly different compared DR-NS at the same stage; c: significantly different compared to BL.

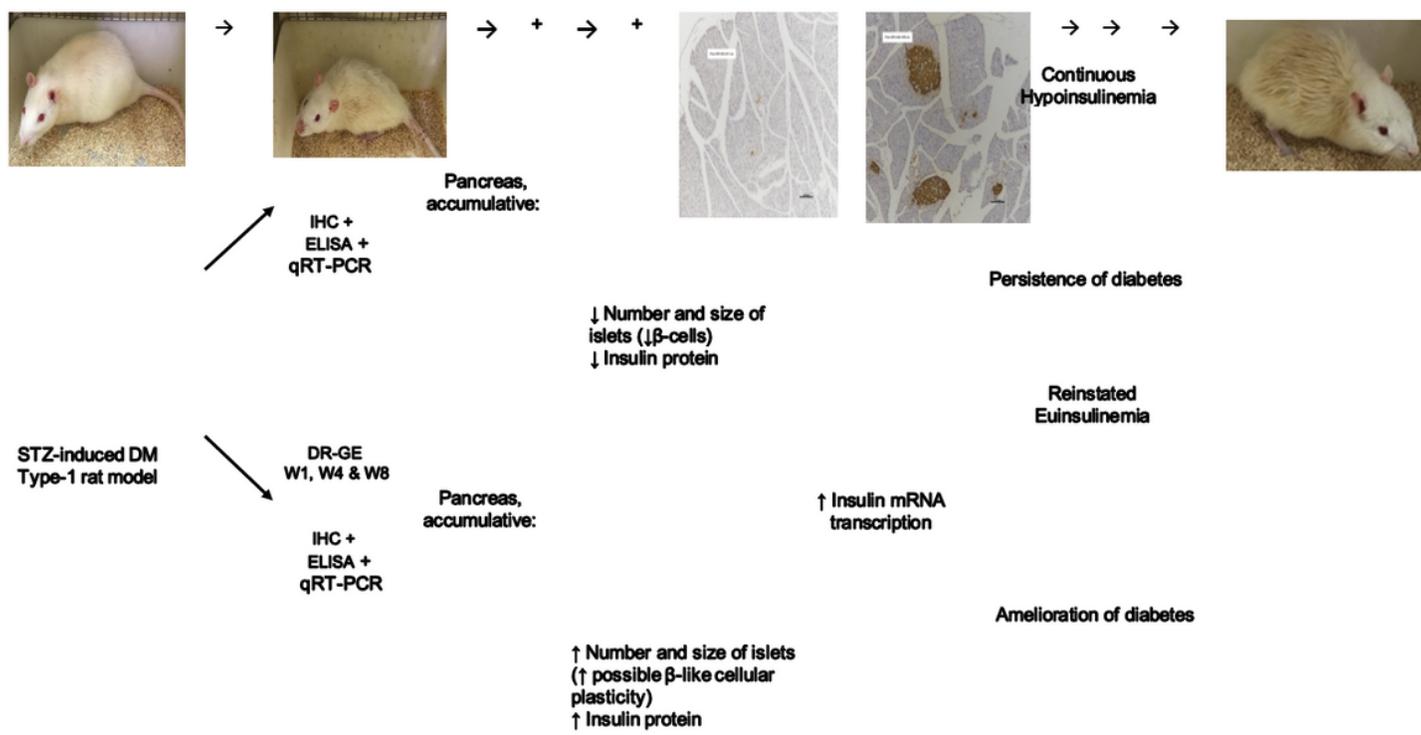


Figure 12

Suggested garlic extract insulinogenesis mechanism in the pancreas of STZ-diabetic rat. This schematic illustration demonstrates the suggested mechanism by which oral treatment with GE for a period of 8 weeks and monitored at the different stages (W1, W4 and W8) leads to progressive augmentations in pancreatic insulin m-RNA expression, insulin concentration and Islets of Langerhans quantity, cellular plasticity and intensity and hence amelioration of monitored diabetic symptoms. STZ: streptozotocin; DM: diabetes mellitus; NS: normal saline, W1: week 1; W4: week 4; W8: week; qRT-PCR: quantities real time – PCR; IHC: immunohistochemistry; GE: garlic extract.